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What dairy veterinarians should know about bovine leukemia virus

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Abstract

Bovine leukemia virus (BLV) is now present in almost all US dairy herds, and the prevalence among cattle in our milking herds is now approaching 50%. Most dairy producers know that BLV can cause tumors in a small percentage of cattle, but the associations now being observed with reduced milk production and cow longevity are not yet appreciated by most dairy producers. Over 20 nations have eradicated BLV from their cattle populations by removing all antibody-positive animals. This is economically impossible for all but a small percentage of farms that have very low BLV prevalence. Management interventions to reduce transmission have not been promising. New diagnostic methods may be helpful to better target culling and segregation to those animals that are most infectious to herdmates. Producers interested in determining BLV prevalence in their herd are advised to conduct a BLV herd profile, which involves milk or serum ELISA testing of the 10 most recently calved cattle in lactations 1, 2, 3, and 4+. More information is available on our website at www.BLVUSA.com.

Key words: BLV, bovine leukemia virus, dairy cattle

Résumé

Le virus de la leucémie bovine (VLB) est maintenant présent dans presque tous les troupeaux de vaches laitières aux États-Unis et la prévalence dans nos troupeaux laitiers s'approche de 50%. La plupart des producteurs laitiers comprennent que le VLB peut causer des tumeurs chez un faible pourcentage des bovins mais l'association entre le virus et la perte de production laitière et la longévité plus courte n'est pas bien évaluée par la plupart des producteurs laitiers. Plus de 20 pays ont éradiqué le VLB de leur cheptel bovin en réformant tous les animaux positifs aux anticorps. Ceci n'est toutefois économiquement possible que dans des cas peu fréquents de fermes qui ont une très faible prévalence du VLB. Les interventions de gestion afin de réduire la transmission ne se sont pas avérées prometteuses. De nouvelles méthodes de diagnostic peuvent être utiles pour mieux cibler la réforme et la ségrégation des bovins qui sont le plus à risque d'infecter les autres animaux du troupeau. On conseille aux producteurs qui sont intéressés à déterminer la prévalence du VLB dans leur troupeau de faire un profil du VLB au niveau du troupeau à l'aide de tests ELISA du lait ou du sérum impliquant les 10 dernières vaches vêlées pour les lactations 1, 2, 3 et 4+. Pour plus d'information, on peut consulter notre site web au www.BLVUSA.com.

Introduction

Bovine leukemia virus (BLV) causes enzootic bovine leukosis, which is a non-curable, progressive disease of cattle. Transmission is usually by intact lymphocytes contained in blood or other fluids. About 1 to 5% of infected cattle eventually develop tumors (lymphoma or lymphosarcoma). These tumors were once seen as the only economic loss associated with BLV infection. As is the case with other retroviruses, BLV infection disrupts the cow's immune system, which may reduce her ability to respond to vaccines and a multitude of opportunistic infections. Researchers from around the world are reporting that BLV infection is associated with milk loss and an increased risk of culling. After decades of avoiding the issue, the US dairy cattle BLV prevalence has increased to where almost all of our herds and almost 50% of dairy cattle are infected. Dairy veterinarians need to be aware of new developments for BLV testing and control.

Prevalence in US and beyond

The animal-specific prevalence of BLV in the US national herd has now surpassed 40% of dairy cattle.[2,11,12,29] In our ongoing study of BLV in 40 cows in each of 103 dairy herds in 11 states, 94% of herds had BLV detected, and in the average herd 47% of all the cows were infected.[21] Japan, Canada, Argentina, Brazil, China, and many other countries are also reporting BLV prevalence similar to the US.[28,43] In contrast, at least 21 nations have eradicated BLV, and more are actively pursuing eradication through national control programs.[10]

Economic impact

There are many cost components to BLV, but reduced milk production, reduced cow longevity and lymphoma/lymphosarcoma are thought to be the most significant.

Reduced milk production

The USDA's National Animal Health Monitoring System[27] determined that 209 lb (95 kg) of milk (per cow/year) were lost for each 10% increase in BLV-infected cows within a herd.[30] Our 2010 study of Michigan dairy herds found nearly identical herd-level production losses[13] (Figure 1). Cow-level milk production decreased in a dose-response as milk BLV ELISA optical density (OD) increased.[13,29,30] Our most recent national study of 103 herds in 11 states showed a 540 lb (245 kg) loss in rolling herd average milk with each 10% increase in BLV prevalence (Figure 1). Recent large studies in China and Canada have confirmed the association between BLV infection and reduced milk production.[28,43]

Cow longevity

In a 2012 Michigan analysis we found that herds with higher BLV prevalence had a significantly lower proportion of older cows.[11] This association led to our study of 3,849 dairy cattle that demonstrated a decreased ($P<0.0001$) survival of cattle with BLV infection as compared to uninfected herd mates[1] (Figure 2). Compared with age-matched herd mates, infected cattle were 23% more likely to be culled over the 19-month monitoring period, and cattle with the highest milk ELISA OD values (> 0.5) were over 40% more likely to be culled. Last year, a large Canadian study corroborated our findings in reporting that BLV-positive cattle had a greater probability of being culled or dying when compared to BLV-negative cows.[28] Others have also reported similar BLV-associated decreases in cow longevity.[7,31,32,39,40]

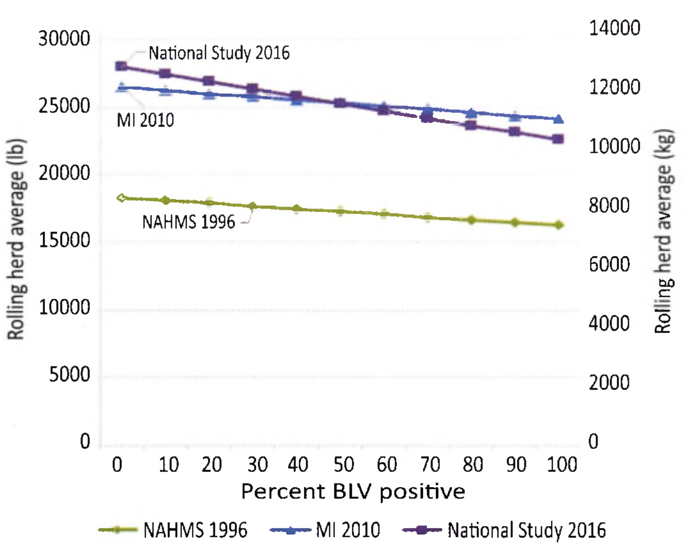

Figure 1. Association between herd prevalence of bovine leukemia virus and rolling herd average milk production.[11,21,27,30]

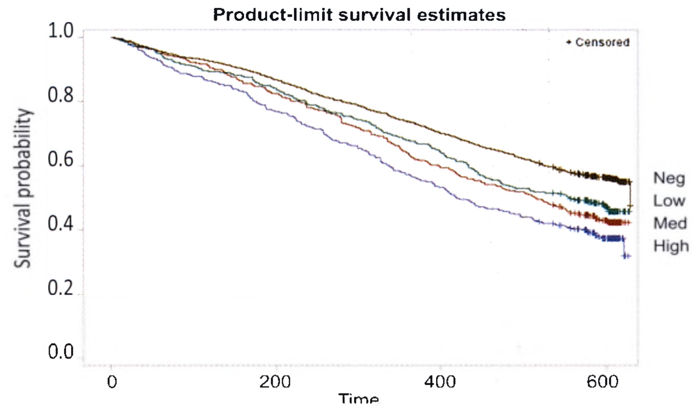

Figure 2. Survival of cows (in days) after ELISA testing for BLV. BLV negative (optical density < 0.1); low positive (0.1 ≤ optical density < 0.25); medium positive (0.25 ≤ optical density < 0.5); high positive (optical density ≥ 0.5).

Other impacts

The economic and societal impacts of BLV reach beyond milk production and cow longevity. BLV-induced malignant lymphosarcoma has become the most common reason for US cattle (beef and dairy) to be condemned during postmortem inspection at slaughter plants, and it accounts for 13.5% of beef and 26.9% of dairy postmortem condemnations, respectively.[41,42] Impacts on animal welfare and human health are more difficult to quantify, but recent publicity in the popular press caused alarm when DNA of BLV origin was reported in human breast cancer tissue.[3,4] Consumer reaction to BLV could one day damage the US dairy industry in a global market where many other nations have prioritized BLV control. Restrictions on trade of animals and animal products will increase as more nations attempt to eradicate BLV. Recent recognition of the multiple and previously hidden economic impacts of BLV warrants a reconsideration of the US dairy industry's decision that BLV is not enough of a problem to warrant control.

Our studies between 2009 and 2015 were used to estimate the total economic impact of BLV for one of our demonstration herds with a BLV prevalence of 62%. We estimated a loss of about $38,000 per 100 milking cows (or $380 per milking cow), largely from decreased milk production and reduced cow longevity. The analysis spreadsheet and discussion are available on our BLV website, www.BLVUSA.com, under the "resources" tab.

How other nations eradicated BLV

Other nations have eradicated BLV from their cattle by culling antibody-positive animals as detected with the older AGID test or the newer ELISA test.[10] This disease control approach was possible because the historical prevalence of BLV was usually under 5%, and small farms typically had only a few infected animals. Most government programs provided an indemnity for positive animals that were culled. Some programs allowed for temporary segregation of positive cattle until it was more economical for animals to be culled. The ELISA test has a major advantage in that it can be run on milk samples, and today's Dairy Herd Improvement (DHI) organizations routinely offer this service even if dairy herds do not get regular DHI testing. Test and cull programs are not economically feasible for most US herds given that almost half of our dairy cows are infected. We need a way to reduce BLV prevalence to perhaps 5 to 10%, at which point removal of all antibody-positive cattle might be economically feasible.

Controlling within-herd transmission with management

Transmission of BLV is thought to occur primarily through the transfer of lymphocytes harboring the infectious BLV provirus.[10] Proposed management methods to reduce BLV transmission involve employing single-use hypodermic needles and reproductive sleeves, control of biting flies, freezing or pasteurizing colostrum and fed milk, avoiding natural breeding, and avoiding blood transfer such as during tattoos, tail docking, extra teat removal, and hoof trims.[2] Probably all of these blood-borne routes of transmission occur, but the relative importance of each route is unknown and may be different for each farm. Many of these proposed interventions were identified as statistically significant risk factors in observational surveys.[12] Direct transmission from the exchange of body fluids, such as nasal secretions, milk, saliva, and feces, can realistically only be controlled by segregation of the infected animals from the rest of the herd.

Switching to single-use needles and obstetric sleeves is probably the most frequently attempted method of control. Anecdotal reports from several herds that switched to single-use needles and/or reproductive sleeves often indicate no measurable decrease in their BLV prevalence. However, there certainly are other reasons for improvement in medical hygiene in that veterinarians need to be confident that they are not in any way causing any disease transmission. Our 3-herd intervention trial to reduce BLV transmission found that the rate of new BLV infections in cattle receiving single-use hypodermic needles and rectal examination sleeves did not differ from herd mate controls.[33] Our 2 extension participatory field trials of 77 herds involved each producer trying at least 1 management intervention to control BLV. Implementing single-use hypodermic needles and obstetrical sleeves was the most commonly used intervention. No particular management intervention had any statistically significant impact on reducing BLV prevalence in the 1 or 2 years in which producers participated.[9] We speculate that specific management interventions by themselves showed little effect due to their inability to control all of the multiple routes of direct and indirect transmission.

Detecting super-shedders for culling or segregation

Proviral load (PVL) is the number of DNA provirus copies per cell or volume of blood, nasal secretions, saliva, milk or other fluids.[16,44] In collaboration with Japanese colleagues, we now routinely perform the qPCR CoCoMo proviral load assay for several of our research projects.[17,36] PVL differs vastly among ELISA-positive cattle. The first herd we tested 2 years ago had 12 ELISA-positive cattle, with PVL ranging from 30 to 48,826 (x 10^4 copies per µl of blood). This means that the cow with the highest PVL had 1,648 times more provirus per unit of blood than did the cow with the lowest PVL. Hence, the term "super-shedder" is used for high-PVL cattle. The standard BLV antibody ELISA test cannot distinguish low-PVL from high-PVL cattle.[33]

For other retroviruses, such as HTLV and HIV, it is widely accepted that viral load or PVL is associated with infectiousness or likelihood of transmission.[6,22,23,24] Field data support the idea that most natural BLV transmission is from high PVL cattle. Juliarena et al[19] found no transmission in the subsequent 20 months after 20 low-PVL cows were introduced into a herd of 105 BLV ELISA-negative cattle. The same paper also notes that the minimum BLV infective dose from low-PVL cattle would require the transfer of such a large volume of blood between animals, that this would rarely happen. Tracking genetically distinct proviral clones based on genomic insertion sites, Mekata et al[25] reported that low-PVL cattle rarely transmit BLV. Cattle infected with less than 3 copies/100 cells (i.e. low PVL) did not transmit BLV to other cattle for more than 30 months.[25] All observed transmission was from cattle with higher PVL. This laboratory and field evidence strongly supports our working hypothesis that PVL is positively associated with infectivity. The many routes of BLV direct and indirect transmission appear to be largely dependent upon transmission from this subset of highly infectious cattle, making their removal from the herd (via culling or segregation) the obvious critical control point.

Recent studies have shown a low BLV proviral load in milk, saliva, nasal secretions, smegma, and semen, especially when blood PVL values are high.[16,17,36,44] Focusing on transmission from so many infectious fluids could prove very difficult compared to removing the high-PVL cows, whose presence in the herd is the common factor and the weakest link in the various chains of transmission.

We are currently conducting a pilot field test of 3 herds using semi-annual CoCoMo PVL testing to identify high-PVL cattle for selective culling or segregation. The initial results show great promise in reducing overall herd BLV prevalence.[a] The herd that most aggressively eliminated high-PVL cows saw a reduction in prevalence from 64% to 30% within the first year, while the second herd reduced prevalence from 58% to 44%. The third herd is very small with no ability to segregate infected cattle. There were only a few new cases in this milking herd, but an influx of infected heifers has prevented the overall herd prevalence from decreasing. The decrease in prevalence in the 3 herds together was significant at $P<0.0000001$ by the extended Mantel-Haenszel chi-square test for trend.

The term "super-shedder" is relative to the distribution of PVL values within each herd. For example, on the 3 pilot farms, every 6 months we provide producers with a list of test results sorted in descending order of PVL. The producers start at the top of the list and prioritize these cattle for culling or temporary segregation until they can be culled. At each semi-annual visit, the cattle at the top of the list have progressively lower values for PVL and lymphocyte count (LC). Therefore, in application, the term "super-shedders" is defined as the highest PVL relative to herd mates. We have therefore resisted giving a firm definition of the term "super-shedder" or to define high PVL or lymphocytosis.

How do we Estimate Infectivity?

ELISA

The ELISA test is most useful for detecting positive versus negative cows, with sensitivity and specificity between 95 and 100%, depending on which commercial test is used, the sample used for testing (milk or serum), and the cutoff for what optical density result is considered positive. However, ELISA is not an effective tool for determining which cows pose the greatest threat to their negative herdmates and should therefore be segregated or culled.

Based on our samples so far, we see a pair-wise correlation between ELISA OD and PVL of r=0.45 (n=583), and of r=0.28 (n=583) between ELISA and LC. The detection of anti-BLV antibodies is therefore not very useful for identifying the ~1/3 of ELISA-positive cows that are the greatest infectious hazard to their herdmates. ELISA is best used as an initial screening to identify infected cows, but a follow-up test is needed to enable dairy producers to avoid culling the ~2/3 of their ELISA-positive cattle that are most likely not an immediate infectious threat to their unexposed herdmates.

qPCR PVL

A quantitative polymerase chain reaction (qPCR) can be used to estimate PVL. The Argentinean[19] and Japanese qPCR tests[26] are not licensed or commercially available in the US. Both are time intensive and not scalable for widespread use. For the past 2 years we have been using the BLV CoCoMo test for PVL[b] in our BLV laboratory. It is very laborious. It still takes a week to test about 150 cows due to a 2-day DNA isolation/normalization procedure prior to single-plex reactions of BLV and a control gene, both of which require standard curves for each run. These tests are acceptable for research, but are too laborious and expensive for widespread commercial use. A fast and inexpensive qPCR test is needed that produces quantitative results that are correlated with PVL and LC measures and, most importantly, are shown in field test results to be predictive of infectivity to herdmates. Fortunately, such tests are now under development.

Blood lymphocyte count

The original test for BLV was the Bendixen Key test, which was basically an age-adjusted lymphocyte count. On-farm, chute-side complete blood count (CBC) with differential white blood count machines are now being marketed.[c] One of these portable machines costs ~$18,000, provides a blood count for $5 per animal, and includes an easy blood collection system for collecting the requisite drop of blood from the jugular. In our pilot project, it takes about 45 seconds to process each sample and another 45 seconds for the analysis. In contrast, the cost of a CBC at our veterinary school's clinical pathology laboratory is $35, and the results are not usually available for several hours after hand-delivery to the laboratory. This remarkable advance in diagnostic technology has suddenly made it economical for the food animal industry to utilize the CBC, which is probably the most common and useful diagnostic test in human and companion animal medicine. Every veterinarian that graduated in the last 40 years is familiar with using CBC data for diagnosis and prognosis. Until now this diagnostic information has never been commonly used in food animal medicine because of the cost, the need to transport blood samples to a laboratory, and the delay in obtaining the results. Virtually no training is needed for the entire population of dairy veterinarians to instantly start employing the CBC as a diagnostic tool.

Lymphocyte count is strongly correlated with PVL, and is usually the most easily measured metric of disease progression and a disrupted immune system. The correlation between PVL and LC was reported at r=0.88,[34] and our data are currently showing a correlation of r=0.78.[33] Field studies would be needed to determine what combination of LC, and PVL are the best predictor of infectivity to herdmates. So far, we have been providing both LC and PVL to the 3 farms participating in our field trial to selectively cull (or segregate) those cattle with the highest PVL and/or LC.

Hope for a vaccine

The search for a BLV vaccine has been long and so far unsuccessful.[15] Retrovirus vaccines are notoriously difficult to develop. Genetically modified vaccines, such as those with deleted genes, would probably face a difficult and lengthy approval process in the US. Additionally, vaccines rarely have 100% efficacy, so detection of highly infectious cattle would still be a necessary component of any future BLV control program.

Genetic selection for resistance

It seems clear that cattle (and many other species) exposed to BLV will develop a detectable antibody titer. The question for geneticists regards whether or not some genes facilitate the progression of infected cattle to high LC and PVL. In some studies, BoLA-DRB3*0902 and BoLA-DRB3*1101 alleles were associated with a low proviral load, and BoLA-DRB3*1601 was associated with a high proviral load in Japanese Black cattle.[26,35,37,38] However, at least 1/3 of cattle with "resistant" alleles can still develop a high PVL, just as some cattle with "susceptible" alleles remain with a low PVL. To elucidate a genetic component to high PVL susceptibility, our laboratory is conducting a genetic comparison between a group of high-PVL cattle and another group that appear phenotypically resistant to becoming high-PVL as demonstrated by being BLV antibody-positive, yet remaining low-PVL with normal lymphocyte counts over at least 2 consecutive semi-annual tests. So far, it appears as though genetic selection may aid in reducing the percentage of BLV infected cows that progress to a high-PVL, but some cattle would still develop a high-PVL, and a diagnostic test for PVL (or LC) will always be needed.

First steps: The BLV herd profile

The first step for a dairy client interested in BLV should be to conduct a BLV herd profile[13] (Figure 3), which can be done via ELISA testing of either blood or milk samples. Milk samples submitted through the local DHI organization are usually the easiest, and can be submitted whether or not the herd uses DHI for routine testing. The 10 most recently calved cows in the first, second, third, and fourth+ lactations are tested. The producer should not be allowed to 'pick and choose' which cattle to test. The prevalence in each lactation group is simply the percentage of tested animals which are positive. The first lactation prevalence is particularly useful because it reflects transmission that occurred in the young stock. An estimate of overall prevalence in the herd is determined by taking the simple average of the 4 lactation-specific measures of prevalence. This average is independent of the herd-age breakdown so it can be used to compare among herds and with historical records from the same herd. Herds with a low estimated prevalence may choose to do a whole-herd test and cull positive cows to be free of the disease, providing they maintain a closed herd and make sure their young stock are also negative.

For herds with higher prevalence that wish to pursue whole-herd testing followed by segregation or culling, hopefully the new diagnostic tools discussed above will soon be available to help identify those animals that are the greatest infectious hazard to their herdmates. Other herds may choose to use management methods to reduce transmission, using the lactation-specific prevalence figures from the herd profile to help identify age groups where management should be targeted. For example, the herd profile in Figure 3 shows the typical pattern of first-lactation cows entering the milking herd at a low prevalence, but then quickly increasing prevalence in later lactations. For a herd with this pattern, management changes should be targeted to reduce transmission within the milking herd. The herd profile in Figure 4 is less common, and demonstrates a pattern where cows are entering the herd already having a high BLV prevalence, which is maintained relatively constantly in later lactations. Such a herd should focus its efforts on eliminating risk factors for calves and growing heifers.

More information about the BLV herd profile, as well as a spreadsheet that can be used to input the test results and generate a herd profile, can be found at www.BLVUSA.com, under the "resources" tab. Once the BLV herd profile is complete, the partial-budget cost estimator worksheet can be downloaded and used to estimate the economic impact of the disease in the herd.

Conclusions

The prevalence of BLV in US dairy cows has increased from about 10% in the 1970s to almost 50%. Along with this increase in prevalence has been a new recognition of the hidden economic impact of this disease on milk production and cow longevity. Successful eradication programs in other countries have relied on culling antibody-positive cattle. Management interventions to reduce intra-herd transmission are often unsuccessful. New diagnostic and disease control

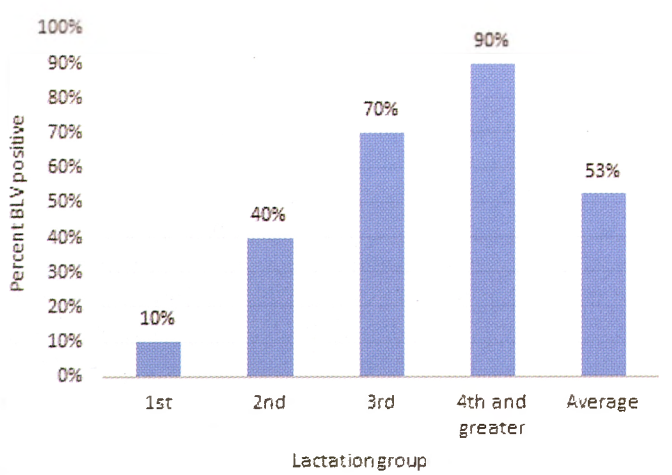

Figure 3. Results of a BLV herd profile taken at 1 point in time is consistent with the explanation that cattle enter the milking herd with low prevalence, but then become infected in subsequent lactations while they are in the milking herd.

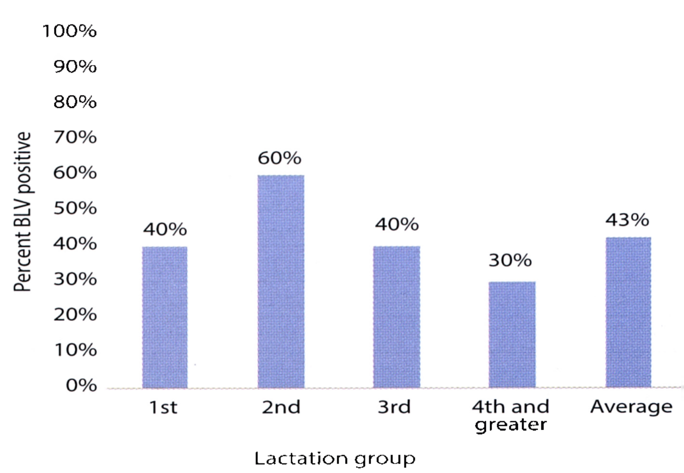

Figure 4. An example of a BLV herd profile taken at 1 point in time which suggests that a high percentage of young stock enter the milking herd already infected with BLV.

approaches are under development to help dairy producers control BLV transmission.

Endnotes

[a]Ruggiero, unpublished data
[b]Riken Genesis, Japan
[c]Advanced Animal Diagnostics, Morrisville, NC

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