



## Associations between reproductive performance and seropositivity for bovine leukemia virus, bovine viral-diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* in Canadian dairy cows

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### ABSTRACT

Our objective was to investigate effects of seropositivity for bovine leukemia virus (BLV), Type 1 bovine viral-diarrhea virus (BVDV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP), and *Neospora caninum* (NC), and their possible interactions, on reproductive efficiency (specifically, first-service conception [FSC], and calving interval [CI]) in dairy cows. The sample population included up to 30 randomly selected animals from 179 randomly selected farms in five provinces in Canada, from which 23 farms did not meet the inclusion criteria for the final dataset. Serum samples were tested for antibodies against the stated pathogens using commercially available diagnostic tests. A Cox proportional hazards model with shared (herd-level) frailty was utilized to analyze the CI data. In this model, BLV-seropositive cows had a 7% lower rate of conception compared to seronegative cows ( $P=0.06$ ). Mixed logistic regression models of  $CI > 484$  days,  $CI > 534$  days, and  $CI > 584$  days were built to explore factors of long CIs. These cut-offs were selected to represent calving-to-conception intervals of  $>200$  days,  $>250$  days, and  $>300$  days. BLV-seropositive cows had higher odds of having a  $CI > 484$  days compared to BLV-seronegative cows, and BLV serostatus interacted with lactation number in this model, with 1st lactation seropositive cows being more likely to have a  $CI > 484$  days than older seropositive cows. NC-seropositive cows had a 1.27 times higher odds of exhibiting a  $CI > 484$  days, a 1.37 times higher odds of a  $CI > 534$  days, and a 1.54 times higher odds of a  $CI > 584$  days, compared to NC-seronegative cows. Neither BVDV nor MAP seropositivity showed any significant effect in these models. For the FSC models, a first service was classified successful (pregnancy = 1) if it was the cow's last service and she calved 270–290 days later. A mixed logistic regression model of FSC revealed an interaction between NC and BVDV-seropositivity at the herd level, with odds ratios of 0.64, 1.06 and 0.85 for NC-seropositive cows (compared to NC-seronegative cows) in BVDV-seronegative, BVDV-seropositive and BVDV-missing herds, respectively. BLV and MAP seropositivity had no significant impact on FSC. All models controlled for herd-clustering effects, and included parity, linear score of somatic cell counts, peak milk, and province to control for confounding. The overall FSC was 51%, the average CI was 393 days, and 18%, 9% and 5% of lactations had  $CI > 484$  days,  $>534$  days, and  $>584$  days, respectively.

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### 1. Introduction

Enzootic bovine leukosis (caused by bovine leukosis virus; BLV), bovine viral-diarrhea (caused by bovine viral-

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diarrhea virus; BVDV), Johne's disease (caused by *Mycobacterium avium* subspecies *paratuberculosis*; MAP), and neosporosis (caused by *Neospora caninum*; NC) are all transmissible diseases that are considered to be of economic importance in the international trade of animals and animal products. Each of these pathogens can lead to clinical disease that can have effects on reproduction (Thurmond and Hietala, 1996, 1997; Thurmond et al., 1997; Moen et al., 1998; Lindberg, 2003; Bartels et al., 2006). However, due to conflicting evidence, it remains unclear whether there is impaired reproductive performance from subclinical infection with BLV (Langston et al., 1978; Huber et al., 1981; Heald et al., 1992; Emanuelson et al., 1992; D'Angelino et al., 1998), BVDV (Baker, 1995; Rufenacht et al., 2001; Waldner, 2005), MAP (Johnson-Ifearulundu et al., 2000), and NC (Romero et al., 2005; Waldner, 2005; Bartels et al., 2006).

Furthermore, it is unclear whether there are any combined reproductive effects from interactions between subclinical infections with these pathogens (e.g. infection with one pathogen making the reproductive effects of infection with another pathogen worse). Possible interactions between these infectious agents (BLV, BVDV, MAP, and NC) have been theorized, but there has been limited published research on these hypotheses. For example, the immunosuppression from concurrent infections with other agents might lead to recrudescence of latently infected cattle with NC. However, an increased risk of abortion was not observed when cows were seropositive to both NC and BVDV infections compared to NC infection alone (Bartels et al., 1999; Bjorkman et al., 2000; Mainar Jaime et al., 2001). Similarly, a Canadian study tested serum for BVDV, IBR and NC antibody at pregnancy diagnosis in beef herds and found no interactions between NC and the other two agents (Waldner, 2005). In Peru, NC-seropositivity significantly affected the hazard of late abortion, but again there was no interaction between NC and circulating BVDV antibody or antigen, however few cows were susceptible to incident infection of BVDV (Stahl et al., 2006). No previous study has ever looked at all four of these agents in the same sample population of cattle.

Our objective was to investigate associations between reproductive efficiency (specifically, first-service conception—FSC and calving interval—CI) and seropositivity for BLV, BVDV, MAP, and NC in the same population of dairy cows, controlling for the effects of seropositivity for other pathogens. Combined reproductive effects from interactions between subclinical infections with these pathogens will also be investigated.

## 2. Materials and methods

The data we utilized were from prevalence surveys of Canadian dairy herds (Keefe and VanLeeuwen, 2000; VanLeeuwen et al., 2001; VanLeeuwen et al., 2005) in New Brunswick (NB), Nova Scotia (NS), Ontario (ONT), Prince Edward Island (PEI), and Saskatchewan (SASK). To assess overall reproductive performance, fetal loss, and conception ability specifically, three different approaches were used, based on the restriction that the only source of reproductive data on the sampled cattle was from monthly

milk testing data from dairy herd improvement (DHI) records. Initially, with observations restricted to 314–484 days, CI was utilized to study the impact of NC, BVDV, BLV, and MAP seropositivity on overall reproductive performance. Second, three dichotomous variables (CI > 484 days, CI > 534 days, and CI > 584 days) were created under the assumption that prolonged CI was a surrogate for fetal loss. These cut-offs were selected to represent calving-to-conception intervals of >200 days, >250 days, and >300 days. Finally, FSC was utilized to assess conception ability.

### 2.1. Serum sample collection

A stratified two-stage random sampling procedure was utilized for herd recruitment. During the summer of 1998, participating dairy herds in PEI, NB, and NS were randomly selected (using computer-generated random numbers) from all herds on monthly milk testing through the regional DHI company until 90 herds were recruited, 30 from PEI, NB, and NS (response rate >87%). These herds met the herd-level recruitment inclusion criteria which included willingness to: provide representative cattle for blood sampling; allow the blood to be tested for antibodies indicating exposure to the four pathogens (BLV, BVDV, MAP and NC); and release DHI data to the research team. Subsequently, similar herd-level inclusion criteria were utilized to recruit 45 herds in ONT in 1999, and 44 herds in SASK in 2001. Additional exclusion criteria were utilized, as described in Section 2.4, to ensure that the selected reproductive data from participating farms were reliable for the specific outcomes and analyses conducted.

Sample sizes for herd sampling within each province were based on calculations first determined and reported in detail for the Atlantic provinces (VanLeeuwen et al., 2001). Briefly, the formula assumed 300 herd owners in each province subscribing to the monthly milk recording service provided by the Atlantic Dairy Livestock Improvement Corporation (for data acquisition ease), an estimated average prevalence of disease of 10% (this low prevalence was used because we were sampling for antibodies to four pathogens and expected low herd prevalences for most of them in Atlantic Canada), an allowable error of 10%, and a confidence level of 95%. The number of sampled herds required from the other provinces was adapted for the number of herds, estimated herd prevalences, and available funds for each province. The sampling proportions ranged from 1% to 10%.

Using computer-generated random numbers, up to 30 (all cows if the total number of cows in the herd was <30) lactating animals were randomly selected for blood collection in each participating herd. The sample-size calculation for cow sampling within herds was also based on calculations first determined and reported in detail for the Atlantic provinces (VanLeeuwen et al., 2001). Input parameters included an average herd size of 40–45 milking cows, estimated average prevalence of disease of 10%, an allowable error of 10%, and a confidence level of 90%. This sample size of 30 cows was adopted in the other provinces because a standard number of sampled cows in each sampled herd was desirable for comparison purposes of herd-level prevalences across provinces.

The goal for BVDV testing was to identify herds with possible immunosuppression from current or recent past exposure to BVDV, either from transiently or persistently infected animals being on the farm. However, with vaccination for BVDV commonplace in Canada, and maternal antibodies for BVDV lasting up to 6 mo, the previously described cow samples could not be utilized from all farms. In BVDV-vaccinated herds, five unvaccinated heifers >6-mo-old in each sampled herd were selected and blood sampled, where available, to ensure that maternal antibodies would no longer be present. In BVDV-unvaccinated herds, five animals of the approximately 30 cows tested for the other three pathogens were selected for BVDV testing because these cows were being blood sampled already and could provide similar evidence of BVDV exposure in the herd compared to the heifer samples. The BVDV sampling protocol was based upon previous studies (Houe, 1992; Pillars and Grooms, 2002) that used five sentinel animals to detect herds with at least one animal persistently infected with BVDV. Within 24 h, the blood samples were centrifuged, and the serum was harvested and stored at  $-20^{\circ}\text{C}$  until all the samples were collected and ready for testing for that province.

## 2.2. Laboratory analysis

The test utilized for BLV antibodies was an enzyme-linked immunosorbent assay (ELISA) from IDEXX (IDEXX Laboratories, Westbrook, MA, USA), which has a sensitivity = 98.5% and specificity = 99.9% (Johnson and Kaneene, 1991). Using the manufacturer's testing instructions, a cow was considered to be seropositive for BLV if the sample-to-positive (S/P) ratio on the ELISA was  $>0.50$ , as recommended by the manufacturer of the test kit. The BLV ELISA test kit also requires a confirmation of positive tests, using a sample-to-negative host-cell ratio  $>1.8$ . The samples were tested in duplicate at the national BLV testing laboratory in PEI (now in Quebec), which is certified to conduct BLV testing for international trade purposes.

The test utilized for BVDV antibodies was virus neutralization to the cytopathic Type-1 Singer strain, which has a sensitivity = 99.6% and specificity = 100% (Deregt et al., 1992). An animal was considered BVDV-seropositive with the presence of any antibodies for BVDV. However, due to many infected animals overcoming and clearing BVDV infections (with a subsequent drop in antibodies when the antigen is no longer present), an animal was considered to have been "recently infected" with BVDV with a titer  $\geq 1:64$  for BVDV. A herd was considered to have been "recently infected" with BVDV if  $>1$  animal had a titer  $\geq 1:64$ . This cut-point was utilized based on an observed natural division in the titers within study farms in Atlantic Canada (VanLeeuwen et al., 2001) where  $>80\%$  of herds with cattle having titers  $\geq 1:64$  also had cattle with titers of 1:256 (the highest dilution tested). Conversely,  $>80\%$  of herds with cattle having titers of 1:32 did not have cattle with titers  $>1:32$ . A similar trend was seen in other provinces as well. Testing for BVDV was conducted at the Animal Diseases Research Institute in Alberta.

The test utilized for MAP antibodies was the IDEXX Johne's Herdchek ELISA (IDEXX Laboratories, Westbrook, MA, USA), which has a sensitivity ranging from 15.4% in subclinical cattle to 88.1% in clinical cases when compared to fecal culture results, and specificity = 98.9% (Dargatz et al., 2001). This ELISA was evaluated (McKenna et al., 2005) based on tissue-culture results producing a sensitivity as low as 8.8%. Using the manufacturer's instructions, an animal was considered to be seropositive for MAP if the S/P ratio on the ELISA was  $>0.25$ , as recommended by the manufacturer. The samples were tested in duplicate at Prairie Diagnostic Services in SASK, which is accredited for MAP-ELISA testing by the United States Department of Agriculture.

The test utilized for NC antibodies was the BIOVET ELISA (BIOVET Inc.; St. Hyacinthe, Quebec, Canada) which has a sensitivity = 89.0% and specificity = 99% (Wapenaar et al., 2007). Using the manufacturer's instructions, a cow was considered to be seropositive for NC if the S/P ratio was  $>0.60$ , as recommended by the manufacturer. The samples were tested in duplicate at Biovet Laboratories in Quebec.

## 2.3. Variables

Reproductive and production data of sampled cows were obtained from the Canadian Dairy Herd Management Services which processes DHI records for all of Canada. We utilized data for all lactations (specifically, the lactation in which the blood sampling was done, plus the lactations before and after sampling) between July 1996 and October 2002, based on the assumption that infection with BLV, MAP or NC likely occurred prior to the first lactation for most seropositive cattle. There were no DHI data on abortions in this database. The data thus derived were structured on four-levels: lactation, cow, herd, and province.

CI was obtained from the DHI data. Inherently, this led to the exclusion of lactations that did not have a subsequent calving. All lactations starting after 1st January 2001 were also excluded to allow a minimum of 22 months of follow-up for each calving to overcome selection bias (otherwise, later lactations could only include those that successfully conceived quickly). Lactations with CIs shorter than 314 days were also excluded, assuming these to be recording errors or outliers because calving-to-conception intervals (CCI)  $<30$  days would be highly unlikely or unrepresentative of normal cattle, assuming a gestation of 284 days.

CIs ranging from 314 to 484 days (corresponding to CCIs between 30 and 200 days) were utilized as an overall assessment of reproductive performance because CIs combine the potential impact of the pathogens and other possible factors on all breedings up to 200 days post-calving. However, we also wanted to separate out impacts potentially related to fetal loss. A lactation with a CI of  $<484$  days was considered unlikely to have had an abortion event. We utilized various cut-point values of CI to determine which cows had an unusually long CI (i.e. CI was transformed into a dichotomous variable). This variable initially utilized 484 days as a cut-point (CI484), where cows with a CI  $>484$  days were considered an

indirect indicator of fetal loss or abortion. Cut-points of 534 days (CI534) and 584 days (CI584) were also investigated, corresponding to CCLs of 250 days and 300 days, respectively, as described in Section 2.4.

For FSC, strict criteria were defined to minimize misclassification errors. Each first breeding was classified as successful (pregnancy = 1) if it was the cow's last service and she calved 270–290 days later. Breedings were classified as unsuccessful (pregnancy = 0) if it was not the last service, or if the interval between first service to next calving did not fall in the range of 270–290 days, or if the interval between service to dry-off was >270 days. Multiple breedings of the same cow within 72 h were considered a single insemination event, with the last breeding being recorded as the date of breeding. Services not meeting the criteria for either successful or unsuccessful breeding remained unclassified and were not utilized in the final analysis. Unclassified breedings were retained in the dataset to determine if classification failure might have lead to a selection bias in the results.

BLV, BVDV, MAP and NC-seropositivity were all coded as positive or negative for each cow sampled. While BLV, MAP and NC-seropositivity were cow-level variables, BVDV was a herd-level variable; cows within a herd were coded as positive for BVDV if the herd was classified as BVDV-seropositive. Lactation number was coded as a three-level (1st, 2nd, and 3+) categorical variable. The 1st lactation was the baseline level for all models. We also coded provinces as a categorical variable, with PEI as the baseline.

The potential effect of linear score (logarithmic transformation) of somatic cell count (LS-SCC) on the reproductive parameters was estimated by determining the median value of the LS-SCC from the observations measured between 30 and 200 days-in-milk (DIM) for that lactation. Peak milk (PM) production was recorded as the highest level of milk production at any of the DHI tests taken between 30 and 200 DIM.

#### 2.4. Statistical analysis

A cow was excluded if: DIM at first service was <30 or >300 days (considered outliers); or milk production was ≤0.1 kg (missing code). Herds with an average services per conception of 1 were excluded from the analyses because only successful breedings were entered into the DHI database for these herds. Also, herds with fewer than five cows remaining in the final dataset were excluded because with so many exclusions, we could not be confident that the remaining cows were representative of the cows on the farm.

For determining the significant predictors of CI, a survival analysis using Cox's proportional hazards models and Aalen's linear hazards models was conducted (Hosmer and Royston, 2002). The latter was utilized to investigate the time-varying influence of the predictors utilized in the Cox model. This linear hazards model produced graphs with time on the X-axis and the cumulative coefficient of each predictor on the Y-axis. If the effect of the predictor was constant over time, the graph would form a straight line. Any change in the slope of the line would be indicative of a time-varying effect.

Two types of Cox proportional hazards models were fit: unconditional models to determine the effect of each predictor on the outcome variable without controlling for other predictors or herd effects (using Wald's test,  $P < 0.10$ , 2-sided); and shared frailty survival models, controlling for other significant predictors through a manual forward stepwise procedure ( $P \leq 0.05$ , 2-sided, based on the likelihood ratio chi-square test) and shared herd effects, assuming a gamma frailty distribution (Gutierrez, 2002). All first-order interactions were evaluated and retained if significant ( $P \leq 0.05$ , 2-sided).

To analyze CI484, CI534, CI584, and FSC, mixed logistic regression models were used, with adjustment for clustering of cows within herds as a random effect. Clustering of herds within provinces and lactations within cows were also investigated as random effects. Initially, unconditional models were developed to determine the effect of each predictor on the outcome variable without controlling for other predictors. Then final multivariable random effects logistic regression models for each outcome variable were developed using a manual backward stepwise process of eliminating least statistically significantly associated variables until all remaining variables were significant at  $P < 0.05$  (2-sided), based on the likelihood ratio chi-square test. All first-order interactions were evaluated and retained if significant ( $P \leq 0.05$ , 2-sided). Model diagnostics were performed, including a normal probability plot of the higher level residuals from each of the logistic models, and Hosmer–Lemeshow statistics.

To determine if unclassified breedings for FSC might have lead to a selection bias, descriptive statistics for classified and unclassified breedings were calculated for the following variables for which data were available for this study: parity (1st, 2nd, and 3+ lactations); production variables (LS-SCC and PM); and a breeding variable (DIM on breeding date) (Table 1). We utilized t-tests ( $P < 0.05$ , 2-sided) to confirm that there were no differences in these variables (except for parity, where a Wilcoxon rank-sum test was used) between the FSC-classified and FSC-unclassified (and therefore excluded) records.

The effects of PM on each of the outcomes evaluated were handled in three different ways. In the survival analysis of CI, the effects of PM were different early in the breeding period (<73 days) compared to later. This can be seen in Fig. 1 (Aalen's linear hazards model – discussed in Section 3) in which PM appears to have a substantial suppressive effect on CI up to 73 days but not thereafter. Consequently, two variables (PM Early and PM Late) were created to estimate separately these two effects. In our logistic regressions with CI484, CI534, and CI584 as the response variables, we utilized a single estimate of PM as a continuous variable on its own. However, in the logistic regression of FSC, we included PM as a 3-level categorical variable (<32 kg, 32–42 kg, >42 kg) because the relationship between PM and logit FSC was not linear. The cut-points were defined based on the change of the behavior of the odds ratio relative to the PM in a lowess smoothing curve (Fig. 2; discussed in Section 3).

All analyses were conducted using the statistical software package STATA (version 9). For handling

**Table 1**

Descriptive statistics of the laboratory test results and outcome variables in the datasets used for each of the reproductive effects models, in Canadian dairy herds in 1999–2001.

| Variables                               | CI survival model:<br>parameter and (95% C.I.) | CI logistic models:<br>parameter and (95% C.I.) | FSC model: parameter<br>and (95% C.I.) |
|---|--|---|--|
| # Of herds                              | 151  | 156   | 147                                    |
| # Of cows (2+ lactation)                | 2391   | 2938  | 2359                                   |
| # Of first lactation heifers            | 485  | 593   | 509                                    |
| Percent test positive–BLV               | 26.7 (25.0–28.3)                               | 27.5 (24.4–27.6)                                | 26.9 (25.3–28.5)                       |
| Percent test positive–BVDV <sup>a</sup> | 40.9 (38.9–42.8)                               | 41.7 (39.8–43.4)                                | 40.5 (38.6–42.3)                       |
| Percent test positive–MAP               | 2.4 (1.8–2.9)                                  | 2.5 (1.7–2.8)                                   | 2.5 (1.9–3.0)                          |
| Percent test positive–NC                | 12.6 (11.4–13.8)                               | 12.1 (11.1–13.2)                                | 12.6 (11.4–13.8)                       |
| Average calving interval (days)         | 449.2 (446.0–452.3)                            | 443.8 (441.5–446.5)                             | 449.6 (446.2–452.6)                    |
| Average first-service conception (%)    | 50.9 (49.0–52.7)                               | 51.0 (49.1–52.8)                                | 50.7 (48.9–52.5)                       |

<sup>a</sup> Herd level.

confounding, variable coefficients were examined with the inclusion (for CI survival analysis) or removal (for CI or FSC) of each variable to determine if the other model variables changed by more than 20%. If a change of more than 20% was observed, then confounding was detected, and the variable was forced to remain in the model, regardless of whether it was statistically significant or not. We also forced seropositivity for the four pathogens into the final models to control for any confounding that the pathogens may have on the outcome variables. Correlation coefficients between variables were calculated to assist in decisions regarding collinearity of predictors. If two or more collinear variables were statistically significantly associated with the outcome variable in unconditional models, the most biologically plausible variable was used in the final models.

### 3. Results

#### 3.1. Descriptive analysis

Table 1 provides sample sizes and descriptive statistics of the frequency of positive test results and outcome variables for the datasets used for each of the models developed. There were 23 herds that did not meet the exclusion criteria, leaving 156 herds in the final dataset for analysis. For the survival analysis, we limited the data to cows with a CI of 314–484 days (30–200 day CCI), leading to five more farms being excluded because they did not

have five or more cows with this low CI. This limitation also led to individual cows and first calf heifers removed from the survival analysis dataset from farms that did have five or more animals with this low CI. However for the logistic regression analyses using different CI cut-points, the entire final dataset was used. For FSC, nine herds were excluded from the final dataset due to the presence of fewer than five cows in the herd that met the inclusion criteria, primarily due to the strict criteria to classify each first breeding as successful or not, but also because labeled cow names and/or farm records sometimes did not match with DHI database names. Farms with fewer than five cows in the dataset were excluded because these herds had undue influence on the final models, with high standardized residuals.

To avoid a substantial reduction of sample size (and associated power), herds that could not be classified as BVDV infected (BVDV = 1) or not infected (BVDV = 0), due to a lack of unvaccinated animals older than 6 months old in the herd, were classified as unknown BVDV status (BVDV = 2) instead of missing. There were two, three, and 15 herds in New Brunswick, Ontario, and Saskatchewan, respectively, requiring this additional classification. The percent of herds vaccinated for BVDV in our sample population was 73.1%. The apparent prevalence of MAP infection was very low, probably due, in part, to the low sensitivity of the diagnostic test (McKenna et al., 2005). There were 18%, 9% and 5% of lactations with CI > 484 days, >534 days, and >584 days, respectively. There were no

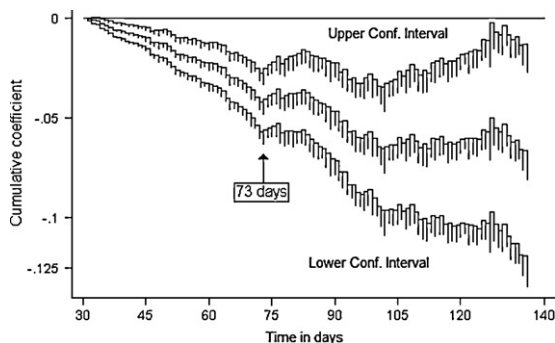


Fig. 1. Aalen's linear hazards models for peak milk production using calving interval as an explanatory variable for 2876 cows in 151 Canadian dairy herds from 1998 to 2001.

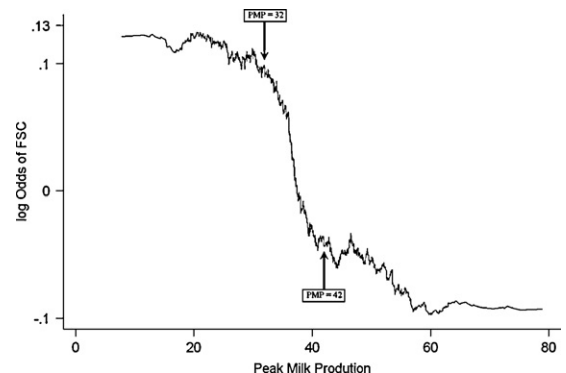


Fig. 2. Smoothed curve of log odds of first-service conception using peak milk production as an explanatory variable for 2868 cows in 147 Canadian dairy herds from 1998 to 2001.

**Table 2**

Descriptive statistics for production variables for 6061 first breedings from 3531 cows in 147 Canadian dairy herds in 1998–2001.

| Variable             | Classified <sup>a</sup> |       |      |      | Unclassified <sup>b</sup> |      |
|----------------------|-------------------------|-------|------|------|---------------------------|------|
|                      | Min                     | Mean  | SD   | Max  | Mean                      | SD   |
| Peak milk production | 7.8                     | 38.3  | 9.2  | 75.2 | 38.6                      | 9.0  |
| Linear score-SCC     | 0.1                     | 2.3   | 1.6  | 8.6  | 2.6                       | 1.7  |
| Parity <sup>c</sup>  | 1                       | 3     | –    | 12   | 3                         | –    |
| DIM at service date  | 40                      | 106.5 | 46.8 | 200  | 109.3                     | 47.7 |

<sup>a</sup> Includes 5038 first breedings among 2868 cows classified as having a successful or unsuccessful service at first breeding.

<sup>b</sup> Includes 1023 first breedings among 663 cows which could not be classified as successful or unsuccessful due to insufficient data.

<sup>c</sup> Parity is not Gaussian and therefore medians (3 and 3 for classified and unclassified cows, respectively) are the appropriate measures of central tendency, not means, and SD is therefore not appropriate for parity either.

differences in test results or outcomes among the datasets or among the excluded records, indicating minimal bias from the exclusions.

Descriptive statistics for FSC-classified and unclassified cows are in Table 2. All *P* values for differences between these two groups of cows were  $\geq 0.06$ , indicating minimal selection bias associated with classification failure.

### 3.2. Analysis of CI

Clustering of lactations within cows was not controlled for in the final CI model because there were only 1.6 lactations/cow, on average, so any clustering effect would have been small. Province was included as a fixed effect in the model because of the small number of provinces in the dataset (attempts to include it as a random effect produced very low variance estimates). The final dataset for the survival analysis of CI contained 4569 lactations from 2876 cows in 151 herds.

In the final CI model (Table 3), only BLV serostatus was marginally ( $P = 0.06$ ) associated with CI; BLV-seropositive cows had a 7% lower rate of conception compared to seronegative cows (Table 3). BVDV, MAP, and NC-seropositivity, lactation and province had no significant effects in the final model in which clustering within herds was controlled, although they were retained to control for the effects that these pathogens may have on reproductive performance. LS-SCC had a significant association with CI, where each unit increase in LS-SCC lead to a 2% lower rate of conception.

In preliminary models, PM had a significant effect on CI, but the Aalen's linear hazards models for peak milk production using CI as an explanatory variable demonstrated that PM had a time-varying effect on CI (Fig. 1), violating an assumption of the Cox proportional hazards model which assumes that the ratios of the hazards should be constant over time. Fig. 1 identifies a substantial detrimental effect of PM on CI, but only up to 73 DIM. The specific test for proportional hazards for PM had *P* values of 0.03 and 0.01 on the time and log (time) scales, respectively. PM was therefore divided, using 73 days as the cut-point (based on the data), into two effects, PM early and PM late (Table 3). After the division of PM into early

**Table 3**

Final Cox proportional hazards model for calving interval (days) for 2876 cows in 151 Canadian dairy herds from 1998 to 2001.

| Variable                                | Hazard <sup>e</sup> ratio              | SE   | <i>P</i> value |
|---|--|------|----------------|
| Fixed effects                           |  |      |                |
| BLV <sup>a</sup>                        | 0.93                                   | 0.04 | 0.06           |
| BVDV <sup>b</sup> –negative             | Baseline—overall <i>P</i> value: 0.177 |      |                |
| BVDV <sup>b</sup> –positive             | 0.91                                   | 0.06 | 0.18           |
| BVDV <sup>b</sup> –missing              | 0.98                                   | 0.07 | 0.74           |
| MAP <sup>c</sup>                        | 0.97                                   | 0.10 | 0.76           |
| NC <sup>d</sup>                         | 0.95                                   | 0.05 | 0.29           |
| Lactation                               |  |      |                |
| 1st lactation                           | Baseline—overall <i>P</i> value: 0.143 |      |                |
| 2nd lactation                           | 1.02                                   | 0.05 | 0.69           |
| 3+ lactation                            | 1.08                                   | 0.05 | 0.09           |
| Province                                |  |      |                |
| Prince Edward Island                    | Baseline—overall <i>P</i> value: 0.231 |      |                |
| New Brunswick                           | 1.02                                   | 0.07 | 0.78           |
| Nova Scotia                             | 1.09                                   | 0.07 | 0.18           |
| Ontario                                 | 1.12                                   | 0.08 | 0.09           |
| Saskatchewan                            | 1.14                                   | 0.08 | 0.06           |
| Linear score–somatic cell count         | 0.98                                   | 0.01 | 0.05           |
| Peak milk (kg/cow/day)—early (<73 days) | 0.98                                   | 0.01 | <0.01          |
| Peak milk (kg/cow/day)—late (>74 days)  | 0.99                                   | 0.01 | 0.05           |
| Random effects                          |  |      |                |
| Herd (variance)                         | 0.03                                   | 0.01 |                |

<sup>a</sup> BLV: bovine leukemia virus.

<sup>b</sup> BVDV: bovine viral–diarrhea virus.

<sup>c</sup> MAP: *Mycobacterium avium* subspecies paratuberculosis.

<sup>d</sup> NC: *Neospora caninum*.

<sup>e</sup> Note that the term hazard is standard within survival analysis, but with the hazard being conception in this case, it is more appropriate to think of it as a probability ratio—the relative probability of conception at a point in time.

and late segments, the *P* values for the tests of proportional hazards for PM were no longer significant. First-level interactions between main effects revealed no significant effects.

### 3.3. Logistic regression using CI484, CI534 and CI584

Clustering of lactations within cows was again not controlled for in the final model because there were only 1.72 lactations/cow. In the logistic regressions of various cut-points of CI, the final dataset contained 6078 lactations from 3531 cows in 156 herds. From the logistic regression diagnostics, normal probability plots of the higher level residuals from each of the logistic models were normally distributed, and the Hosmer–Lemeshow statistics were not significant, confirming a good fit of the models to the data.

BLV-seropositive cows had a higher odds of having a CI > 484 days compared to seronegative cows (Table 4). BLV status also interacted with lactation number, but only in the CI484 model, indicating that the effect of BLV-seropositivity on CI484 was dependent on lactation number, and vice versa. To determine the odds ratios for BLV-seropositivity in each lactation category, or for lactation number in each BLV-seropositivity category, the odds ratios for the main effect variables and interaction variables in Table 4 must be multiplied together, with the baseline values of odds ratios = 1. Therefore, the CI484 odds ratios for BLV-seropositive cows in 1st, 2nd, and 3rd plus lactations were 1.66 (1.66 × 1), 0.83 (1.66 × 0.5) and

**Table 4**

Final logistic mixed models for three lengths of calving intervals for 3531 cows in 156 Canadian dairy herds from 1998–2001.

| Variable                         | Calving interval > 484 days     |      |              | Calving interval > 534 days |      |         | Calving interval > 584 days |      |         |
|----------------------------------|---------------------------------|------|--------------|-----------------------------|------|---------|-----------------------------|------|---------|
|                                  | Odds ratio                      | SE   | P value      | Odds ratio                  | SE   | P value | Odds ratio                  | SE   | P value |
| <b>Fixed effects:</b>            |                                 |      |              |                             |      |         |                             |      |         |
| BLV <sup>a</sup>                 | 1.66                            | 0.28 | <sup>e</sup> | 0.97                        | 0.12 | 0.83    | 1.12                        | 0.18 | 0.48    |
| BVDV <sup>b</sup> –negative      | BL–overall P value: 0.57        |      |              | BL–overall P value: 0.37    |      |         | BL–overall P value: 0.65    |      |         |
| BVDV <sup>b</sup> –positive      | 1.23                            | 0.26 | 0.32         | 1.40                        | 0.38 | 0.21    | 1.22                        | 0.40 | 0.54    |
| BVDV <sup>b</sup> –missing       | 1.14                            | 0.25 | 0.56         | 1.49                        | 0.43 | 0.16    | 1.36                        | 0.47 | 0.37    |
| MAP <sup>c</sup>                 | 1.05                            | 0.24 | 0.84         | 0.99                        | 0.32 | 0.98    | 0.98                        | 0.43 | 0.96    |
| NC <sup>d</sup>                  | 1.27                            | 0.14 | 0.03         | 1.37                        | 0.19 | 0.03    | 1.54                        | 0.28 | 0.02    |
| 1st lactation                    | BL–overall P value <sup>e</sup> |      |              | BL–overall P value: 0.27    |      |         | BL–overall P value: 0.06    |      |         |
| 2nd lactation                    | 1.01                            | 0.13 | <sup>e</sup> | 0.99                        | 0.15 | 0.96    | 1.08                        | 0.21 | 0.68    |
| 3+ lactation                     | 0.96                            | 0.12 | <sup>e</sup> | 0.84                        | 0.12 | 0.23    | 0.76                        | 0.15 | 0.17    |
| BLV <sup>a</sup> × 1st lactation | BL–overall P value: <0.01       |      |              |                             |      |         |                             |      |         |
| BLV <sup>a</sup> × 2nd lactation | 0.50                            | 0.12 | <0.01        |                             |      |         |                             |      |         |
| BLV <sup>a</sup> × 3+ lactation  | 0.61                            | 0.12 | 0.01         |                             |      |         |                             |      |         |
| Prince Edward Isle               | BL–overall P value: 0.67        |      |              | BL–overall P value: 0.43    |      |         | BL–overall P value: 0.36    |      |         |
| New Brunswick                    | 0.77                            | 0.15 | 0.19         | 0.68                        | 0.17 | 0.13    | 0.57                        | 0.18 | 0.07    |
| Nova Scotia                      | 0.82                            | 0.16 | 0.30         | 0.71                        | 0.18 | 0.16    | 0.66                        | 0.20 | 0.16    |
| Ontario                          | 0.78                            | 0.16 | 0.21         | 0.69                        | 0.18 | 0.15    | 0.65                        | 0.20 | 0.17    |
| Saskatchewan                     | 0.87                            | 0.17 | 0.48         | 0.88                        | 0.22 | 0.60    | 0.83                        | 0.24 | 0.52    |
| Linear score–somatic cell count  | 1.00                            | 0.02 | 0.89         | 0.98                        | 0.03 | 0.52    | 0.93                        | 0.04 | 0.11    |
| Peak milk (kg/cow/day)           | 1.02                            | 0.01 | <0.01        | 1.02                        | 0.01 | 0.01    | 1.02                        | 0.01 | 0.08    |
| <b>Random effects</b>            |                                 |      |              |                             |      |         |                             |      |         |
| Herd (variance)                  | 0.35                            | 0.07 |              | 0.51                        | 0.11 |         | 0.58                        | 0.16 |         |

<sup>a</sup> BLV: bovine leukemia virus.<sup>b</sup> BVDV: bovine viral–diarrhea virus.<sup>c</sup> MAP: *Mycobacterium avium* subspecies paratuberculosis.<sup>d</sup> NC: *Neospora caninum*.<sup>e</sup> No P value is given because the variable is involved in an interaction variable, making it impossible to interpret this P-value.

1.01 (1.66 × 0.61), respectively, showing that the odds of a CI > 484 days in BLV-seropositive cows were higher in 1st lactation heifers compared to older cows, with no effects in older cows. In the BLV-seronegative group of cows, the 1st, 2nd, and 3rd plus lactation cows also had CI484 odds ratios equal to or close to one (1.00, 1.01 and 0.96, respectively), demonstrating minimal effect of lactation on CI484 in BLV-seronegative cows.

A significant dose:response relationship was observed for NC-seropositivity when comparing the 3 CI logistic models. NC-seropositive cows had a 1.27 ( $P=0.03$ ) times higher odds of having a CI > 484 days compared to NC-seronegative cows, and this odds ratio increased to 1.37 ( $P=0.03$ ) times in the CI534 model and 1.54 ( $P=0.02$ ) times in the CI584 model. PM was also associated with CI484 and CI534. Province, LS-SCC, and BVDV and MAP seropositivity did not exhibit a significant impact in our models, although again they were retained to control for the effects of these pathogens and factors on reproductive performance.

#### 3.4. Logistic regression using FSC

Clustering of lactations within cows was again not controlled for in the final model because it was unnecessary. The final dataset for the logistic regression model of FSC contained 5038 lactations from 2868 cows in 147 herds.

An interaction between NC and BVDV-seropositivity was observed (Table 5), indicating that the effect of NC-seropositivity on FSC was dependent on BVDV serostatus,

and vice versa. To determine the odds ratios of FSC for NC-seropositivity in each BVDV-seropositivity category, or for BVDV-seropositivity in each NC-seropositivity category, the odds ratios for the main effect variables and interaction variables in Table 5 must be multiplied together. The FSC odds ratios for NC-seropositive cows (compared to NC-seronegative cows) in BVDV-seronegative, BVDV-seropositive, and BVDV-missing herds were 0.64 (0.64 × 1), 1.06 (0.64 × 1.65) and 0.85 (0.64 × 1.32), respectively, showing that the effect of NC-seropositivity on FSC was primarily within the BVDV-seronegative herds, with minimal effects in BVDV-seropositive herds. In NC-seronegative cows, the odds ratio of FSC for BVDV-seropositivity was 1.23.

The PM variable divided into three categories using two cut-points, 32 and 42 kg/cow/d, was associated with FSC. As PM increased, the odds of conception on first service decreased. These categories were based on the lowest smoothing curve in Fig. 2, which shows the non-linear effect of PM on the log odds of FSC. Province and LS-SCC were also associated with FSC ( $P \leq 0.05$ ), although the direction of the association for LS-SCC was opposite (albeit weak with an odds ratio of 1.04) to the effect seen in the CI survival model. Parity, and MAP and BLV-seropositivity were not significantly associated with FSC, though again they were retained to control for the effects of these pathogens on reproductive parameters.

#### 4. Discussion

This is the first study that has investigated the reproductive effects of these four pathogens in the same

**Table 5**  
Final logistic mixed model for first-service conception for 2868 cows in 147 Canadian dairy herds from 1998 to 2001.

| Variable                                     | Odds ratio                      | SE   | P value      |
|--|---------------------------------|------|--------------|
| <b>Fixed effects</b>                         |                                 |      |              |
| BLV <sup>a</sup>                             | 1.15                            | 0.09 | 0.08         |
| BVDV <sup>b</sup> —negative                  | Baseline—overall P value: 0.14  |      |              |
| BVDV <sup>b</sup> —positive                  | 1.23                            | 0.13 | <sup>e</sup> |
| BVDV <sup>b</sup> —missing                   | 1.13                            | 0.20 | <sup>e</sup> |
| MAP <sup>c</sup>                             | 0.98                            | 0.21 | 0.91         |
| NC <sup>d</sup>                              | 0.64                            | 0.08 | <sup>e</sup> |
| NC <sup>d</sup> × BVDV <sup>b</sup> negative | Baseline—overall P value: 0.03  |      |              |
| NC <sup>d</sup> × BVDV <sup>b</sup> positive | 1.65                            | 0.32 | <0.01        |
| NC <sup>d</sup> × BVDV <sup>b</sup> missing  | 1.32                            | 0.50 | 0.47         |
| Lactation 1st                                | Baseline—overall P value: 0.10  |      |              |
| Lactation 2nd                                | 1.09                            | 0.10 | 0.33         |
| Lactation 3+                                 | 1.21                            | 0.11 | 0.04         |
| Prince Edward Island                         | Baseline—overall P value: <0.01 |      |              |
| New Brunswick                                | 1.42                            | 0.22 | 0.02         |
| Nova Scotia                                  | 0.80                            | 0.12 | 0.15         |
| Ontario                                      | 0.68                            | 0.11 | 0.02         |
| Saskatchewan                                 | 0.91                            | 0.14 | 0.53         |
| Linear score-somatic cell count              | 1.04                            | 0.02 | 0.06         |
| Peak milk 1 (<32 kg/cow/day)                 | Baseline—overall P value: 0.01  |      |              |
| Peak milk 2 (32–42 kg/cow/day)               | 0.85                            | 0.07 | 0.06         |
| Peak milk 3 (>42 kg/cow/day)                 | 0.75                            | 0.07 | <0.01        |
| <b>Random effects</b>                        |                                 |      |              |
| Herd (variance)                              | 0.20                            | 0.04 |              |

<sup>a</sup> BLV: bovine leukemia virus.

<sup>b</sup> BVDV: bovine viral diarrhoea virus.

<sup>c</sup> MAP: *Mycobacterium avium* subspecies paratuberculosis.

<sup>d</sup> NC: *Neospora caninum*.

<sup>e</sup> No P-value is given because the variable is involved in an interaction variable, making it impossible to interpret this P-value.

sample population of dairy cattle. We utilized a number of outcome variables to assess reproductive performance, based on available DHI data: overall reproductive performance (CI); fetal loss (CI484, CI534, and CI584); and the ability to conceive (FSC). On some farms, complete breeding information was recorded by DHI technicians, however this practice was not consistent across all farms and all technicians. We considered CI to offer a more unbiased estimate of reproductive performance than other reproductive parameters based on available DHI data. CI only requires two calving dates, which are consistently reported, whereas days-to-first service, overall conception rate or services per conception require accurate entry of breeding data beyond the successful breeding. Three outcome variables (CI484, CI534, and CI584) were utilized as surrogate measures of fetal loss, because abortion records were not available. We utilized FSC as a measure of conception ability, though early fetal loss might also show up as failure to conceive.

BLV-seropositive cows exhibited a borderline significant lower probability of conception in lactations with CI < 484 days. Furthermore, there was an interaction between BLV and lactation number in the CI484 logistic model (but not in the CI534 and CI584 models), with 1st lactation seropositive cows being more likely (odds ratio = 1.66) to have a CI > 484 days than older seropositive cows. It is unclear how to interpret these findings.

Management of first lactation animals is considerably different from heifers. Heifers often undergo minimal handling and health management interventions, and have much less disease incidence compared to cows. Conversely, cows have more opportunities for virus transfer in the form of shared needles or syringes for treating or preventing disease, and shared rectal sleeves during multiple heat checks and pregnancy checks. Therefore, first lactation cows might be getting infected during this first lactation, with the effects of this new infection reducing reproductive performance. Also, the clinical effect of BLV, the development of lymphosarcoma, is more likely to occur in older cows, but then these cows would not likely have a subsequent calving, and therefore not be included in the CI dataset. Our study corroborates findings of an increased CI in seropositive cows in Ontario, even after controlling for age and milk production, although no interaction between age and BLV status was found (Heald et al., 1992). Alternatively, we might have detected a spurious interaction in the CI484 model. With 18%, 9% and 5% of lactations having CI > 484 days, >534 days, and >584 days, respectively, the CI534 and CI584 models had reduced power to detect significant associations among BLV-seropositivity, lactation number, and CI534 or CI584.

The BVDV results were surprising. While controlling for NC status, they revealed a slightly higher likelihood of conception at first service in BVDV-seropositive herds (odds ratio = 1.23) than in BVDV-seronegative herds, suggesting that the presence of BVDV might improve FSC rates, contrary to findings elsewhere. Infection with BVDV from 46 to 210 days resulted in a statistically significant increase in the abortion rate (Baker, 1995). Elsewhere, BVDV infection increased the risk of embryonic and fetal death (Robert et al., 2004). Furthermore, 7% of fetal deaths have been associated with infection with BVDV (Rufenacht et al., 2001). Seroconversion for BVDV after 2 years of age had a negative effect on the herd average time to first calving by 14–16 days (Valle et al., 2001). One possible reason for the unexpected finding of a beneficial effect of BVDV in this study was a possible selection bias. Herds that did not have five or more unvaccinated cattle >6 months old were not included in the study. Herds with aggressive vaccination schedules due to on-going BVDV problems, leading to vaccination of calves and heifers, would have been ineligible for inclusion in the study, and these are the herds in which negative effects of BVDV might be expected.

We found no impact of MAP seropositivity on the outcome variables in any of our models, contrary to findings in the USA (Johnson-Ifeorunludu et al., 2000). However, with only 2% of cows testing MAP seropositive in our study, we had low power to detect a significant association between MAP seropositivity and CI. Also, due to the lower sensitivity and specificity of the ELISA tests compared to fecal culture (Sockette et al., 1992), this association might be spurious because of misclassification bias. Additional evidence is required to determine if MAP seropositive cows have longer CI or impaired FSC compared to MAP-seronegative cows.

Effects of seropositivity for NC on CI < 484 days in our survival model were not significant, and this is consistent

with other studies (Bjorkman et al., 1996; Jensen et al., 1999). However, we discovered a negative effect of NC on FSC, the probability of conceiving at first service. Also, using outcomes of CI484, CI534 and CI584, we consistently found a positive association between NC-seropositivity and CI for each cut-point. Although there were fewer cows with CI > 584 days than cows with CI > 484 days, and hence the power of the model to detect an association was expected to fall, there was no decrease in the statistical significance of the observed odds ratio, due to the increased strength (odds ratio) of the observed association as the cut-point was raised. We speculate that this increased strength of association was due in part to fewer “problem breeder” cows in this CI584 group that were erroneously assumed to be suffering fetal loss than in the CI484 group, although we have no data to confirm this speculation. NC-seropositivity also had an increased risk of non-pregnancy and abortion in a Canadian study of 66 beef herds and 2516 cows and heifers (Waldner, 2005).

We did discover an interaction between NC and BVDV-seropositivity in our FSC final model, with the odds of conception on first service for NC-seropositive cows being dependent on the BVDV herd status. In BVDV-seronegative herds, NC-seropositivity reduced the odds of FSC, however this negative effect of NC-seropositivity was not seen in BVDV-seropositive herds. This finding is somewhat different from other results where there was no difference in risk of abortion due to NC-seropositivity when stratifying on BVDV serostatus (Bartels et al., 1999; Bjorkman et al., 2000; Mainar Jaime et al., 2001; Stahl et al., 2006). The difference between the studies may be due to the different outcome variable being examined, because unlike the other studies, our study did not look specifically at abortion. Further research is needed to corroborate these findings.

Milk production (represented by peak milk—PM) and parity behaved as expected in the final model, as seen by others (Stevenson et al., 1983; Coleman et al., 1985). We also found that a unit increase in LS-SCC (from clinical or subclinical mastitis) was associated with a 2% lower rate of conception in the CI survival model, but 1.04 times higher odds for FSC in the logistic regression. No relationship was found between CI and SCC in dairy cattle in Northern Ireland (McCoy et al., 2006). However, clinical mastitis either prior to or after first postpartum breeding decreased reproductive efficiency in high producing Holstein dairy cows (Santos et al., 2004). It may be possible that the energy associated with fighting clinical or subclinical mastitis may be reducing the quality of the egg, or leading to reabsorption of the embryo. Clinical mastitis with concomitant fever may also lead to reabsorption of the embryo.

One limitation of this study was the definition of a positive herd for BVDV exposure. Herds only required one animal with a titer  $\geq 1:64$  for type-1 BVDV to be considered recently infected with BVDV. This definition is less stringent than that used by Houe (1992) or Pillars and Grooms (2002). However those studies were reporting on a cut-off to detect farms with persistently infected (PI) cattle, whereas we wanted to include herds with either transiently or PI cattle in our definition of BVDV-positive

because both circumstances would likely lead to some immunosuppression of cattle. Furthermore, in Pillars and Grooms Michigan study, which would represent Canadian conditions better than Houe’s Danish study, only 66% of PI herds were detected using a cut-off of at least three of five animals having  $>1:128$  titer. Therefore, it is probable that the less stringent cut-off used in the present study, which was based on a natural division in the titers within study farms, would provide a better sensitivity than the Michigan study at detecting transiently and PI herds, which was the goal of our study. Also, it is certainly biologically plausible that a farm that no longer has BVDV but had BVDV in the recent past would have animals with high but waning titers compared to farms with active transmission of BVDV. Therefore, we decided to define herds that met our cut-off, which was derived from our data but respective of other research cut-offs, as “recently infected”, not currently infected or PI infected.

A second concern was that the herds were not tested for antibodies against type-2 BVDV due to insufficient funding. Therefore, some herds that were negative for type-1 BVDV might have been false negatives if the herd had type-2 BVDV that was not cross-reactive with the type-1 BVDV test used. The implication of these possible misclassifications is that the actual strength of association of the interaction between NC-seropositivity and BVDV-seropositive herds might be even larger than what was observed, assuming that any errors were non-differential.

Our reproductive indices were derived from DHI data, and particularly on the identification of a subsequent calving to confirm the success of a particular breeding. Cows that were bred but subsequently culled prior to another calving would not be included in our datasets, potentially leading to a selection bias. If culling were associated with pathogen presence (meaning that the pathogen had some effect on the animal, reproductive or otherwise, leading to premature culling) then a stronger reproductive effect would be expected if this culling did not occur. Therefore, culling would only lead to a bias toward the null, even if it were differential misclassification. Therefore the associations found could actually be stronger than what was reported.

For FSC, the 1023 unclassified first services represented 17% of all first services, providing some opportunity for selection bias. However, we believe that this bias was minimal for two reasons. First, there were no differences between classified and unclassified cattle in Table 2. Second, based on the classified cattle, the overall FSC in our study was 51% which is similar to what has been reported elsewhere. For example, from a 1-yr study of 17 herds in Virginia (Dransfield et al., 1998), a first-service conception probability of 46% was reported. In a 3-yr study of 45 herds in southwestern and eastern Ontario, there was a first-service conception probability of 48% (Kinsel and Etherington, 1998). Conversely, in 19 commercial herds in New York (Butler et al., 1996), a 41% overall first-service conception probability was reported, based on 1800 first breedings (heifer 51% vs cows 38%). If we were to include our unclassified services into the unsuccessful services group (where they likely belong because most would be from culled cows), then our first-service conception

probability would be 42%. It is likely that our true FSC is somewhere between 42% and 51%.

## 5. Conclusion

BLV-seropositive cows had a borderline ( $P=0.06$ ) negative association with CI compared with BLV-seronegative cows, and had a higher risk of prolonged CI in first lactation cows in particular. Seropositivity for NC was associated with two reproductive performance measures. NC-seropositive cows had a 1.27 times higher odds of having a CI > 484 days compared to NC-seronegative cows, and this odds ratio increased to 1.37 times in the CI534 model and 1.54 times in the CI584 model. Also, an interaction between NC and BVDV was observed with respect to FSC, with odds ratios of 0.64, 1.06 and 0.85 for NC-seropositive cows (compared to NC-seronegative cows) in BVDV-seronegative, BVDV-seropositive and BVDV-missing herds, respectively. MAP-seropositive cows were not associated with any of the reproductive parameters examined.

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