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Carryover of bovine leukemia virus antibodies in samples from shared milk meters

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ABSTRACT

Screening for infectious diseases of cattle using milk from the dairy herd improvement (DHI) sampling process is very convenient. However, when samples from shared milk meters are used, carryover of antibodies or other diagnostic targets can complicate the interpretation of the diagnostic test results for diseases, including bovine leukosis. The objectives of this study were (1) to assess the potential for carryover of antibodies against bovine leukemia virus (BLV) in milk samples obtained from shared meters, and (2) to determine if adjustment of the diagnostic test cut-off value would improve the test characteristics for meter-collected milk ELISA results. Eight dairy farms were randomly selected from herds with a wide range of BLV prevalence levels in Prince Edward Island, Canada. Within each chosen farm, 2 to 4 milk meters were randomly selected. During the routine procedures of DHI sampling, 2 simultaneous milk samples, 1 hand-collected at the beginning of milking (after udder preparation) and the other from the corresponding milk meter, were taken from all lactating cows (n = 236) that were milked at the selected meters (n = 26). The sequence of cows using each meter was recorded. All samples were tested for BLV antibodies using a commercial indirect ELISA. Antibody carryover potential was assessed in meter-collected samples that were preceded by other cows using the same meters. Applying the hand-collected sample results as our reference standard, a new cut-off was defined for meter-collected samples to optimize the test characteristics. At the standard cut-off value of the diagnostic test, 110 (46.6%) of the hand-collected and 136 (57.6%) of the meter-collected samples were positive. For low-titer cows (e.g., true negatives), the likelihood of antibody carryover significantly increased as the titer of preceding cows increased, whereas this change was not substantial for high-titer cows. The odds of obtaining false diagnoses in meter-positive samples became larger with increase in the titer of preceding cows. A suspicious category for meter ELISA results was defined, and a retest was recommended for the cows falling into this category. This strategy effectively assisted in reducing the number of consequent false-positive results. When DHI-collected samples are used, carryover can affect the interpretation of dichotomous test results and may require adjustment of assay cut-off values.

Key words: carryover, milk meter, dairy herd improvement, bovine leukemia virus

INTRODUCTION

Enzootic bovine leukosis (**EBL**) is an economically important infection of dairy cattle worldwide, which is caused by bovine leukemia virus (**BLV**). In North America, prevalence of the infection has been high and appears to have a rising trend (Samagh and Kellar, 1982; Richardson and Macaulay, 1992; Sargeant et al., 1997; VanLeeuwen et al., 2001; VanLeeuwen et al., 2005; VanLeeuwen et al., 2006; Bartlett et al., 2014). For instance, in Prince Edward Island, Canada, herd-level prevalence of BLV was 49.2% in 1989 (Richardson and Macaulay, 1992), increased to 63.3% in 1998 (VanLeeuwen et al., 2001), and is currently at 90% based on a survey of bulk tank milk in all dairy herds in the province completed in 2013 (O. A. Nekouei, unpublished data). However, no broad-based national program for controlling EBL in Canada and the United States has been implemented.

Several studies have recently been conducted to estimate the prevalence of BLV infection and define cost-effective screening tools to be applied in control programs. Monitoring meter-collected milk samples, obtained from the DHI process has become one of the standard and economically efficient procedures for screening for important infectious diseases in dairy cattle, such as bovine viral diarrhea, Johne's disease, and EBL (Houe et al., 1995; Attalla et al., 2010; Sorge et al., 2011). Among the available commercial tests for detection of antibodies against BLV, milk ELISA is a desirable method in large-scale herd surveillance

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Table 1. Herd characteristics and milk-ELISA results for antibodies against bovine leukemia virus (BLV) on 8 study farms from Prince Edward Island, Canada

Farm	Prevalence level[1]	Lactating herd size	Selected meters	Tested cows	Positives in meter[2]	Positives in hand[2]
1	Low	49	3	27	0	0
2	Low	83	4	28	4	4
3	Medium	57	4	22	15	11
4	Medium	46	2	12	5	4
5	High	62	3	30	24	23
6	High	126	4	48	35	25
7	Very high	76	3	37	27	23
8	Very high	64	3	32	24	20
Total	—	563	26	236	136	110

[1]Predicted levels of within-herd prevalence of BLV based on a companion study (O. A. Nekouei, unpublished data).

[2]Number of cows testing positive at the recommended ELISA cut-off of 10 (percent positivity).

because milk sampling during the DHI process is much more convenient and cost-effective than serum collection (Erskine et al., 2012).

With respect to sequential milk samples, "carryover" denotes the risk for inclusion of some residual milk from the previous cows in the subsequent samples (Ordolff, 1997; Løvendahl and Bjerring, 2006). With increasing utilization of DHI diagnostic services on meter-collected samples, a legitimate concern exists regarding carryover or cross contamination of milk samples from shared milking equipment. The objectives of our study were (1) to assess the potential for carryover of BLV antibodies in milk samples obtained from shared meters, and (2) to determine if adjustment of the diagnostic test cut-off value would improve the test characteristics for meter-collected milk ELISA results.

MATERIALS AND METHODS

Sample Collection

Based on a companion study using bulk tank milk samples from all dairy farms in Prince Edward Island, Canada, all DHI-participant farms were assigned into 5 separate categories of BLV infection level: (1) assumed uninfected, (2) low prevalence, (3) medium prevalence, (4) high prevalence, and (5) very high prevalence farms (O. A. Nekouei, unpublished data). In July 2013, 2 farms were randomly selected from each of the categories 2 to 5 (8 farms total). Within each selected farm, 2 to 4 milk meters were randomly selected based on the lactating herd size (Table 1). During one round of the routine DHI sampling procedure, 2 simultaneous milk samples (30 mL each), one hand-collected at the beginning of milking (after udder preparation) and the other from the corresponding milk meter, were taken from all lactating cows (n = 236) that were milked at the selected meters (n = 26). The sequence of the cows milked using each meter was precisely recorded by the project personnel.

Laboratory Testing

Meter-collected samples were submitted to the Maritime Quality Milk (**MQM**) laboratory located at the Atlantic Veterinary College, University of Prince Edward Island, in Charlottetown, Canada, after undergoing the standard quality and components analyses in a local DHI laboratory (PEI Analytical Laboratory, Charlottetown, Prince Edward Island, Canada). Hand-collected samples were directly submitted to the MQM laboratory. All samples were tested for BLV antibodies using a commercial indirect ELISA kit (Svanovir BLV gp51-Ab, Svanova, Uppsala, Sweden). The test results were reported as percent positivity (**PP**) values [$PP = (OD_{corrected}$ sample$/OD_{corrected}$ positive control$) \times 100$, where OD = optical density], and the recommended cut-off value of the kit for individual milk samples was 10.

Statistical Analyses

All of the statistical analyses were conducted in Stata 13.1 (StataCorp, College Station, TX).

Agreement. To evaluate the overall agreement between hand ($\mathbf{PP_{hand}}$) and meter ($\mathbf{PP_{meter}}$) test results, a scatter diagram was produced and the concordance correlation coefficient was calculated. In addition, the overall agreement of the dichotomized results (at the recommended cut-off of 10) from the 2 types of samples was explored using McNemar's Chi-squared test and Cohen's kappa coefficient (Dohoo et al., 2009).

Carryover Effects. To determine the potential carryover effects of BLV antibodies, all cows that had been preceded by other cows at the same meters contributed to building a multivariable linear regression