A Serological Survey of Bovine Syncytial Virus in Ontario: Associations With Bovine Leukemia and Immunodeficiency-Like Viruses, Production Records, and Management Practices

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ABSTRACT

Of the 920 cows tested, 56.7% showed antiretroviral serological reactivity. Prevalence rates (95% confidence interval) of antiretroviral antibodies among individual dairy cows in Ontario were: BIV 5.5% (4.0–7.0), BLV 25.7% (22.9–28.6), and BSV 39.6% (36.4–42.8). The following percentages of cows showed serological reactivity against the specified retroviruses: BIV 2.3%, BLV 14.0%, BSV 27.5%, BIV and BSV 1.3%, BIV and BLV 0.9%, BLV and BSV 9.9%, BIV and BLV and BSV 0.9%. These rates of seropositivity are similar to those found in other countries. Serological test results were not adjusted for sensitivity and specificity. The prevalence rates of antibodies to the three retroviruses (BIV, BLV, and BSV) were significantly different, but no associations were observed between specific retroviral serological test results among individual cows. The prevalence rates of BIV and BSV seropositivity were constant across Ontario, whereas, there was a significant trend for the prevalence rate of BLV seropositivity to decrease going from southwestern to eastern Ontario; cows in eastern Ontario had approximately half the prevalence rate of those in southwestern Ontario. Cows that were seropositive for BSV were significantly older than BSV seronegative cows. There was no association between culling rate and BSV serology. Significant negative associations were found with winter or summer housing of calves separate from adults and summer outdoor exercise for dry cows. The use of calf hutches in the summer had a significant positive association with BSV seropositivity.

Regression analyses were done to assess the association of retroviral (BIV, BLV, and BSV) seropositivity on calving interval, milk somatic cell count, and milk production. Serological test results for BIV, BLV, and BSV were entered into all models and all models were adjusted for intra-cluster (intra-herd) correlation. Herd size and age were found to be important confounding variables. BIV seropositivity was not associated with any changes in production using this approach, however when considered in isolation BIV seropositivity remained associated with decreased milk production. BLV seropositivity was significantly associated with longer calving intervals and higher somatic cell counts in older cows. As well, in older cows, BSV seropositivity was significantly associated with higher milk production.

RÉSUMÉ

À partir de sérums recueillis chez 920 vaches ontariennes, 56,7 % étaient séropositives aux rétrovirus suivants : BIV (5,5 %); BLV 25,7 %; BSV 39,6 % et de façon plus précise : BIV (2,3 %), BLV (14,0 %); BSV (27,5 %); BIV + BSV (1,3 %); BIV + BLV (0,9 %); BLV + BSV (9,9 %); BIV + BLV + BSV (0,9 %). Cette prévalence est comparable à celle retrouvée dans les autres pays. Les taux de prévalence pour chacun des virus étaient significativement différents et aucune association n'a pu être établie entre les animaux séropositifs. La prévalence du BIV et du BSV était constante à travers la province mais diminuait de moitié d'ouest et est pour le BLV. Les vaches BSV séropositives étaient significativement plus vieilles que les négatives. Aucune relation entre la séropositivité au BSV et le taux de réforme n'a pu être obtenue. Une corrélation négative fut notée entre la stabulation des veaux gardés séparés des adultes et l'exercice extérieur des vaches taries en période estivale. L'utilisation de huttes à veaux durant l'été augmenterait les niveaux de séropositivité au BSV.

Des analyses de régression entre la séropositivité des vaches aux trois rétrovirus et la période vêlage-vêlage, le comptage des cellules somatiques (CCS) et la production laitière ont été réalisées. Les résultats des tests sérologiques étaient intégrés dans tous les modèles et ces derniers ajustés pour les corrélations intra-troupeau. La taille et l'âge du troupeau constituaient d'importantes variables confondantes. In a ainsi été démontré que la séropositivité au BIV n'affecte pas la production, mais de façon isolée, elle diminue la production laitière. La séropositivité au BLV était associée à une augmentation de la période vêlage-vêlage et à une augmentation du CCS chez les vieilles vaches. Par contre, la séropositivité au BSV chez les vieilles vaches était associée à une augmentation de la production laitière.

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INTRODUCTION

Bovine syncytial virus (BSV), a member of the spumavirus or foamy virus subfamily of retroviruses, was first isolated from lymphoid tissue and milk (1,2). BSV is present in many areas of the world; the seroprevalence rates range from 20% to greater than 80% (2–9). Infected cattle have persistent serum anti-BSV antibodies and BSV is recoverable from the peripheral blood of most seropositive cattle. However, BSV may be found occasionally in seronegative cattle and experimentally-inoculated sheep (8,9). Among laboratory animals, rabbits were the only ones susceptible to BSV infection (9). The mechanism(s) of transmission are not known with certainty but in utero or perinatal transmission is suspected to account for most infections (3,8,10,11). The virus can be easily recovered from the female bovine genital tract (12,13).

There have been no associations of disease or decreased fertility with BSV infection; thus, BSV is currently considered nonpathogenic, as are most spumaviruses (14). However, a simian foamy virus caused transient immunosuppression in rabbits (15) and other foamy viruses have been associated with polyarthritis (16), gingivitis/stomatitis (17), and thyroiditis (18,19). More recently, a high prevalence of the human foamy virus was found among patients with Grave's disease (autoimmune hyperthyroidism) (20), and a progressive encephalopathy and myopathy occurred in mice transgenic for the partial or complete genome of the human foamy virus (21,22). Most of the interest in BSV revolves around its seemingly ubiquitous nature in the laboratory which often results in contamination of cell cultures where it causes syncytia formation, vacuolization, and cytolysis. BSV may be a contaminant in some lots of fetal bovine sera (23).

Recent molecular biological studies show that BSV has a relatively large and complex genome (24) as is typical of other members of this subfamily (25–28). All retroviruses have in common genomic regulatory elements that may be of cellular origin or derived from autologous, homologous, or heterologous viruses. These genomic regulatory elements are important in viral replication, infectivity, and pathogenesis of disease. Host cellular factors can be potent inducers of bovine and human lentiviruses (29,30). Viruses unrelated to the human immunodeficiency virus type 1 (HIV-1) transactivate the HIV-1 long terminal repeat (LTR) (29–32) and may play a role in the progression of the acquired immunodeficiency syndrome. Similarly, the bovine immunodeficiency-like virus (BIV) LTR can be transactivated at low levels by the other bovine retroviruses, the bovine leukemia virus (BLV) and BSV (30), as well as by an unrelated virus (33). Despite experimental support for cross transactivation between the bovine retroviruses, there is no significant association of BIV and BLV seropositivity in individuals and there is no observed interaction of these two retroviruses resulting in decreased measurements of production although BIV seropositivity has been associated with decreased milk production in 1 observational study (34).

Multiple retroviral infections occur in cattle (35,36). Infections with BSV may be the most frequent bovine retroviral infection and, although considered nonpathogenic, BSV may be an important cofactor, through such a mechanism as cross transactivation, in producing disease or production deficits in concert with BIV and BLV. The present study was designed to determine the prevalence of BSV seropositivity in Ontario and to investigate potential associations of the three bovine retroviruses with production levels and farm management practices.

MATERIALS AND METHODS

SERA

Sera were obtained from an existing serum bank that had previously been established as part of an epidemiological study of paratuberculosis in Ontario dairy cattle (37). Briefly, only herds enrolled in milk recording through the Ontario Dairy Herd Improvement Corporation and participating in the Brucellosis Free Listed Program of Agriculture Canada were included in the sampling frame. A random-sampling design, stratified in proportion to the number of herds in each county within the province was used to select study herds from which blood samples were collected between September 1986 and April 1987, from all dairy cows over two years-of-age. The resultant serum bank consisted of approximately 12,000 samples that were stored at −20°C. A subset of 920 samples from 263 herds was randomly (computerized random-number generator) selected and tested for antibodies to BIV (34), BLV (38), and, in the present study BSV. The number of samples per herd included in BSV testing ranged from 1 to 13 with a median of 3. The geographical locations of the herds were divided into four regions including southwestern, western, central, and eastern Ontario.

SEROLOGICAL TESTS

Testing for anti-BSV antibodies was conducted at the Department of Pathology, University of Guelph, Guelph, Ontario. Antigen production and the protocol for the immunodiffusion test for the detection of anti-BSV antibodies has been described in detail elsewhere (2). Briefly, immunodiffusion tests were done in 100 mm plastic Petri dishes filled with 8 mL Noble's agar. Every set of wells included positive and negative control sera and the results were read at 24 and 48 hours. Samples that exhibited lines of partial identity or very weak lines were repeated. The detection of anti-BLV antibodies was done at Agriculture Canada, Animal Diseases Research Institute, Nepean, Ontario using the standard agar gel immunodiffusion test (Leukassay-B, Pitman Moore, Washington Crossing New Jersey) while anti-BIV antibodies were detected using a chemiluminescence Western blot assay (36) in the Department of Pathology, University of Guelph. All serological test results were scored dichotomously, as either positive or negative.

PRODUCTION AND MANAGEMENT DATA

The serum bank was collated with electronic data files of selected production indices and with farm management data that were collected previously (37). Official production records were obtained from the Ontario Dairy Herd Improvement Corporation following

the written permission of the animal owners. The production indices included breed class average (BCA) for milk production, calving interval in months (CI), and the mean of the natural logarithm of up to six of the most recent individual milk somatic cell counts (SCC). Not all herds registered with the Ontario Dairy Herd Improvement Corporation participated in SCC monitoring. Therefore, SCC data were available from 150 of the 263 herds included in the present study. The farm management data were collected by mail survey during the Paratuberculosis study and included herd level information concerning calving and calf management, contact between heifers and adult herdmates, adult cow housing, lactating cow management, and general farm information (37).

STATISTICAL TESTS

Laboratory test results, production records, and management data were collated in electronic data files and analyzed using the Statistical Analysis System (PC-SAS, SAS Institute Inc., Cary, North Carolina) and BMDP (BMDP Statistical Software Inc., Los Angeles, California) software programs. Files were screened electronically for data that were potentially out of an acceptable range. Subsequently, 25% of all of the farm management files, selected by a computerized random-number generator, were manually verified with their original hard copy questionnaires. All of the data were kept confidential by using codes to identify and cross reference records.

The original serum samples were collected during the fall and winter of 1986–1987. To insure that the production outcomes being measured occurred temporally after serological status was determined, the production indices were recorded as of January 1988. Each of the production indices was measured in units of the individual cow deviation from the mean of that index, within the respective herd of origin (e.g., BCA). This approach attempts to control for many herd-level factors such as feeding, housing, and management. Analyses were conducted at the cow level of organization.

The prevalences of BIV, BLV, and BSV reactors were estimated at the cow-level of organization. The proportions of positive test results for each of the retroviruses were compared with one another using the Pearson chi-square test and the association between pairs of test results was assessed using a contingency table (39). Contingency tables were used to study the association of retroviral serological status with culling rates, geographical region, and the association of management characteristics with individual cow BSV serological status. Differences in age, milk production, calving interval, herdsmans' years of dairy farming experience, milk somatic cell count, and the number of cows in the herd were compared between BSV-positive and BSV-negative cows. The significance of differences was determined with the robust standard error after accounting for intra-cluster (intra-herd) correlation using a Generalized Estimating Equation (GEE1, SAS Macro, M. Rezual Karim, Department of Biostatistics, The John Hopkins University, 1989). The logit link function, binomial variance and exchangeable correlation matrix were used for dichotomous outcomes and identity link function, Gaussian variance, and exchangeable correlation matrix were used for continuous outcomes.

Backward step-wise least squares regressions were used to investigate associations between production indices and retrovirus serological test results for BIV, BLV, and BSV while controlling for cow age and herd size. Variables were removed if the P-value was greater than 0.10 for the change in F statistic. The final models were established after accounting for intra-cluster correlation using a Generalized Estimating Equation (identity link function, Gaussian variance, and exchangeable correlation matrix) and removing variables not significant at the 0.05 level. Age and herd size were assessed as indicator variables as non-linear associations were noted; cut-points were established using Walter's technique (40). The interaction of age and retroviral serological status was also included since retroviral infections generally have long incubation periods.

RESULTS

Of the 920 cows tested, 56.7% showed antiretroviral serological reactivity. Prevalence rates (95% confidence interval) of antiretroviral antibodies among individual dairy cows in Ontario were: BIV 5.5% (4.0–7.0), BLV 25.7% (22.9–28.6), and BSV 39.6% (36.4–42.8). The following percentages of cows showed serological reactivity against one or more retroviruses: BIV 2.3%, BLV 14.0%, BSV 27.5%, BIV and BSV 1.3%, BLV and BIV 0.9%, BLV and BSV 9.9%, BIV and BLV and BSV 0.9%.

TABLE I. Summary of BIV, BLV, and BSV serological test results among dairy cattle in Ontario

		BIV		Totals
		+	−	
BLV	+	16	220	236
	−	33	651	684
Totals		49	871	920

Pearson chi-square $P = 0.249$ (OR = 1.435)

		BSV		Totals
		+	−	
BLV	+	99	137	236
	−	265	419	684
Totals		364	556	920

Pearson chi-square $P = 0.385$ (OR = 1.143)

		BSV		Totals
		+	−	
BIV	+	20	29	49
	−	344	527	871
Totals		364	556	920

Pearson chi-square $P = 0.854$ (OR = 0.588)

Table I summarizes the BIV, BLV, and BSV serological test results. There were no significant associations between the frequencies of any of the retroviral serological reactions; however, the prevalence rates among individuals for all serological reactions were significantly different from one another.

Dairy cows in eastern Ontario had a significantly ($P = 0.006$) lower prevalence rate of BLV seropositivity compared with other regions (Table II). Interestingly, there was a significant ($P = 0.003$) trend for the frequency of BLV seropositivity to decrease progressing from southwestern to eastern Ontario while the rates of BIV and BSV seropositivity remained relatively constant across all regions.

In simple one way comparisons, age was significantly different between BSV+ve and BSV−ve cows (Table III). Differences in the BCAs for milk production, calving intervals, and milk somatic cell counts were not

TABLE II. The association of retroviral serological status of cows and geographical region in Ontario

Seropositivity	Geographical Region in Ontario				
	Southwest	West	Central	East	*n*
BIV	4.68[a]	5.49	7.64	4.15	49[b]
BLV	32.7	28.9	25.7	15.8[c]	236
BSV	36.8	37.9	43.1	41.9	364
n	171[d]	364	144	241	920

[a] Percentage of cows in a geographical region with a serologically positive test result regardless of coreactivity against other retroviruses
[b] Number of serologically positive cows
[c] Significantly different at $P = 0.006$ when compared with the rates of the same retrovirus but in different geographic regions and significant trend ($P = 0.003$) after accounting for intra-cluster (intra-herd) correlation
[d] Number of cows tested in a geographical region

TABLE III. Selected descriptive statistics in cows tested for anti-BSV antibodies

		Anti-BSV Antibody					
		Positive			Negative		
	Total *n*	*n*	Mean	SD	*n*	Mean	SD
Age (years)	913	359	5.81*	2.25	554	5.49	2.31
BCA milk deviation[a,b]	439	177	3.80	20.6	262	2.18	19.8
Calving interval deviation[a]	414	164	−0.05	2.04	250	0.06	2.28
In SCC deviation[a,c]	527	197	0.02	0.75	330	0.11	0.80
Herd size	920	364	54.8	35.2	556	52.4	28.9
Herdsman's years of farming experience	914	362	21.6	11.4	552	21.4	11.0

[a] Units of deviation from the herd mean; the expected mean deviations will be the same for BSV +ve and BSV −ve cows if there is no association
[b] Breed class average index for milk
[c] Mean of the natural logarithm of up to 6 of the most recent individual milk somatic cell counts
* Significantly different from serologically negative cows at $P < 0.035$

TABLE IV. Annual culling rates (%)[a] of cows tested for various anti-retroviral antibodies

Serological Status	% Culled	*n*
Negative	20.1	398
BLV	25.6	129
BIV	19.1	21
BSV	17.8	253
BLV and BIV	25.0	8
BLV and BSV	22.0	91
BIV and BSV	25.0	12
BLV, BIV, and BSV	12.5	8

Pearson chi-square = 3.94; df = 7; $P = 0.786$
[a] Percentage of cows present in January 1987 but not present in January 1988

significant (Table III). Culling rates did not differ significantly between cows grouped by serological test results for individual retroviruses or for combinations of retroviruses (Table IV). Culling rates were also examined in relationship to age and serological test results (Table V). There was a trend for BLV seropositive cows less than 3.5 years old to be culled at a lower rate, while those greater than 3.5 years old were culled at a greater rate than BLV seronegative cows; however, the differences in the rates were not significant at $P < 0.05$.

Dependent variables examined using backward step-wise least squares regressions were cow-level deviations from herd means for milk production (breed class average, BCA), calving interval (months), and the natural logarithm milk somatic cell count (Table VI). Serological test results for BIV, BLV, and BSV, cow age, herd size, and age interactions were offered into all models. All models were adjusted for intra-cluster correlation. Herd size and/or age were important confounding variables. There were significant interactions of age and seropositivity to BLV and BSV. Seropositivity for BLV was significantly associated with higher BCAs for calving intervals and somatic cell counts at older ages. In older cows, BSV seropositivity was significantly associated with higher BCAs for milk production; however, lower BCAs for milk production were found in younger aged cows with BSV seropositivity.

Table VII summarizes the distribution of management factors hypothesized to be potentially associated with individual cow BSV serological status. Only the use of calf hutches in the summer had a significant positive association with BSV seropositivity. Significant negative associations were observed with winter or summer housing of calves separate from adults and summer outdoor exercise for dry-cows.

DISCUSSION

Due to the nature of the retroviral life cycle, it is likely that all individuals with positive serological test results are persistently infected. If the serological tests were 100% sensitive and specific then almost 60% of dairy cows in Ontario were infected with retroviruses. Unfortunately, it is difficult to determine the sensitivity and specificity since the efficiency of virus isolation varies between retroviruses and virus isolation or molecular biological approaches are labor intensive. For these reasons, the prevalence rates reported here and elsewhere were not adjusted for sensitivity and specificity; the interpretation of statistical tests could change depending on these parameters. The data in other studies are not as comprehensive as those presented here, but overall the prevalence rates in Ontario are consistent with the those in other countries (2–9, reviewed in 34,38).

The most prevalent retroviral infection was BSV, which accounted for approximately 70% of retroviral infections. Most retroviral infections were due to a single agent although multiple infections did occur. There was no significant association between serological test results for different retroviruses within individuals. Retrovirally-infected individuals did not appear to be predisposed to additional retroviral infections, a phenomena that could be due to either viral determinants or constitutive host characteristics.

Assuming that these retroviral infections are endemic, then unrelated transmission mechanisms or differing infectious potentials of retrovirally-infected individuals might explain the significant differences observed between the prevalence rates of the three retroviruses. We know that

different transmission mechanisms are important; BSV is transmitted mostly congenitally (3,8,10,11), while BLV is most often spread postnatally and horizontally (39). BIV is the least prevalent of the three bovine retroviruses and very little is known about its mechanism of natural transmission. However, the number of BIV-infected cells in peripheral blood is exceedingly small (42), a factor which might account for the difficulty in isolating BIV from the blood of chronically infected individuals. In contrast, BLV is relatively easily isolated from peripheral blood and the number of BLV-infected cells in circulation appears related to the infectivity of the individual (43–46). Although nothing is known about the amount of BSV in circulation, BSV was often isolated from peripheral blood cells in attempts to recover BLV (2,6) indicating that BSV may be as prevalent in peripheral blood cells as BLV or more easily isolated. Further study is needed to establish an association between the relative infectious potentials of the 3 bovine retroviruses, the prevalence of virus in peripheral blood cells, and the persistence of anti-retroviral antibodies in serum.

As this study used cross-sectional sampling, the serological test results represent results from cows that have "survived" in the herd to the time of sampling and determination of their serological status. We cannot rule out absolutely the possibility that cows with multiple retroviral infections were under-represented because cows with multiple retroviral infections may have been removed from the herd at a faster rate than those cows with single retroviral infections. The data in Table IV suggests that this is not the case if one can assume that the serological status did not change from the time of sampling to January 1988 and that these "survivors" are representative of all cows that seroconverted.

We have previously reported on studies of BIV and BLV in this same data set (34,38). In these studies, BIV seropositivity was associated with significantly lower than herd average milk production, while BLV seropositive cows had a slight, but statistically significant, increase in calving interval. Here we have considered the 3 bovine retroviruses together and adjusted the data for intra-cluster (intra-herd) correlation. It seems reasonable to consider the influence of the three retroviruses together since there is potential for cross transactivation; similarities in modes of transmission, genetic structure, replication, and host–virus interactions; and the potential for this group of viruses to cause chronic disease. As a result of these considerations, in the present study BIV serological test results did not appear in any of the final models. Hence, BIV serological test results, when considered along with BLV and BSV serological test results and adjusting for intra-cluster (intra-herd) correlation, had no significant association with changes in calving interval, somatic cell count, or milk production. Other than our initial report (34), in which BIV was considered as a solitary agent, there have been no associations of BIV seropositivity with deficits in production or disease. BIV seropositivity appears associated with lower than herd average milk production when considered in isolation of the other retroviruses. We found that the association of BLV seropositivity and calving interval greater than the herd average did

TABLE V. Annual culling rates (%)[a] of cows with serological evidence of retroviral infections at different ages

		BSV		BLV		BIV		
Age (yrs)	*n*	+	−	+	−	+	−	Total
<3.5	125	17.7	18.7	5.3[b]	20.8	40.0[d]	17.5	18.4
3.5–6.0	469	16.6	18.8	23.6[c]	16.2	18.2	17.9	17.9
>6.0	319	22.7	25.7	26.7	23.4	18.2	24.9	24.4
Total	913	18.9	21.1	23.5	19.1	20.4	20.2	20.3

[a] Percentage of cows present in January 1987 but not present in January 1988

[b] Trend for BLV seropositive cows less than 3.5 years-of-age to be culled at a lower rate ($P = 0.19$, two-tailed; $P = 0.09$, one-tailed) than seronegative cows. One of 19 seronegative cows in this age group was culled

[c] Trend for BLV seropositive cows between 3.5–6.0 years-of-age to be culled at a higher rate ($P = 0.088$, two-tailed; $P = 0.052$, one-tailed) than seronegative cows. Twenty-six of 110 seropositive cows in this age group were culled

[d] Low prevalence of BIV with small number in the positive group; 2 cows culled of 5 seropositive cows in this age group

TABLE VI. Results of backward stepwise least squares regression of deviation from herd mean for calving interval (months), in somatic cell count (the mean of up to six of the most recent determinations), and BCA milk production. Serological test results of the 3 retroviruses, herd size, and age were offered into all models

Variable	Parameter estimate	Standard error[a]	*P*
Calving Interval (n = 414)			
Intercept	−0.64	0.18	<0.001
BLV serological status	0.57	0.29	0.050
Herd size > 70	0.62	0.27	0.021
Age (yrs) > 5.0	0.45	0.21	0.029
Somatic Cell Count (n = 522)			
Intercept	−0.31	0.08	<0.001
BLV serological status	−0.49	0.22	0.027
BLV-age interaction	0.09	0.04	0.011
3.5 < Age (yrs) < 5.0	0.22	0.10	0.029
5.0 < Age (yrs) < 11.0	0.59	0.10	<0.001
Age (yrs) > 11.0	1.14	0.22	<0.001
Milk Production (n = 439)			
Intercept	−0.03	1.29	0.810
BSV serological status	−6.67	4.68	0.153
BSV-age interaction	1.57	0.77	0.041
Herd size > 50	5.64	1.96	0.004
9.0 < Age (yrs) < 11.0	−15.71	6.32	0.013
Age (yrs) > 11.0	23.61	8.60	0.060

The r^2 values were not available once intra-cluster correlation was considered. Without intra-cluster correlation the values were 0.04, 0.14, and 0.07 for calving interval, somatic cell count, and milk production, respectively

[a] Represents the robust standard error from the Generalized Estimating Equation procedure

TABLE VII. Descriptive summary of management characteristics potentially associated with BSV serological status

Variables within subgroups	% of individuals with characteristics	
	BSV +ve (n = 364)	BSV −ve (n = 556)
winter calving location:		
cows calve in separate pen vs other	63.8	68.0
summer calving location:		
cows calve in separate pen vs other	46.2	49.1
cows calve on pasture vs other	42.9	37.6
feeding after colostrum and before weaning:		
raw milk or soured colostrum only from dam vs other	9.9	11.3
pooled raw milk or soured colostrum vs other	81.0	79.1
separation of calf from dam:		
most calves separated from dam between 48 hr and 1 wk vs other, mostly <48 hr	3.6	6.3
winter housing of calves less than 4 mo old:		
room or barn separate from adults vs other	28.3	36.7*
calf hutches vs other	14.0	10.5
summer housing of calves less than 4 mo old:		
room or barn separate from adults vs other	28.6	37.4*
calf hutches vs other	20.2	13.1*
contact between heifers (>4 mo old) and adults:		
routine contact in winter vs other	43.7	42.3
routine contact in summer vs other	49.2	48.2
winter dry-cow housing:		
free-stall or loafing barn vs other	37.6	38.4
dry-cows tied but exercised at least 3 times weekly vs other	24.5	25.2
summer dry-cow housing:		
free-stall or loafing barn vs other	4.6	6.9
outdoor exercise lot, average size 2.3 ha vs other	37.8	46.9*
winter milking-cow housing:		
free-stall or loafing barn vs other	15.1	13.7
tie-stall but exercised 3 times weekly vs other	45.6	46.0
summer milking-cow housing:		
tie-stalls or stanchion, rarely loose vs other	0.9	1.8
outdoor exercise lot, average size 3.0 ha vs other	50.0	55.4
frequency of additions from other herds:		
frequent introductions from other herds vs other	25.6	26.9
some replacements, at least one in the last 3 yr vs other	33.1	33.1
dry cow treatment:		
use dry-cow mastitis preparation on >70% of cows vs other	55.2	59.4
use dry-cow mastitis preparation only on cows with history of mastitis vs other	31.0	29.8
teatdip:		
apply teat-dip after milking vs other	87.4	88.7

* Significantly different at $P < 0.05$. Where the differences were significant, the Odds Ratios were all less than 1.000 except for summer housing of calves in hutches in which case the Odds Ratio was 1.68

persist in the present study. In addition, we found that BLV was associated with milk somatic cell counts greater than the herd average in older cows. Others have found BLV seropositivity to be unassociated with any deficits in production (47), or to be associated with increased culling rates and calving intervals (38,48,49) and decreased (50,51) or increased (49,52,53) milk production. It is interesting to note that the culling rate of BLV-seropositive cows increased relative to seronegative cows as cows aged, although the differences in culling rates did not achieve significance at P = 0.05. This observation was consistent with the significant association of higher culling rates and BLV seropositivity found in previous studies by other researchers (38,48–50,54). Currently, we do not have a definitive biological explanation for the observed association between BLV seropositivity and increased calving interval, but we hypothesize that subclinical disease, possibly resulting from immunosuppression, could be responsible.

Cows seropositive for BSV were significantly older than BSV seronegative cows. Seroreactivity for BSV was not associated with changes in culling rate. As we found for BLV (47), it seems that a tendency for cows to produce more milk of better quality leads to those cows being maintained in the herd, hence the slightly greater age of BLV and BSV seropositive cattle. It is likely that the longer the time spent in the herd, the greater the chance there is to become seropositive. This is reasonable for BLV since we expect that most infections result from horizontal transmission; however, prior data suggest that BSV is mostly spread congenitally (3,8,10,11). In the present study, we found BSV to be significantly and negatively associated with housing calves in the winter or summer separate from adults and providing outdoor exercise in the summer for dry-cows. These management practices could conceivably decrease opportunities for transmission. The data suggest that horizontal transmission of BSV may occur which could explain the similarity of age distributions of BLV and BSV. We have no satisfactory explanation for the positive association of BSV seropositivity and the use of calf hutches in the summer.

We found that the rates of BIV and BSV seropositivity remained constant across Ontario, but BLV seropositivity was significantly lower in eastern Ontario. There was a significant trend for the rate of BLV seropositivity to decrease when moving from southwestern to eastern Ontario. Currently, we have no biological explanation for this geographical trend. Variations in farm management practices between regions could potentially account for this observation.

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