PEER REVIEWED

Single-use hypodermic needles and obstetric sleeves failed to reduce bovine leukemia virus transmission in three dairy herds

Vickie J. Ruggiero, PhD, MPH, LVT; **Paul C. Bartlett,** MPH, PhD, DVM, ACVPM, DACVPM
Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824
Corresponding author: Dr. Vickie J. Ruggiero, 784 Wilson Road, Rm F107, East Lansing, MI 48824, 517-355-6509, woodsvi1@msu.edu

Abstract

The objective of this study was to determine the utility of single-use hypodermic needles and reproductive examination sleeves in reducing the transmission of bovine leukemia virus (BLV) in dairy herds. Numerous epidemiological studies have identified re-use of needles and exam sleeves as significant risk factors for BLV, therefore adopting a practice of single-use needles and sleeves is a commonly suggested management change for reducing BLV transmission. We conducted a field trial on 3 midwestern commercial dairy herds that had not been employing single use of needles or sleeves as part of their herd health protocol. Additionally, each of the herds had a BLV prevalence among adult cows of at least 20%. BLV milk-ELISA negative cows were randomly assigned to always receive a new single-use needle and new exam sleeve (intervention group). We also monitored and tested BLV milk-ELISA negative cows that received the standard management practice of needles and sleeves that were shared with ELISA-positive herd mates (controls). Cumulative incidence of new infections was determined by semiannual BLV milk-ELISA testing. The cumulative incidence of new BLV infections was not statistically different between the 2 groups. Medical hygiene to prevent bloodborne transmission is still recommended, but in these 3 herds, re-use of needles and sleeves did not appear to be a major route of BLV transmission.

Key words: enzootic bovine leukosis, BLV, incidence, medical hygiene, bloodborne, seasonal

Résumé

L'objectif de cette étude était de déterminer si l'usage unique d'aiguilles hypodermiques et de gants d'examen reproducteur pouvait réduire la transmission du virus de la leucémie bovine (BLV) dans les troupeaux laitiers. Plusieurs études épidémiologiques ont déterminé que la réutilisation d'aiguilles et de gants d'examen est un important facteur de risque dans la transmission du BLV. Par conséquent, on suggère couramment un usage unique pour les aiguilles et les gants d'examen pour réduire la transmission du BLV. Nous avons mené un essai sur le terrain dans trois troupeaux laitiers commerciaux du Midwest qui ne préconisaient pas l'usage unique des aiguilles et des gants d'examen dans leur protocole de santé du troupeau. De plus, la prévalence du BLV chez les vaches adultes devait excéder 20% dans chaque troupeau. Des vaches dont le lait était négatif au BLV suite à un test ELISA ont été assignées au hasard à un groupe où les aiguilles étaient jetées après chaque usage et où de nouveaux gants étaient utilisés à chaque examen (groupe d'intervention). Nous avons aussi surveillé et testé les vaches dont le lait était négatif au BLV suite au test ELISA mais soumises aux normes de gestion habituelles incluant la réutilisation des aiguilles et le partage des gants avec les autres vaches du troupeau dont le lait était positif à l'ELISA (groupe témoin). L'incidence cumulative de nouvelles infections a été déterminée en testant le lait pour la présence du BLV par ELISA deux fois par année. Il n'y avait pas de différence statistiquement significative au niveau de l'incidence cumulative de nouvelles infections au BLV entre les deux groupes de vaches. Bien qu'on recommande quand même l'hygiène médicale afin de réduire la transmission par le sang, la réutilisation des aiguilles et des gants ne semblait pas être une voie de transmission importante pour le BLV dans ces trois troupeaux.

Introduction

Bovine leukemia virus (BLV) is a retrovirus of cattle that causes significant economic losses in the US dairy industry. In addition to causing cancer in about 5% of infected cattle,[6] the virus disrupts the immune system and results in decreased milk production,[7,12,35,52] decreased cow longevity in the herd,[3,12,14,34] and decreased response to vaccines.[16,17,40] Over 20 nations worldwide have eradicated the virus, and many other nations have ongoing eradication programs.[11] In the US,

however, BLV is present in over 90% of dairy herds,[30] and the average herd prevalence has increased to about 43%.[30,49] The economic impact of BLV in the US was estimated to be $525 million in 1995,[38] and a recent estimate of BLV costs revealed a loss of $283/milking cow annually.[1,2]

Bovine leukemia virus is transmitted by transfer of infected cells, primarily lymphocytes.[32,39] Epidemiological studies have identified a number of common management practices as risk factors for BLV transmission in commercial dairy herds.[13,23,33] Most identified risk factors relate to bloodborne transmission; transmission of BLV via rectal exam sleeves has been demonstrated in both experimental[21,25] and observational studies.[10,20] Direct evidence for BLV transmission by re-use of needles is perhaps less clear. For example, Roberts et al reported no transmission from re-use of tuberculin needles during routine TB testing, but the same authors were able to transmit BLV in this way when the needle was intentionally contaminated with "a minute quantity" of blood.[42]

Although adopting single-use needles and exam sleeves incurs costs both in time and supplies, both are commonly recommended to control BLV transmission.[5,29,43] We hypothesized that changing to single-use needles and exam sleeves would decrease the cumulative incidence of new BLV infections. To determine the impact of re-use of hypodermic needles and reproductive examination sleeves in the transmission of bovine leukemia virus, a field trial was implemented in 3 midwestern commercial dairy herds to compare the new BLV infection rates among 2 randomly assigned treatment groups.

Materials and Methods

Herd Enrollment and Study Design

Herd enrollment requirements were as follows: 1) current management practices where common needles and sleeves were re-used regularly, 2) herd prevalence of antibodies against BLV by testing of milk by ELISA ≥ 20%, and 3) herd managers who were willing to follow the study protocol described below.

Individual cow milk-testing to detect antibodies against BLV by ELISA was conducted on all lactating cows at enrollment to identify BLV-negative cows, and thus susceptible to infection. BLV-negative cows identified at this test in each herd were stratified on days-in-milk and randomly assigned to the control or intervention group. The control group cows received the standard management practice of needles and sleeves shared with other control cows and with BLV-positive cows. The intervention group cows were marked by the herd manager with additional ear tags, leg bands, and/or chalk, and always received a new single-use needle and sleeve. All cows intermingled freely with each other and with their ELISA-positive herd mates as per the herd's standard management practices.

Subsequent individual cow milk-testing was conducted semiannually, as close as possible to November 1 and May 1 each year. Cows that became BLV-positive were removed from the study. After each semiannual test, the protocol was reviewed with the herd managers and asked if any mistakes had been made in the single-use needle and sleeve protocol. We had 1 report of an inadvertently re-used needle in an intervention group cow; this cow was not BLV-positive on any subsequent test. When possible, BLV-negative cows identified at the semiannual test that were not previously enrolled in the study were enrolled and randomly assigned to a study group in order to replace cows that had been removed.

Herd "P". Herd P was a Michigan free-stall dairy that milked about 220 cows with a starting BLV ELISA prevalence of 25.3% (58/223) at enrollment in fall 2014. This herd participated for 1 year until fall 2015.

Herd "W". Herd W was a Wisconsin free-stall/pasture organic dairy milking about 350 cows with a starting BLV ELISA prevalence of 74.4% (262/352) at enrollment in the fall of 2014. This herd participated for 2.5 years, until spring 2017; however, the semiannual test scheduled for May 2016 was inadvertently missed due to a communication failure with the laboratory.

Herd "F". Herd F was a Michigan free-stall dairy (with non-lactating cows on pasture) milking about 320 cows. The starting BLV ELISA prevalence was 53.5% (169/316) at enrollment in spring 2015. This herd participated for 2 years, through the spring of 2017.

Milk Sample Collection

Routine milk samples were collected by DHI technicians into containers with standard DHI preservative (bronopol/natamycin), transported to the NorthStar Cooperative Michigan Laboratory (NorthStar), and tested for antibodies against BLV via ELISA. Procedures for this study were reviewed and approved by the MSU Institutional Animal Care and Use Committee.

ELISA Test for Anti-BLV Antibodies

A modified ELISA test to detect antibodies directed against BLV, as described by Erskine et al,[15] was performed at NorthStar. Aliquots of milk samples were diluted (1:30) and added to 96-well BLV-coated ELISA plates. After washing, BLV antibodies were detected by reaction with horseradish-peroxidase-labeled antibodies to bovine immunoglobulin with addition of an enzyme substrate. Standardized reaction times were determined by color development of positive controls, and the reaction was stopped by addition of 0.5 NH_2SO_4. Results were reported as corrected 450nm optical density (OD) measurements (raw sample OD - negative control OD). Milk samples with a corrected OD > 0.1 were considered positive for anti-BLV antibodies.

Data Analysis

The principal study endpoint based on ELISA results was cumulative incidence of new infections, calculated for the previous 6 months from semiannual test results. Cumulative

incidence (proportion) was calculated at each inter-test period of approximately 6 months. For each inter-test period, the population was closed. Only enrolled, ELISA-negative cows at the beginning of a period were considered at risk of acquiring a new infection. A new infection was defined as an at-risk cow that converted to a positive ELISA result, and positive cows were excluded from all future at-risk populations. The at-risk population for each inter-test period was estimated by calculating a corrected at-risk population:[44] the number of enrolled, BLV ELISA-negative cows at the semiannual test at the start of the period, minus one-half the number of these cows absent at the semiannual test at the end of the period. In rare cases where cows were inadvertently not sampled at a semiannual test point (despite remaining in the herd) and subsequently identified as BLV-positive on the following semiannual test (e.g. cow X tested negative at test 1, was not tested at test 2, and tested positive at test 3), the standard population correction was made to the 12-month period. To avoid bias, each 'missed' newly BLV ELISA-positive cow was considered to have contributed 1 new infection over the total inter-test period (e.g. one-half new infection per 6-month period). The cumulative incidence for new infections between groups was evaluated using the Fisher mid-P 2-tail exact test in individual herds (each herd considered a stratum), and in all 3 herds combined in a stratified analysis.[8]

Each period between semiannual tests approximated summer (May through October) or winter (November through April) months at risk. The cumulative incidence was calculated for each season using the corrected at-risk population and the definitions of a new infection as described above. Due to the missed semiannual test in Herd W, only 1 summer season could be measured in that herd. The cumulative incidence of new infections in the summer and winter exposure periods that could be measured were compared using the Fisher mid-P 2-tail exact test as described above.[8]

Results

Semiannual Cumulative Incidence of New Infections between Groups

The ELISA test result was used to calculate the cumulative incidence of new infections during each inter-test period for each group (Table 1). The cumulative incidence of new infections was slightly higher numerically in the intervention group compared to the control group in both Herd W (37.7 vs 26.9) and Herd F (24.5 vs 23.9), while the opposite was true for Herd P (7.7 vs 8.2). None of these differences were statistically significant. The groups also did not differ significantly in the cumulative incidence of new BLV infections for all groups combined (P=0.378), which were 20.0 (88/440) for the controls and 22.7 (102/448.5) for the intervention group.

Seasonal Cumulative Incidence of New Infections

The combined cumulative incidence of new infections for the 3-herd combined analysis by season was higher during summer periods of exposure (P=0.036; Table 2). This appears to be primarily driven by Herd W, where the cumulative incidence was more than twice as high in the 1 summer exposure period that was measured (40.8 vs 16.8; P=0.001). The seasonal cumulative incidence was not significantly different between summer and winter in Herd F (24.5 and 23.6, respectively; P=0.788) and Herd P, although the risk in Herd P was numerically higher in the winter (9.4 vs 6.2 in the summer; P=0.357).

Discussion

This field trial examined the impact of a commonly recommended management procedure for BLV control in dairy herds. Single-use needles and rectal exam sleeves to prevent BLV transmission in dairy herds is a "common sense" approach because BLV is known to be transmitted by infected blood, and re-use of needles and sleeves has been identified as a risk factor for BLV prevalence in epidemiologic studies.[13,23,33] However, there are few field studies into the true impact of this management change, and most studies have examined re-use of needles or sleeves alone using a mixture of methodologies. A 1991 study by Hopkins et al found no difference in the rate of BLV seroconversion in cows palpated with re-used versus single-use exam sleeves under field conditions, but found a lower rate in cows palpated with washed sleeves in a teaching hospital setting.[20] In contrast, Divers et al found a 2.8-fold increase in risk of seroconversion in seronegative cows palpated with a re-used sleeve immediately

Table 1. Cumulative incidence of new bovine leukemia virus infections in enrolled groups overall and by herd.

		Herd P			Herd W	
Group	**New infections**	**Cows at risk**	**Cum. incidence***	**New infections**	**Cows at risk**	**Cum. incidence***
Control	11	134.5	8.2	35	130	26.9
Intervention	11	142.5	7.7	46	122	37.7
		Herd F			**All Herds Combined**	
Group	**New infections**	**Cows at risk**	**Cum. incidence***	**New infections**	**Cows at risk**	**Cum. incidence***
Control	42	175.5	23.9	88	440	20.0
Intervention	45	184	24.5	102	448.5	22.7

*Cumulative incidence: Mid P exact 2 tail P value: Herd P, P=0.893; Herd W, P=0.134; Herd F, P=0.921; all herds, P=0.378

Table 2. Seasonal cumulative incidence of new bovine leukemia virus infections in enrolled groups overall and by herd.

	Herd P			Herd W		
Season	**New infections**	**Cows at risk**	**Cum. incidence***	**New infections**	**Cows at risk**	**Cum. incidence***
Winter	14	148.5	9.4	17.5	104	16.8
Summer	8	128.5	6.2	37.5	92	40.8
	Herd F			**All Herds Combined**		
Season	**New infections**	**Cows at risk**	**Cum. incidence***	**New infections**	**Cows at risk**	**Cum. incidence***
Winter	29.5	125	23.6	61	377.5	16.2
Summer	57.5	234.5	24.5	103	455	22.6

*Cumulative incidence: Mid P exact 2 tail *P* value: Herd P, *P*=0.357; Herd W, *P*=0.001; Herd F, *P*=0.788; all herds, *P*=0.036

after a BLV positive cow.[10] Studies on the re-use of needles are similarly mixed, such as the Roberts et al report cited earlier, which showed no transmission under field conditions, but successful transmission when needles were deliberately contaminated.[42] Most BLV control programs recommend implementing both single-use needles and single-use sleeves, therefore our study was designed to evaluate the impact of this management change under field conditions in a manner that was minimally disruptive to participating commercial dairy operations.

Because the intervention was the use of single-use needles and exam sleeves, herd managers and staff knew which cows were assigned to the intervention group, thus preventing blinding during the study. We used within-herd controls to ensure other management practices were consistent between control and intervention cows, and we discussed the protocol with herd managers regularly to ensure compliance. However, because the study was not blinded, it is impossible to rule out that the results may have been inadvertently biased by herd managers' awareness of which cows were enrolled in the intervention. Another limitation of this study is that it followed 3 dairy herds in the midwestern United States, so the results may not be applicable to other geographic regions. In addition, all 3 herds were free-stall or free-stall/pasture; other management systems may have significant impact on transmission risk. For example, in a study of tie-stall herds in Japan, having a BLV-positive neighbor resulted in a significantly higher risk of seroconversion.[24] The cumulative incidence of BLV infections varied among herds and tended to be higher in the herds with higher BLV prevalence, consistent with other reports.[9,19,31]

The seasonal cumulative incidence was higher in the summer period of exposure, consistent with reports that associate the presence of blood-sucking insects with BLV incidence.[4,37] Herd W was the major contributor to this result, while seasonal cumulative incidence was essentially equivalent in Herd F and numerically higher in winter in Herd P. Herd W was an organic dairy, and cows spent much of their summer on pasture, therefore both of these management approaches may have influenced seasonal cumulative incidence. In contrast, our research group reported a slightly higher (but not statistically significant) cumulative incidence in winter in a different field trial, except for 1 tie stall/pasture herd which had numerically higher cumulative incidence in summer.[44] Individual herd management practices, including calving practices and housing, as well as the presence of blood-sucking insects, likely impact BLV infection and may explain the inconsistency in reported seasonal patterns of BLV infection.[46,48,50] We also used semiannual milk ELISA testing to determine BLV status. ELISA is a highly sensitive and specific test method used for BLV status certification by the World Organisation for Animal Health (OIE),[51] but it relies on the production of anti-BLV antibodies by an infected animal. In cattle, these antibodies typically appear ~3 to 10 weeks post-infection.[28] There is therefore a risk that some animals may have been infected but did not yet have detectable antibody levels on the test day, but this risk was similar for all animals and experimental groups, and any infected animals should, if still in the herd, be identified on the subsequent test. This may have affected the season in which we observed the 'new infection'. Again, however, this risk was similar for all groups.

Although the present study data did not find a statistically significant difference in the cumulative incidence of BLV infections between the 2 treatment groups, the use of single-use needles and exam sleeves, along with other interventions, has been part of several successful BLV control programs.[22,26,45,47] In addition, blood transmission via needles and sleeves is a plausible mechanism of BLV transmission in cattle.[10,20,42] However, this management practice is rarely the sole change implemented. It is possible that other practices associated with BLV incidence, such as housing practices[36,46] and feeding of pooled milk,[9,27] may pose greater risk of BLV transmission than re-use of needles and sleeves, or there could be a synergistic effect when multiple risk-associated practices are in use. Recent reports indicate that BLV transmission may be disproportionately attributable to a small number of highly infectious cows.[18,43,44] The presence (or absence) of these cows may have affected the overall transmission risk in each herd and in each exposure period, but because cows were free to intermingle, regardless of group assignment, these cows are unlikely to have affected the risk to each group within each herd.

Even though we did not see an effect on BLV transmission in this trial, implementing a practice of single-use needles

and exam sleeves improves medical hygiene and likely reduces blood transfer of many pathogens, such as anaplasmosis.[41] The cost of each needle and sleeve has decreased to less than US $0.10 each, so implementing single-use needles and sleeves is an affordable practice. Adopting single-use needles and exam sleeves should be recommended as part of most comprehensive disease control programs regardless of the impact on BLV transmission, which still requires further investigation to determine if the results reported here are repeatable.

Endnotes

[a] NorthStar Michigan Laboratory, Grand Ledge, MI

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