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HEALTH: *Short Communication*

Controlling bovine leukemia virus in a large dairy herd by selective culling based on diagnostic testing

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ABSTRACT

Objective: This intervention field trial aimed to reduce bovine leukemia virus (BLV) from a large dairy herd using a new diagnostic test to identify the most infectious cows for segregation or culling. Bovine leukemia virus infects over 40% of all dairy cattle in the United States. Similar prevalence has been reported in many other countries. Previously hidden economic impacts of BLV on the industry have only recently been recognized. The traditional method of controlling this disease was by culling all antibody-positive cattle. The availability of a new diagnostic test can identify the most infectious cows for removal.

Materials and Methods: A 3,000-cow dairy farm conducted a 4-yr BLV disease control program using blood lymphocyte count (LC), BLV ELISA antibody test, and quantitative-PCR proviral load (PVL) to identify the most infectious animals for segregation or culling.

Results and Discussion: The percentage of cows with a high LC was significantly decreased from 4.22 to 1.04% after the first year of selective culling of cows with high LC. Thereafter, BLV ELISA screening with PVL follow-up assays were used to target segregation and culling of cows with the highest PVL. After 3 yr, only 0.85% of the tested cows were BLV ELISA positive. The prevalence of animals with detectable PVL decreased from 284 to zero animals.

Implications and Applications: When coupled with the results of an intervention trial in 3 small dairy farms, our findings from a large dairy herd demonstrate that BLV may be eradicated without the simultaneous culling of all ELISA-positive cows.

Key words: proviral load, bovine leukosis, ELISA, lymphocyte counts

INTRODUCTION

Bovine leukemia virus (**BLV**) infects approximately 40% of dairy cattle within the United States, and many other nations have similarly high prevalence (LaDronka et al., 2018; Bartlett et al., 2020). Beef cattle are also infected but usually at lower prevalence. Research in the last 15 yr shows that BLV reduces milk production, shortens cow lifespan, and predisposes animals to diseases such as lymphoma and mastitis (Frie et al., 2016; Bartlett et al., 2020).

Over 20 countries eradicated BLV by simultaneously removing all animals with detectable BLV-specific antibodies (Bartlett et al., 2014; EFSA, 2015). However, these countries generally started their control when their BLV prevalence was very low. The high prevalence now existing in many herds makes it economically difficult to simultaneously cull all their ELISA-positive milking cows and youngstock. Fortunately, a diagnostic test allows producers to control BLV by identifying the most infectious cattle for segregation or culling while retaining the majority of ELISA-positive cattle, which are lowly infectious to their herd mates (Juliarena et al., 2016; Ruggiero et al., 2019; Hutchinson, 2020; Taxis et al., 2020).

The 3 diagnostic tests used for this study measure different effects of BLV infection. Blood lymphocyte count detects animals with high blood concentrations of lymphocytes (lymphocytosis). The BLV ELISA detects BLV-specific antibodies as evidence of previous life-long infection. Last, BLV quantitative-PCR (**qPCR**) enables the measurement of blood proviral load (**PVL**), which is the concentration of detectable BLV provirus (Bartlett et al., 2020). The BLV provirus within infected lymphocytes is the infectious agent for transmission to new hosts. Cattle with greater PVL are thought to be responsible for most transmission (Rodriguez et al., 2011; Mekata et al., 2015; Juliarena et al., 2016; Bartlett et al., 2020; Hutchinson, 2020).

This report of an intervention field trial describes how one herd effectively controlled BLV using the 3 available

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diagnostic assays to direct segregation or culling of the most infectious cattle while avoiding the need to cull all antibody-positive cattle as has traditionally been necessary.

MATERIALS AND METHODS

This intervention study was approved by the Michigan State University's Animal Use Committee under MSU IACUC approval number PROTO201900271 on July 29, 2019.

The herd used for this study was an approximately 3,000-head milking Holstein dairy herd in northeast Wisconsin. This farm used a computerized system for all record keeping such as animal health and events, lameness, milk production, diagnostic results, medical treatments, and culling data. Additionally, barns on this farm were either sprayed or tunnel ventilated for fly control, and horizontal translation of the virus was prevented through the use of pulse guns for injections, single-use palpation sleeves, and paste for dehorning, and all hoof trimming equipment was sterilized at the end of each day. Furthermore, all cows were bred through AI, and calves only received their own dam's colostrum. These management protocols remained constant throughout the entirety of the study.

This herd raised its own replacement heifers and had already been screening heifers for BLV for its active embryo transplant program. The farm manager sought to implement a BLV-control program that would be less costly and disruptive than the traditional method of simultaneously culling all ELISA-positive cows. Blood was collected from cattle at parturition during the first quarter of the study and at mid-lactation points through the second, third, and fourth quarters of the study. Blood was collected through tail vein or artery.

Removal of cattle with a lymphocyte count over 10,000 cells/μL was accomplished during the first year. As these high lymphocyte cows became scarcer, the herd transitioned to using ELISA assays to test for BLV antibodies in milk or blood, with all ELISA-positive samples being further tested for PVL determination by qPCR at CentralStar Laboratories, Lansing, Michigan (Taxis et al., 2020).

Antibodies to BLV were detected through the use of an ELISA test using individual milk samples from routinely collected DHIA tests. Sample aliquots were diluted in sample buffer and pipetted into 96-well plates coated with BLV antigen. Horseradish-peroxidase-labeled bovine anti-immunoglobulin antibodies were added and incubated. Plates were washed after each incubation and before adding an enzyme substrate. Reaction times were standardized using color development of positive controls and stopped by adding 0.5 M NH_2SO_4. Results were reported as adjusted 450-nm optical density measurements with a corrected optical density >0.3 considered antibody positive.

To obtain PVL measurements, DNA was extracted from whole blood samples via the Qiagen DNeasy blood and tissue kit to consistently isolate DNA >30 ng/μL for use in the proviral qPCR assay. This extraction method was changed to the King Fisher MagMAX Core magnetic bead-based automated nucleic acid system, as this was preferred by CentralStar Cooperative Inc., after performing comparison validations 9 mo into the study. The SS1 qPCR assay, developed by CentralStar Cooperative Inc., is a multiplex probe-based qPCR assay that targets the BLV proviral polymerase gene, bovine β-actin gene, and an internal amplification spike-in control ultramer to quantify PVL. Briefly, 4 μL of extracted DNA, 12.5 μL of 2× PrimeTime Gene Expression Master Mix (Integrated DNA Technologies), 1.25 μL of a 20× primer mix, 1 μL of an internal spike-in control (10,000 copies/μL), and 7.25 μL of DNA-free water were combined for each qPCR reaction. All qPCR was performed on a Applied Biosystems 7500 Fast Real-Time PCR system with qPCR conditions as follows: 95°C for 10 min, 40× (95°C) for 15 s, (60°C) for 1 min. Bovine leukemia virus and β-actin (measure of bovine genomes) copy numbers were derived using a standard curve consisting of linearized plasmids containing respective target sequences previously quantified and normalized by digital droplet PCR. Amplification efficiency and manual thresholds were established from initial qPCR machine calibration and used for the duration of the study. Proviral load was calculated and expressed as the ratio between proviral BLV copies and bovine β-actin copies.

This testing was performed on each heifer upon entering the milking herd and again about 2 mo after each calving. Once a cow was found to be BLV ELISA positive, no subsequent BLV ELISA was ever performed, and only PVL was monitored annually thereafter. Bovine leukemia virus PVL thresholds for segregation or culling were continually reduced according to the herd's stocking needs and as the herd's PVL values decreased over time. As was true for the previous 3 herds using this control method (Ruggiero et al., 2019), producers culled the cows with the highest PVL, but these culling decisions were always tempered by the need to maintain the desired stocking rate, the availability of replacement heifers, the market for milk and meat, the availability of segregation facilities, and undoubtedly many other factors. As the control program progressed, cows with the highest PVL values had already been removed, so cows with progressively lower PVL values became targeted for culling. As such, the criteria for culling changed on a weekly basis but generally became more stringent. Sometimes culling was preceded by temporary segregation in a pen of animals that were not to be inseminated and were destined for culling after delivery of a calf or after milk production diminished.

Data were compiled and the results discussed with the farm manager on a quarterly basis. By the end of the second year, thresholds had been adjusted such that any animal with detectable PVL was immediately culled from the herd.

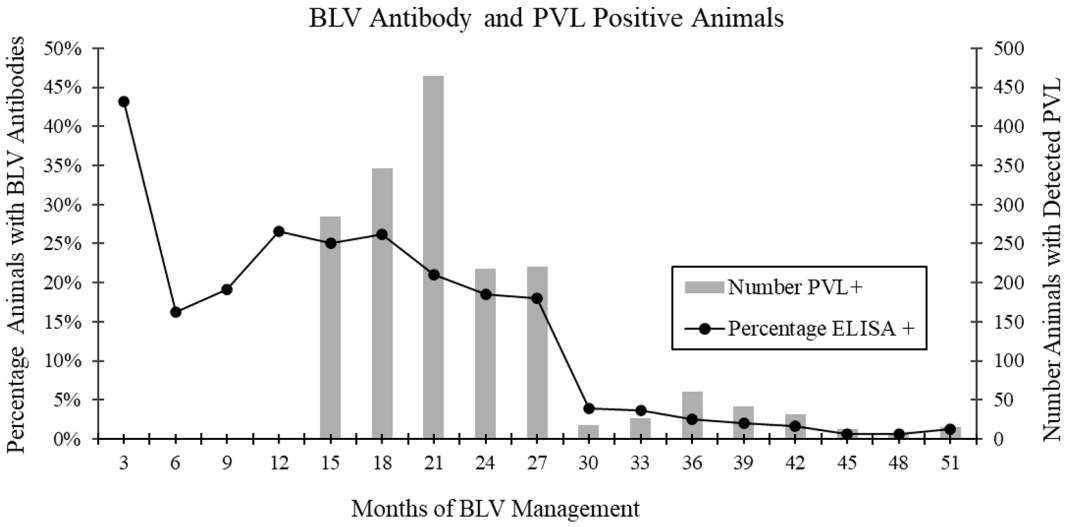

Figure 1. An intervention study was conducted to control bovine leukemia virus (BLV) by selectively culling or segregating those cows most likely to transmit BLV to their herd mates. In 4 yr, the percentage of BLV ELISA-positive cows on a 3,000-head Wisconsin dairy farm declined, and the number of cows with a detectable proviral load (PVL) was also reduced.

RESULTS AND DISCUSSION

Within the first year of BLV management, we measured a 20.8% BLV prevalence with a 95% CI (19.43, 22.28) using the Clopper-Pearson method (Taxis et al., 2020). Additionally, the mean PVL decreased from 1.74 ± 0.17 to 0.14 ± 0.02 for first-lactation cows ($P < 0.001$), 1.96 ± 0.13 to 0.27 ± 0.04 for second-lactation cows ($P < 0.001$), and 2.42 ± 0.11 to 0.21 ± 0.02 for third- and higher-lactation cows ($P < 0.001$).

At the end of the second year of BLV management, 3% of tested cows (n = 204) were BLV ELISA positive, only 6 of which had detectable PVL. At the end of the third year, 0.85% of the tested cows were BLV ELISA positive (Figure 1), and animals with detectable PVL had decreased from 284 at the start of the project to zero animals at the end of the project.

The use of milk BLV ELISA assays reduced labor costs by eliminating the need for labor-intensive blood collection.

Using this new approach to BLV control, the long-term economic benefit of eliminating BLV from a herd may likely outweigh the temporary disease-control costs (Rhodes et al., 2003; Erskine et al., 2012; Kuczewski et al., 2019; Bartlett et al., 2020). Additional research is needed for a cost-benefit analysis of BLV control in both dairy and beef herds operating under widely divergent management systems with a varied range of BLV prevalence.

APPLICATIONS

This intervention study was conducted in one large dairy farm, and there can be no assurances that our findings would be repeatable elsewhere. The control methods we used evolved over time, and there is no indication that the optimal control protocol was ever reached. However, the methods we used may inform other herd managers and help achieve a similar outcome with timeline and thresholds being adjusted to their needs. Clearly, we need multiherd, controlled intervention trials once reasonable BLV-control protocols can be proposed based on individual experiences such as the one described here. Study of this large Wisconsin herd and the 3 small herds described by Ruggiero et al. (2019) showed that BLV diagnostic tests could be used to reduce or eliminate BLV infections without the costly simultaneous culling of all antibody-positive cattle. This breakthrough in control was made possible by the development of the BLV qPCR test and recent revelations, summarized by Bartlett et al. (2020), that most ELISA-positive cattle have low PVL and rarely transmit to their herd mates. It may now be possible for more herds and nations to control this disease for the improvement of farm profitability as well as animal health and welfare.

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LITERATURE CITED

Bartlett, P. C., V. J. Ruggiero, H. C. Hutchinson, C. J. Droscha, B. Norby, K. R. B. Sporer, and T. M. Taxis. 2020. Current developments in the epidemiology and control of enzoonotic bovine leukosis as caused by bovine leukemia virus. Pathogens 9:1058. https://doi.org/10.3390/pathogens9121058.

Bartlett, P. C., L. M. Sordillo, T. M. Byrem, B. Norby, D. L. Grooms, C. L. Swenson, J. Zalucha, and R. J. Erskine. 2014. Options for the control of bovine leukemia virus in dairy cattle. J. Am. Vet. Med. Assoc. 244:914–922. https://doi.org/10.2460/javma.244.8.914.

EFSA Panel on Animal Health and Welfare. 2015. Enzootic bovine leukosis. EFSA J. 13:4188. https://doi.org/10.2903/j.efsa.2015.4188.

Erskine, R. J., P. C. Bartlett, T. M. Byrem, C. L. Render, C. Febvay, and J. T. Houseman. 2012. Association between bovine leukemia virus, production, and population age in Michigan dairy herds. J. Dairy Sci. 95:727–734. https://doi.org/10.3168/jds.2011-4760.

Frie, M. C., K. R. Sporer, J. C. Wallace, R. K. Maes, L. M. Sordillo, P. C. Bartlett, and P. M. Coussens. 2016. Reduced humoral immunity and atypical cell-mediated immunity in response to vaccination in cows naturally infected with bovine leukemia virus. Vet. Immunol. Immunopathol. 182:125–135. https://doi.org/10.1016/j.vetimm.2016.10.013.

Hutchinson, H. C. 2020. Transmission and progression of bovine leukemia virus. PhD Thesis, Michigan State Univ., East Lansing.

Juliarena, M. A., C. N. Barrios, M. C. Ceriani, and E. N. Esteban. 2016. Hot topic: Bovine leukemia virus (BLV)-infected cows with low proviral load are not a source of infection for BLV-free cattle. J. Dairy Sci. 99:4586–4589. https://doi.org/10.3168/jds.2015-10480.

Kuczewski, A., H. Hogeveen, K. Orsel, R. Wolf, J. Thompson, E. Spackman, and F. van der Meer. 2019. Economic evaluation of 4 bovine leukemia virus control strategies for Alberta dairy farms. J. Dairy Sci. 102:2578–2592. https://doi.org/10.3168/jds.2018-15341.

LaDronka, R. M., S. Ainsworth, M. J. Wilkins, B. Norby, T. M. Byrem, and P. C. Bartlett. 2018. Prevalence of bovine leukemia virus antibodies in US dairy cattle. Vet. Med. Int. 2018:5831278. https://doi.org/10.1155/2018/5831278.

Mekata, H., S. Sekiguchi, S. Konnai, Y. Kirino, Y. Horii, and J. Norimine. 2015. Horizontal transmission and phylogenetic analysis of bovine leukemia virus in two districts of Miyazaki, Japan. J. Vet. Med. Sci. 77:1115–1120. https://doi.org/10.1292/jvms.14-0624.

Rhodes, J. K., K. D. Pelzer, and Y. J. Johnson. 2003. Economic implications of bovine leukemia virus infection in mid-Atlantic dairy herds. J. Am. Vet. Med. Assoc. 223:346–352. https://doi.org/10.2460/javma.2003.223.346.

Rodriguez, S. M., A. Florins, N. Gillet, A. de Brogniez, M. T. Sanchez-Alcaraz, M. Boxus, F. Boulanger, G. Gutierrez, K. Trono, I. Alvarez, L. Vagnoni, and L. Willems. 2011. Preventive and therapeutic strategies for bovine leukemia virus: Lessons for HTLV. Viruses 3:1210–1248. https://doi.org/10.3390/v3071210.

Ruggiero, V. J., B. Norby, O. Benitez, H. Hutchinson, K. Sporer, C. Droscha, C. Swenson, and P. Bartlett. 2019. Controlling bovine leukemia virus in dairy herds by identifying and removing cows with the highest proviral load and lymphocyte counts. J. Dairy Sci. 102:9165–9175. https://doi.org/10.3168/jds.2018-16186.

Taxis, T. M., T. N. DeJong, C. L. Swenson, K. R. B. Sporer, C. Droscha, D. Niles, and P. C. Bartlett. 2020. Reducing bovine leukemia virus prevalence on a large midwestern dairy farm by using lymphocyte counts, ELISA antibody testing, and proviral load. Bov. Pract. 54:136–143.

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