Bovine leukemia virus in Michigan beef bulls

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Abstract

Bovine leukemia virus (BLV) is a contagious retrovirus of cattle. Little is known about the prevalence, risk factors, or potential health impact of BLV in beef cattle. Michigan beef bulls receiving breeding soundness examinations (BSE) were tested for BLV antibodies in serum using ELISA to determine the crude and age-specific prevalence of BLV in Michigan beef bulls. Our main objective was to measure the association between BSE results and BLV seropositivity. Additionally, management surveys were administered to the bull owners to identify potential risk factors for BLV infection. The crude BLV prevalence was 24.7% with an age-specific prevalence increasing with age from 1.5% to 67%. This indicates that these animals have a very low rate of BLV infection before they reach breeding age and then experience a very high rate of new infections once they begin natural breeding. Fertility, as measured by a BSE, did not appear to be affected by BLV infection. Scrotal circumference was the only measured BSE component that was associated with BLV infection. Exposure to cows through natural breeding and the frequency of changing hypodermic needles were the only management risk factors associated with BLV infection. The significant association between BLV infection and the reuse of hypodermic needles supports the findings of others in suggesting that needle reuse may be an important means of BLV transmission.

Keywords: Cattle, leukosis, management, fertility, prevalence

Introduction

Enzootic bovine leukosis is a contagious retroviral disease of cattle caused by BLV and is clinically characterized by a persistent lymphocytosis. In dairy cattle, infection becomes more prevalent with increasing age.[1] Most cattle remain asymptomatic, but between 30-40% of BLV infected cattle will develop persistent leukocytosis. Fewer than five percent of infected cattle develop malignant lymphosarcoma, which in the U.S. is the most common neoplastic disease of cattle discovered at slaughter.[2]

At least 20 nations have successfully eradicated BLV from their cattle herds.[3] Surveys in locations outside of Europe have reported a within-herd cow prevalence of 23-46% in dairy cattle.[4-7] In Michigan, the cow-level prevalence of BLV in dairy cattle averaged 32.8%.[1] Few reports have studied the prevalence of BLV in U.S. beef cattle, but they have estimated the animal-level prevalence to be 1.2-10.3%.[8-11]

In the 1960's and1970's when many European countries undertook BLV eradication programs, animal health agencies in the U.S. and Canada decided that BLV was not a sufficiently costly disease to warrant national control. However, recent studies have suggested that BLV prevalence has since increased dramatically, and that BLV has a previously unappreciated subclinical economic impact on the cattle industry. According to Roeber et al., 1.1% of market cow and bull carcasses were condemned at slaughter, and 14.9% of these were condemned due to malignant lymphosarcoma.[12] In comparison, in 1979 only 0.8% of carcass condemnations were due to malignant lymphosarcoma.[13] Bovine leukemia virus infection has also been shown to decrease cow longevity and milk production in dairy cows.[1,14] In addition, the presence of BLV infection precludes the sale of exported animals, semen, embryos, and animal products to many countries.

Programs to control the spread of BLV in cattle have largely focused on segregation of positive animals and other management interventions.[15] However, management changes are only effective if they can be easily and inexpensively implemented by the producer and target modes of transmission that account for a significant proportion of the new infections. In a recent study of dairy cattle in Michigan, reusing hypodermic needles, gouge dehorning, uncontrolled stable flies, reusing obstetrical (OB) sleeves, and using natural breeding instead of artificial insemination (AI) were all found to be significantly associated with BLV infection.[15] These risk factors all relate to hematogenous transmission.

Trauma to the vagina and penis during natural breeding may facilitate blood-borne transmission of BLV between cows and bulls and may be responsible for the decreased prevalence of BLV in herds which only use AI for breeding.[15] Bulls used for natural breeding have the potential to spread BLV to previously BLV negative herds, and may also be responsible for increasing a herd's overall BLV prevalence. It has been reported that about one in three cow-calf operations (30.7%) borrowed, leased, or purchased a bull for use during the breeding season, and over half of the operations (53.3%) added bulls over 18 months of age; on average, 16.3 cows were mated to each yearling bull while 23.7 cows were mated to each mature bull.[16] Consequently, bulls could serve as a source of infection for cows if natural breeding is a common method of transmission.

The objectives of this study were to determine the crude and age-specific prevalence of BLV among beef bulls in Michigan, to assess the possible impact of BLV on bull fertility, and to identify risk factors for BLV seroprevalence.

Materials and methods

Beef bulls that presented for BSE's as part of the Michigan State University Extension BSE program were enrolled in this study. The BSE's were performed on 363 bulls from 113 owners in the spring of 2009 (156 bulls) and 2012 (207 bulls). Bulls were categorized into six age groups (1-12 months, 13-24 months, 25-36 months, 37-48 months, 49-60 months, 61+ months) and six breed groups (Angus, Red Angus, Polled Herefords, Maine-Anjou, Simmental, Other) for analysis.

The BSE's were performed by one of the authors (DLG). Established protocols for the BSE's were followed as described by Hopkins and Spitzer.[17] A physical examination, scrotal circumference (SC) measurement, sperm motility, sperm morphology (normal, primary defects, and secondary defects), and the presence of white blood cells (WBC) in semen were recorded for each of the bulls. Also indicated was whether the bull passed the BSE, failed, or was deferred based on the specifications in Hopkins and Spitzer.[17]

Blood samples from the median caudal vein (tail vein) were collected into serum separator tubes, centrifuged, and then submitted to the Michigan State University Diagnostic Center for Population and Animal Health. Antibodies for BLV were detected using a commercially available antibody capture ELISA (Bovine Leukemia Virus Antibody Test Kit, Veterinary Medical Research and Development, Pullman, WA). The optical density (OD) for each sample was measured and recorded. Bovine leukemia virus status was based on the OD of the sample; those samples with an OD value of greater than or equal to 1.0 were considered positive for BLV.

An eight question survey (Appendix 1) was administered to the owner or manager of those bulls that were enrolled in the study in 2012 (n=207). The questions targeted previously identified risk factors for BLV, including the number of heifers or cows to which the bull was exposed during the breeding season, whether the bull was tattooed or dehorned, whether fly control was utilized on the farm, and the extent of hypodermic needle reuse.[15]

Statistical analysis was performed using SAS (SAS PC, Cary, NC) to test for the association between BLV status and OD measurement and each of the management risk factors included on the questionnaire (type of fly control, number of herds serviced, number of cows serviced, whether the bull was polled, whether the cows to which the bulls were exposed were polled, the number of injections received per year, frequency of changing hypodermic needles, history of tattoos) as well as each of the measures of fertility comprising the BSE (scrotal circumference, sperm motility, presence of WBC and normal, primary defect and secondary defect sperm counts). Herd was included as a random effect in the analysis of each risk factor, and age group was also included as a confounder. The SAS PROC MIXED was used for the analysis of BLV ELISA OD and PROC GLIMMIX was used for the analysis of BLV status.

The age-specific prevalence of BLV in beef bulls in this study was compared to that of dairy cows studied by Bartlett et al.[14] and fitted with a linear trendline (Figure). The apparent difference in slope was tested using a GLMMIX model to predict BLV status and a MIXED model to predict OD. Age group was included as a significant predictor of BLV status.

Results

The overall prevalence of BLV was 24.7% (95/385). Age group was associated with BLV status ($P < 0.004$; Table 1), but breed group was not associated with BLV status ($P < 0.4505$) or BLV OD ($P < 0.9726$; Table 2). The age-specific prevalence of BLV in beef bulls increased with each age group from 1.5% to 66.7% (Figure).

The SC was the only measured BSE component associated with BLV status (Table 3). Further analysis of SC was undertaken to adjust for breed group and to use age as a continuous variable instead of age group. Regardless, SC continued to be significantly associated with both BLV status and OD.

Exposure of bulls to cows for natural breeding and frequency of changing hypodermic needles were the only studied management risk factors significantly associated with BLV status (Table 4). All but one of the bulls in the youngest age group had not yet been exposed to cows, and all of the older bulls had been exposed to cows. Therefore, age and exposure to cows were inseparably confounded. Frequency of changing hypodermic needles was analyzed to adjust for breed and age groups and was still found to be significant. Bulls whose owners changed hypodermic needles between every animal had no BLV (0/19) while those that changed needles about every ten animals had a prevalence of 17.5% (10/57) and those that changed about every 20 animals had a prevalence of 43.5% (37/85; $P < 0.0317$).

The age-specific prevalence determined in this study does not directly reflect the rate of acquiring new infections because these data were collected at a single point in time rather than from a longitudinal study in which animals were followed forward in time and the rate of new infections directly measured. However, if transmission in the herds had reached an equilibrium 'steady-state', the slope of the lines in the Figure suggest that the rate of acquiring new infections was almost twice as great among the beef bulls in this study as it was in dairy cows studied by Bartlett et al.[14] The interaction terms between animal type (beef or dairy) and age group were significant at $P < 0.001$ indicating that the relationship between age and BLV in beef bulls was significantly different from that observed for dairy cows.

Table 1. Age-specific prevalence of BLV in Michigan beef bulls

Age group*	**n**	**Prevalence (%)**
0	65	1.5
1	140	14.3
2	75	32.0
3	35	40.0
4	18	50.0
5	30	66.7

* 0 = 1-12 months, 1 = 13-24 months, 2 = 25-36 months, 3 = 37-48 months, 4 = 49-60 months, 5 = 61+ months)

Table 2. Breed group-specific prevalence of BLV in Michigan beef bulls

Breed group	**n**	**Prevalence (%)**
Angus	178	29.2
Red Angus	68	22.1
Polled Hereford	33	0.00
Maine-Anjou	16	31.3
Simmental	27	29.6
Others	41	19.5

Table 3. Association between BLV ELISA results and measures of bull fertility.

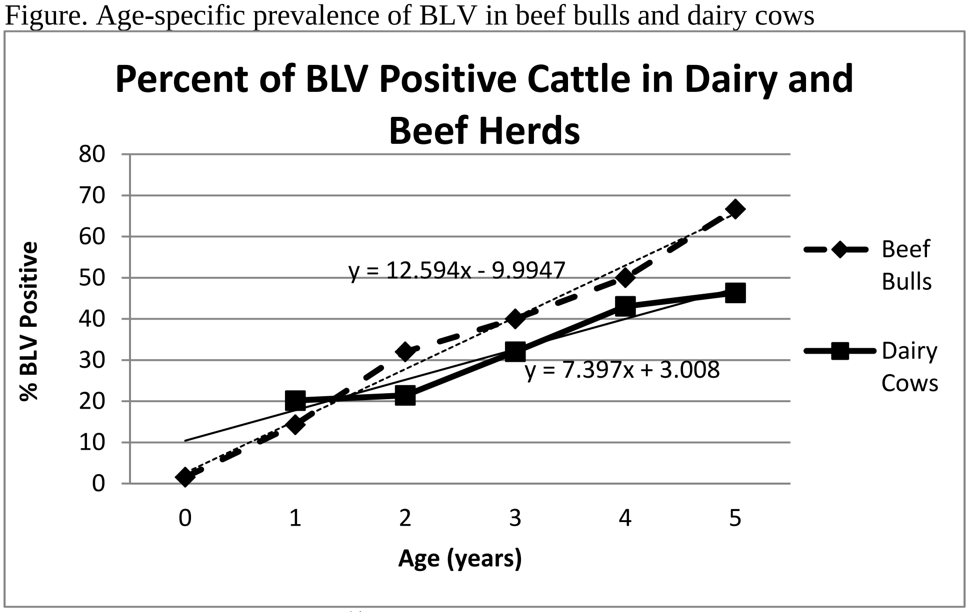
Potential outcome	BLV positive	BLV negative	P value for BLV	P value for OD
Scrotal circumference (cm)	41.6	38.9	0.0198	0.0866
Percent with fair or better motility	98.9	98.5	0.7570	0.8736
Mean % normal sperm morphology	81.7	80.3	0.3823	0.8266
Mean % sperm with primary defects	4.8	7.0	0.9989	0.9859
Mean % sperm with secondary defects	13.1	12.4	0.4098	0.6926
Mean WBC (#/hpf)	0.4	0.3	0.4055	0.5630
Percent with passing BSE	80.9	82.8	0.7296	0.8270

*Above data are adjusted for age group and includes herd as a random effect variable

Table 4. Association between BLV ELISA results and BLV risk factors

Risk Factor	BLV+	BLV-	P value for BLV	P value for OD
Percent bulls exposed to cows for breeding	97.8	53.3	<0.0001	<0.0001
Mean number of herds to which the bull was exposed	1.0	0.7	0.3042	0.4316
Percent using routine fly control	63.3	77.9	0.8668	0.8464
Percent that reuse hypodermic needles	100.0	83.6	0.0317	0.0708
Percent that used tattoos	49.1	70.9	0.4299	0.4682

Figure. Age-specific prevalence of BLV in beef bulls and dairy cows



*Dairy cow data from Bartlett et al.[14]

Discussion

In general, beef herds reportedly have a lower prevalence of BLV than do dairy herds.[4-10,18,19] A recent study of six Michigan beef feedlot herds and six Michigan dairy herds found that the BLV seropositivity in dairy cattle was 48% while it was only 10% in beef cattle (Cristina Venegas, unpublished data). This difference may be partially due to the younger average age of most feedlot beef cattle and to differences in management. Regardless, since the prevalence of BLV has generally been found to be lower in beef cows than in dairy cows, it is unexpected that the prevalence of BLV in beef bulls is comparatively high (Figure).

The comparison of age-specific prevalence of BLV in beef bulls compared to dairy cows outlined in the Figure suggests that the rate of acquiring new infections was almost twice as great among beef bulls as it was in dairy cows. Many possible reasons could exist for this apparent difference. Beef bulls typically receive fewer injections with hypodermic needles and reproductive examinations with OB sleeves, which are both important risk factors for BLV transmission in dairy cows.[15] However, beef bulls might have greater exposure to biting flies and more natural sexual contacts than do dairy cows, both of which could have increased BLV transmission in beef bulls.

Since BLV is a retrovirus, prospects for an effective BLV vaccine are problematic. Due to the high prevalence of BLV in the U.S., transmission of BLV is probably best controlled by segregation and management interventions, as was done in Europe, until the prevalence is sufficiently low that 'test and removal' procedures are economically feasible. Therefore, identifying the most significant routes of BLV transmission is important for establishing effective control measures. For example, one BLV management program in a dairy herd involved the use of single-use needles and OB sleeves, disinfection of tattoo and dehorning equipment, and feeding of milk replacer and heat-treated colostrum.[20] The prevalence of BLV-infected heifers on the farm decreased from 44% to 17% in two years.

Several methods of hematogenous transmission have been suggested to have an association with BLV prevalence, but it is difficult to determine the percent of new cases that can be attributed to each possible route of transmission.[15, 21-25] This study did not find an association between BLV and tattooing (Table 4). This procedure is done at an early age, and transmission by such means should have been reflected by ELISA positivity before one year of age. The BLV prevalence in bulls that were one-year-old and younger was only 1.5% (Table 1), so transmission occurring early in the bull's life from tattooing, appears to be relatively rare. In addition, calves receiving colostrum from BLV-positive cows have a greatly reduced risk of becoming infected with BLV.[26] This could further reduce their likelihood of contracting BLV from procedures occurring early in life, such as tattooing. This study also failed to find an association between BLV prevalence and the use of a fly control program as was found for a recent dairy cow study.[15]

The results of this study agreed with those of earlier studies in finding that the reuse of hypodermic needles was significantly associated with an increase in BLV seropositivity.[15,23] In addition, the dose-response demonstrated in this study supports the theory that this association might be causal in nature, as does the biological plausibility that BLV-infected lymphocytes could be transferred via the immediate reuse of a hypodermic needle on multiple cattle. While it may be prudent to advise beef farmers to use new needles for every animal, acceptance of single-use needles may conflict with labor efficiency and economic return especially in larger herds that currently realize some economy of scale by vaccinating large numbers of cattle at the same time. Further research will be required to determine if BLV transmission via reuse of hypodermic needles is more expensive than the cost of labor and supplies needed for employing single-use needles.

The bulls in this study had a very low prevalence of BLV infection prior to reaching breeding age and then experienced a high rate of new infections once they begin natural breeding (Table 1). Therefore, natural breeding may play a significant role in BLV transmission among beef bulls. This study found that BLV prevalence was associated with increasing age in beef bulls, which may be due to the cumulative number of sexual contacts. However, other hematogenous exposures, such as biting stable flies and hypodermic needle use, also accumulate as the bull ages. Therefore, this increasing rate of BLV seropositivity in older cattle cannot necessarily be attributed to an increasing number of sexual contacts any more than to other exposures that also accumulate as a bull ages. However, this population of breeding bulls is unique in having a very large number of sexual contacts compared with either dairy or beef cows. Natural breeding must be investigated as a potential route of transmission accounting for the apparently high rate of new infections in beef bulls. Attribution studies are needed to compare the relative importance of the various routes of BLV transmission.

Erskine et al. found that natural service breeding of dairy heifers and cows was significantly associated with an increase in BLV prevalence compared with herds that only utilized AI.[15] According to the USDA, an average of 16.3 beef cows are mated to each yearling bull while 23.7 cows are mated to each mature bull.[16] As a beef bull grows older, he is exposed to more cows through natural service and, therefore, is potentially at a greater risk of contracting BLV. If natural breeding was an important route of BLV transmission within beef herds, one would expect much higher rates of infection among the beef bulls than among beef cows, because the bulls have many times more sexual partners than do the beef cows. This explanation is consistent with the relatively low rate of BLV reported among the beef cow population.

Yeon Choi, Monke, and Stott found that BLV was not transmitted in the semen of seropositive bulls.[27] However, penile and vaginal trauma could allow for hematogenous transmission during natural breeding. A previous study showed that the association between BLV and natural breeding was more significant in heifers than in cows, which could be attributed to a greater likelihood of vaginal trauma in the smaller heifers.[15] If future research confirms natural breeding as an important route of BLV transmission, the increased use of AI on beef farms would be a potential disease control intervention.

Confirming that a breeding bull is BLV-negative could also help to reduce transmission. Since 30.7% of cow-calf operations borrow, lease, or purchase a bull for use during the breeding season, the use of a BLV-infected bull for natural breeding could be a significant source of BLV exposure to the herd.[16] In addition, any bull owner wishing to maintain the BLV-negative status of their bull may wish to prevent exposure via natural breeding to any BLV-infected cows.

An important objective of this study was to determine whether BLV infection impacted the fertility of a bull as determined by a standard BSE. The SC was significantly associated with BLV positivity, and this association remained after adjusting for age and breed of the bull. Those bulls that were positive for BLV had a SC that averaged almost 3cm larger than those bulls that were negative. It is not clear why BLV positive bulls may have a larger SC; this finding requires further investigation and analysis. Further research into the bull's ability to successfully breed a cow or heifer and produce a live calf is needed to more fully evaluate the impact of BLV on fertility.

Bartlett et al. showed that dairy cow longevity was negatively impacted by BLV infection.[14] However, cows were not significantly impacted until after their first lactation when they were about three to four years of age. Therefore, while young feedlot steers may not be significantly impacted by BLV due to their truncated lifespan, older beef breeding cows and bulls may experience reduced longevity and productivity due to BLV infection. Similarly, impacts of BLV on milk production are reportedly greater among older cows.[28] If older beef cattle exhibit immune suppression, this might necessitate an increased rate of replacement. Consequently, those farms that need to replace their bulls and cows more frequently may enjoy less discretionary culling.[14]

Conclusion

Approximately 25% of beef bulls that presented for BSE's in Michigan were BLV positive. Older bulls were more likely to be infected than younger bulls, and their apparent rate of new infections increased rapidly once they began functioning as breeding animals. Bovine leukemia virus status did not appear to have a significant impact on fertility as measured by the BSE. The reuse of hypodermic needles was associated with an increased prevalence of BLV in beef bulls.

Acknowledgement

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Appendix
2012 Breeding Soundness Exams

Please give us you mailing address, Email **or** Fax so that we can send you the Bovine Leukemia Virus serologic results when they are available in a few weeks.

Email: _______________________ Fax: ____________________
(preferred) (preferred)

Mailing address:

Name__________________ Address _______________________________

City _______________________________ State _____ Zip _________________

1. About how many cows or heifers has this bull serviced in its life?

__ none __ 1-49 __ 50-199 __ 200-500 __ over 500

2. About how many herds of cattle has this bull serviced in its life?
_____ herds

3. Does this bull's current herd use a fly control program?

__ No __Yes (Please describe what is used and how often)

____

4. This bull was: ___ Polled (dehorning not needed) ___ Gouge dehorned

___ Burn dehorn ___ paste dehorn __ other (describe _____________)

5. The cows to which this bull is exposed are mostly: ___ No exposure yet

___ Polled ___ Gouge dehorned ___ Burn dehorned ___ paste dehorned

__ other (describe _____________)

6. Describe how many total injections (vaccination, supplements, etc.) this bull has received in his lifetime

About _____ per year for each of _____ years.

7. About how many tattoos has this bull received? ________