The effects of viral vaccination of dairy heifer calves on the incidence of respiratory disease, mortality, and growth

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ABSTRACT

Bovine respiratory disease (BRD) is one of the most common infectious causes of morbidity and mortality in young dairy cattle. The objective of this randomized clinical trial was to determine the effectiveness of 1 or 2 doses of a 5-way, modified-live viral vaccine, administered to heifer calves before weaning to aid in the prevention of BRD. The hypotheses were that vaccination would reduce the incidence of BRD and mortality, and that 2 doses would be more effective than 1. A total of 2,874 heifer calves from 19 commercial dairy farms in Minnesota and Ontario were enrolled at 1 to 7 d of age and were followed until 3 mo of age. Calves were randomly assigned to receive a commercial, intramuscular, modified-live vaccine against bovine viral diarrhea virus types 1 and 2, bovine respiratory syncytial virus, bovine herpesvirus type 1, and parainfluenza virus type 3 at 15 to 21 d of age (2 wk only), 35 to 42 d (5 wk only), both 2 and 5 wk, or sterile saline at both times (unvaccinated controls). The incidence of failure of passive transfer was 11 or 32%, using cut-points of serum total protein of 5.2 and 5.7 g/dL, respectively. Overall, 22% of calves were treated at least once for BRD. The incidence risk of naturally occurring BRD was 7.7% before 2 wk of age, 8.0% between 2 and 5 wk, and 9.5% between 5 wk and 3 mo of age, and was not different between vaccination groups. Overall mortality throughout the 3-mo study period was 3.5%. Mortality was 1.6% before 2 wk of age, 0.5% between 2 and 5 wk, and 1.2% between 5 wk and 3 mo of age. The risk of mortality was not affected by vaccination. Mean average daily gain of 1.07 kg/d from 5 wk to 3 mo of age was not different between vaccine groups. In this population of commercial, home-raised calves, with an overall low incidence of failure of passive transfer, intramuscular vaccination with a multivalent, modified live viral vaccine at 2 or 5 wk of age or both was not

associated with a decreased risk of BRD or mortality, or with growth until 3 mo of age. Reasons for these findings may include interference by maternal antibodies, unresponsiveness of the neonatal immune system, timing of immunity relative to pathogen exposure, disease caused by pathogens other than the viruses in the vaccine, or herd immunity. However, in populations with higher incidence of failure of passive transfer or risk of BRD, calves with low levels of specific antibodies may respond differently to vaccination.

Key words: dairy calf, vaccination, bovine respiratory disease, mortality

INTRODUCTION

Bovine respiratory disease (BRD) is an important illness of young dairy replacement heifers. The incidence risk of disease may range from 2 to 39% (Van Donkersgoed et al., 1993; Gulliksen et al., 2009b). Calves treated for BRD have reduced survival and growth compared with their herd mates (Rossini, 2004; Stanton et al., 2010). Viral pathogens potentially associated with BRD include bovine viral diarrhea virus types 1 and 2 (**BVD-1** and **BVD-2**), bovine respiratory syncytial virus (**BRSV**), bovine herpesvirus type 1 (**BHV-1**), and parainfluenza virus type 3 (**PI-3**; Radostits et al., 2007). It is common for dairy producers to vaccinate breeding-age heifers and mature cattle with a multivalent viral vaccine against BVD-1 and 2, BRSV, BHV-1, and PI-3. Traditionally, replacement heifers have not been vaccinated until they are at least 6 mo old, due to the notion that the maternal antibodies the calf receives in colostrum prevent the vaccine from being effective (Woolums, 2007). Unfortunately, dairy calves most often suffer from BRD before, or shortly after weaning, at a time when the titer of maternal antibodies may have decreased to low levels in some individual calves. For this reason, some dairy producers vaccinate calves at or before weaning in an attempt to reduce the occurrence of BRD. However, minimal field research has been done to evaluate the effectiveness of this practice.

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High levels of maternal antibodies may interfere with a calf's humoral response to exposure to viral antigens as measured by seroconversion, defined as a 4-fold increase in antibody titer (Van Donkersgoed et al., 1991; Ellis et al., 2001). However, despite a lack of seroconversion, calves that are exposed to viral antigens in the face of maternal antibodies may have active immune responses. These may be characterized by an apparently slower rate of decline of antibody titer, an anamnestic response upon later exposure, the development of cell-mediated immunity, or clinical protection against experimental viral challenge (Endsley et al., 2003; Fulton et al., 2004). These studies suggest that although vaccination in the face of maternal antibodies may not result in seroconversion, beneficial effects may exist that may reduce the incidence of BRD and associated mortality.

The objective of this research was to determine the effectiveness of 1 or 2 doses of a multivalent, modified live viral (MLV) vaccine, administered to heifer calves before weaning to aid in the prevention of BRD. The hypotheses were that vaccination would reduce the incidence of BRD and mortality, and that 2 doses would be more effective than 1. Furthermore, it was hypothesized that vaccination might reduce both clinical and subclinical disease and, thus, improve growth.

MATERIALS AND METHODS

Herds and Animals

A total of 2,874 calves were enrolled from 19 herds (15 herds in southwestern Ontario, 4 herds in Minnesota). Herds were selected as a convenience sample of commercial dairy farms within a 2-h radius of the University of Guelph (Guelph, ON, Canada) or the University of Minnesota (St. Paul). A wide spectrum of management styles was represented, from 40-cow herds housed in tiestall barns, to 2,400-cow herds housed in freestall barns. Calves were all home-raised throughout the study period and were reared according to routine farm management practices. All heifer calves born from the time the herd was enrolled in the study, between January and May 2008, to December 2008, were eligible for the study and were followed until 3 mo of age. The Animal Care Committee at the University of Guelph and the Institutional Animal Care and Use Committee at the University of Minnesota approved the study.

Vaccination Groups

At enrollment between 1 and 7 d of age, calves were randomly assigned to 1 of 4 vaccination groups using a block randomization method. Vaccination groups were

balanced within each block of 4 calves. They received a label dose (2 mL) of multivalent, MLV vaccine against BVD-1 and -2, BHV-1, BRSV, and PI-3 (Bovi-Shield Gold 5; Pfizer Canada, Kirkland, QC, Canada) by intramuscular injection at (1) 15 to 21 d of age (2 wk only), (2) 35 to 42 d old (5 wk only), (3) both 2 and 5 wk, or (4) sterile saline at both times (unvaccinated controls). The vaccine was stored and used according to the manufacturer's recommendations. Sample size was calculated to detect a difference between an incidence risk of BRD of 30% in unvaccinated calves, 15% in calves vaccinated once, and 10% in calves vaccinated twice, with 95% confidence and 80% power (Dohoo et al., 2009).

Morbidity, Mortality, and Growth

Records of disease events and treatments were maintained by farm personnel using standardized forms and case definitions. A case of BRD was defined as having an increased resting respiratory rate, sound or effort, and fever ($>39.5^{\circ}\text{C}$) with one or more additional signs such as coughing, nasal discharge, depression, decreased appetite, or rough hair coat. Treatments with a long-acting antibiotic had to be more than 7 d apart, and treatments with other medications had to be more than 3 d apart to be considered separate disease events. Farm personnel were blinded to the vaccination status of all calves to reduce bias.

Research technicians visited farms weekly. Calves were examined at 1 to 7 d (enrollment), 15 to 21 d (2 wk), 35 to 42 d (5 wk), and 90 to 120 d of age (3 mo). Height was measured at the withers with the head in a neutral position. Weight was measured using a heart-girth measuring tape pulled snugly around the thorax, just caudal to the forelimb (calves up to 5 wk: Dairy Calf Tape; calves between 90 to 120 d: Weight-By-Breed Dairy Cow Tape; The Coburn Co. Inc., Whitewater, WI).

Failure of Passive Transfer

At each of the 4 examinations, approximately 10 mL of whole blood was collected from the jugular vein into a sterile blood collection tube without anticoagulant (BD Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ) and chilled until further processing. Samples were centrifuged at $970 \times g$ for 10 min at approximately 20°C . Serum total protein (STP) concentration was estimated from samples collected at enrollment (1 to 7 d of age) using a digital refractometer (model 300027; Sper Scientific Ltd., Scottsdale, AZ). Remaining serum was frozen at -20°C . A subset of samples was used for serology, to be reported elsewhere. The sensitivity and

specificity for predicting BRD and mortality before 5 wk of age was calculated from STP cut-points ranging from 4.2 to 6.2 g/dL. The STP value with the highest sum of sensitivity and specificity was used to determine a dichotomous variable of failure of passive transfer (**FPT**). The cut-points of STP used for BRD and mortality were 5.7 and 5.2 g/dL, respectively. Failure of passive transfer was considered as STP <5.2 g/dL for calculating herd-level incidence.

Statistical Analyses

Seasons were defined as winter (December to February), spring (March to May), summer (June to August), and fall (September to November). Herds were categorized as small (<150 milking cows), medium (150 to 350), large (350 to 900), and very large (>900). Herds were categorized as having above median and top quartile incidence of FPT and BRD. The 3 herds that had no occurrences of BRD were excluded from the analyses because the efficacy of vaccination could not be assessed in the absence of disease. Each of these herds contributed fewer than 25 calves to the study. The incidence of FPT (STP cut-point of 5.2 g/dL), BRD between 5 wk and 3 mo of age, and mortality between 5 wk and 3 mo of age were compared between vaccination groups using a chi-squared test.

Multivariable logistic regression models were used to determine the association of vaccination status with BRD and mortality between 5 wk and 3 mo of age as dichotomous outcomes (GLIMMIX procedure in SAS 9.2; SAS Institute Inc., Cary, NC). Variables associated with the outcomes at $P < 0.10$ in univariable analysis were offered to a multivariable logistic regression model of each outcome. Potential covariates included herd size, region, season, STP using cut-points of 5.2 or 5.7 g/dL, and herd-level status for above median or top quartile incidence of FPT and BRD. Farm was included as a random effect to account for clustering of calves within a farm. Vaccination status was forced into the models because this was the primary variable of interest. Covariates with $P > 0.05$ were removed by backward elimination. Remaining covariates were checked for confounding effects by removing each one individually and observing for changes in the coefficients of the other variables. Interaction terms between vaccination and significant covariates were then offered to the model one at a time. When potential covariates were correlated, models were generated using each covariate separately and compared using the Akaike information criterion (**AIC**). The model with the lowest AIC value was selected as the final model. Only calves that survived to the end of the study were included in the BRD analysis.

Survival analyses were used to determine the time to BRD and mortality in the 4 vaccination groups. To include covariates and to adjust for clustering by farm, Cox proportional hazard ratios were estimated (PHREG procedure in SAS 9.2; Lee et al., 1992). The same potential covariates used in the logistic regression models were tested for univariable associations ($P < 0.10$) in the survival analysis. Those that were significant were offered to the multivariable model and removed by backward elimination ($P > 0.05$). Vaccination group was forced into the models. Interaction terms were tested in the survival analysis of BRD, but the data were too sparse in the mortality analysis.

Average daily gain from 5 wk to 3 mo of age in the 4 vaccination groups was assessed using linear regression models (MIXED procedure in SAS 9.1). Model building was similar to that of the logistic regression models for incidence of BRD and mortality. For each calf, ADG was calculated as the difference in weight between 5 wk and 3 mo of age divided by the number of days between these 2 examinations. Covariates were required to be significant at a P -value of 0.02 to remain in the model to reduce the potential of a type 1 error. When potential covariates were correlated, models were generated using each of variables separately and compared using the AIC. Residuals were assessed to evaluate how well the model fit the data and if it met the ANOVA assumptions (Beeler-Marfisi et al., 2010).

RESULTS

Enrollment

The majority of calves came from herds categorized as large (26% of calves) or very large (53%). Six hundred seventy-eight calves (24.7%) were vaccinated at 2 wk only, 664 (24.2%) at 5 wk only, 644 (23.4%) at 2 and 5 wk, and 764 (27.8%) were not vaccinated. The unvaccinated control group contained more calves because on 2 occasions the shipment of vaccines was delayed, and therefore, all calves were given the saline placebo.

Failure of Passive Transfer

The median STP value was 6.0 g/dL (range: 3.6 to 10.0 g/dL; SD = 0.7). The overall incidences of FPT using cut-points of 5.2 and 5.7 g/dL were 11 and 32%, respectively. The median herd-level FPT (cut-point of 5.2 g/dL) was 11.2% (range: 0 to 53.1%; SD = 12.9). The incidence of BRD before 5 wk of age in calves with STP >5.7 g/dL was 13% (254/1,947) and in calves with STP <5.7 g/dL was 18% (168/927). Mortality before 5 wk of age was 1.8% (47/2,552) in calves with STP >5.2 g/dL and 5.3% (17/322) in calves with STP <5.2 g/dL.

Table 1. Logistic regression model of the risk of bovine respiratory disease (BRD) between 5 wk and 3 mo of age in 2,750 dairy calves from 19 herds in Ontario and Minnesota randomly assigned to receive a multivalent modified-live viral vaccine at 2 wk only, 5 wk only, 2 and 5 wk of age, or to be an unvaccinated control

Item	Estimate	SE	P-value	Odds ratio (95% CI)	LSM (%; SEM)
Intercept	-0.62	0.41	0.15		
Vaccination group					
Control	Reference	—	—	—	9.0 (0.02)
Vaccine at 2 wk	0.15	0.18	0.43	1.2 (0.8–1.7)	11.3 (0.02)
Vaccine at 5 wk	0.08	0.19	0.66	1.1 (0.8–1.6)	10.6 (0.02)
Vaccine at 2 and 5 wk	0.08	0.19	0.66	1.1 (0.8–1.6)	10.7 (0.02)
Age at enrollment (d)	-0.07	0.03	0.02	0.9 (0.9–1.0)	
Season of birth					
Winter	Reference	—	—	—	14.8 (0.03)
Spring	-0.10	0.25	0.64	0.9 (0.6–1.4)	13.6 (0.03)
Summer	-0.91	0.23	<0.001	0.4 (0.3–0.6)	6.6 (0.01)
Fall	-0.53	0.25	0.03	0.6 (0.4–1.0)	9.3 (0.02)
Top quartile herd incidence of BRD					
No	Reference	—	—	—	4.7 (0.01)
Yes	1.74	0.40	<0.001	5.7 (2.4–13.3)	22.1 (0.06)

dL. The incidence of FPT (cut-point of 5.2 g/dL) was 11.6% in calves vaccinated at 2 wk of age (78/672), 10.1% in calves vaccinated at 5 wk (67/663), 10.1% in calves vaccinated at 2 and 5 wk (65/643), and 9.2% in unvaccinated calves (70/760). It did not differ between vaccination groups ($P = 0.52$).

Bovine Respiratory Disease

Overall, 21.9% (630/2,874) of calves were treated at least once for BRD. Median herd-level incidence risk of BRD was 21.6% (range: 0 to 44.4%; SE = 0.25). Over 19% (122/620) of calves that were treated for BRD had more than one occurrence of BRD. Median age at first diagnosis of BRD was 30 d (range: 1 to 111 d). The incidence risk of BRD was 7.7% (221/2,874) before 2 wk of age, 8.0% (229/2,874) between 2 and 5 wk, and 9.5% (273/2,874) between 5 wk and 3 mo. The incidence of BRD after 5 wk of age was 10.6% in calves vaccinated at 2 wk of age (72/678), 9.9% in calves vaccinated at 5 wk (66/664), 9.9% in calves vaccinated at 2 and 5 wk (64/644), and 9.0% in unvaccinated calves (69/764). It did not differ between vaccination groups ($P = 0.79$).

The least squares means risk of BRD between 2 and 5 wk of age was not different ($P = 0.40$) between calves vaccinated at 2 wk of age (7.5%; SEM = 0.02) and unvaccinated controls (8.3%; SEM = 0.02), when controlling for significant covariates. In the final multivariable model, the risk of BRD between 2 and 5 wk of age was significantly greater in calves from a herd that was in the top quartile of incidence of BRD [odds ratio (OR) = 3.8; $P = 0.05$], and in calves that experienced BRD before 2 wk of age (OR = 1.7; $P = 0.02$). Interactions between vaccination at 2 wk and significant covariates were assessed, but none were significant ($P > 0.30$).

Vaccination did not affect the incidence of BRD between the ages of 5 wk and 3 mo ($P = 0.89$), when controlling for significant covariates (Table 1). Having a disease other than diarrhea or dullness was also a significant covariate (OR = 43; $P < 0.001$). However, because it had undue influence on the least squares means resulting from a small number of individuals in this category, this variable was removed from the model. Interactions between vaccination group and covariates were not significant ($P > 0.10$). In survival analysis, the age at which BRD occurred for the first time (median = 30 d; $P = 0.48$) did not differ by vaccination group.

Mortality

Overall mortality throughout the entire study period was 3.5% (98/2,830). Median herd-level mortality was 3.2% (range: 0 to 10.0%). The average and median ages at death were 35 and 19 d, respectively. Of calves that survived to enrollment at 1 to 7 d of age, 1.8% (51/2,830) died before 2 wk of age, 0.5% (13/2,830) died between 2 and 5 wk of age, and a further 1.2% (34/2,830) died between 5 wk and 3 mo. The incidence of mortality after 5 wk of age was 1.0% in calves vaccinated at 2 wk of age (7/678), 1.5% in calves vaccinated at 5 wk (10/664), 1.2% in calves vaccinated at 2 and 5 wk (8/644), and 0.9% in unvaccinated calves (7/764). It did not differ between vaccination groups ($P = 0.75$).

The least squares means mortality risk between 2 and 5 wk of age was not different in calves vaccinated at 2 wk (0.7%; SEM = 0.003) and controls (1.0%; SEM = 0.004; $P = 0.45$). The risk of mortality was significantly increased in calves treated for BRD before 2 wk of age (OR = 7.9; $P = 0.0003$). No interaction existed

Table 2. Logistic regression model of the risk of mortality between 5 wk and 3 mo of age in 2,750 dairy calves from 19 herds in Ontario and Minnesota randomly assigned to receive a multivalent modified-live viral vaccine at 2 wk only, 5 wk only, 2 and 5 wk of age, or to be an unvaccinated control

Item	Estimate	SE	P-value	Odds ratio (95% CI)	LSM (%; SEM)
Intercept	-2.55	0.68	0.002	—	—
Vaccination group					
Control	Reference	—	—	—	1.6 (0.008)
Vaccine at 2 wk	-0.04	0.55	0.95	1.0 (0.3–2.8)	1.6 (0.008)
Vaccine at 5 wk	0.39	0.51	0.45	1.5 (0.5–4.0)	2.4 (0.01)
Vaccine at 2 and 5 wk	0.18	0.53	0.74	1.2 (0.4–3.4)	1.9 (0.009)
BRD ¹ before 2 wk of age					
No	Reference	—	—	—	0.9 (0.003)
Yes	1.42	0.44	0.001	4.1 (1.7–9.9)	3.7 (0.02)
BRD between 5 wk and 3 mo					
No	Reference	—	—	—	0.8 (0.003)
Yes	1.68	0.38	<0.001	5.4 (2.5–11.4)	4.2 (0.02)

¹Bovine respiratory disease.

between vaccination and BRD before 2 wk of age ($P = 0.99$).

Mortality after 5 wk of age was not affected by vaccination at 2 or 5 wk or both ($P = 0.49$), when controlling for significant covariates (Table 2). Having a disease other than diarrhea or dullness was a significant covariate ($P < 0.001$), but because it had undue influence on the least squares means resulting from a small number of individuals in this category, it was removed from the model. Interactions between vaccination group and covariates were not significant ($P > 0.50$). In survival analysis, the time to death (median = 19 d; $P = 0.55$) did not differ between the vaccination groups.

Growth

On average, calves weighed 126 kg (SD = 20.1) at 3 mo of age. The unadjusted mean ADG from 5 wk to 3 mo of age was 1.07 kg/d (SD = 0.29). Vaccination group was associated with the mean ADG from 5 wk to 3 mo of age in univariable analyses ($P = 0.03$). However, when accounting for the significant effects of the herd-level incidence of FPT (cut-point of 5.2 g/dL), month of birth, BRD between 2 and 5 wk or after 5 wk, weight at 2 wk of age, and a random effect of farm, vaccination group was not associated with ADG ($P = 0.40$; Table 3).

DISCUSSION

To our knowledge, this is the largest randomized clinical trial of the efficacy of multivalent, MLV vaccination to prevent BRD in young dairy calves reported to date. Many dairy operations in North America routinely vaccinate neonatal and preweaned calves repeatedly against a wide variety of pathogens (NAHMS, 2007).

This practice has been adopted despite the belief that vaccinating calves in the face of maternal antibodies is ineffective (Woolums, 2007). Under the conditions of this study, the hypothesis that preweaning vaccination would reduce the incidence of BRD was not supported. Vaccination of heifer calves at 2 or 5 wk of age or both with a multivalent, MLV vaccine did not influence the incidence of BRD before 3 mo of age. The effectiveness of vaccination did not vary by farm, herd size, region, or season of birth, or by herd-level incidence of FPT or BRD. Reasons for this finding may include interference by maternal antibodies, unresponsiveness of the neonatal immune system, timing of immune protection (lagging behind disease challenge), disease caused by pathogens other than the viruses in the vaccine, or herd immunity resulting from the vaccination of three-quarters of the calf herd. These reasons will each be discussed briefly.

It has been repeatedly shown that calves with sufficient passively transferred antibodies respond differently to vaccination than those deprived of passive immunity. Seronegative, unvaccinated calves and seropositive calves vaccinated at 10 to 14 d of age succumbed to BVD challenge at 4.5 mo of age, whereas seronegative calves vaccinated at 10 to 14 d and seropositive calves vaccinated at 4 mo of age did not (Ellis et al., 2001). Seroconversion occurred less frequently in calves vaccinated with a multivalent, MLV vaccine between 50 and 183 d of age that were seropositive for BVD-1 and BRSV (Kirkpatrick et al., 2008). In the presence of pre-existing viral antibodies, only 2 to 14% of young beef calves seroconverted to BVD-1 and 2, PI-3, and BRSV (Fulton et al., 2004). However, in that study, vaccination did result in slower rates of antibody decline for BVDV-1 and 2, BHV-1, PI-3, *Mannheimia haemolytica*, and *Pasteurella multocida*, suggesting these calves were potentially capable of producing new antibodies.

Table 3. Linear regression model for ADG (kg/d) after 5 wk of age of 2,750 dairy calves from 19 herds in Ontario and Minnesota randomly assigned to receive a multivalent modified-live viral vaccine at 2 wk only, 5 wk only, 2 and 5 wk of age, or to be an unvaccinated control

Item	Estimate	SE	P-value	LSM	SEM
Intercept	0.76	0.07	<0.001		
Vaccination group					
Control	Reference	—	—	1.02	0.03
Vaccine at 2 wk	−0.02	0.01	0.12	1.00	0.03
Vaccine at 5 wk	−0.006	0.01	0.66	1.01	0.03
Vaccine at 2 and 5 wk	−0.001	0.01	0.93	1.02	0.03
Herd-level incidence of FPT ¹	−0.004	0.002	0.02		
Month of birth					
November	Reference	—	—	1.13	0.04
December	−0.16	0.04	<0.001	0.97	0.04
January	−0.11	0.05	0.02	1.02	0.05
February	−0.30	0.03	<0.001	0.83	0.04
March	−0.21	0.03	<0.001	0.92	0.03
April	−0.16	0.03	<0.001	0.97	0.03
May	−0.13	0.03	<0.001	1.00	0.03
June	−0.09	0.03	0.001	1.04	0.03
July	−0.11	0.03	<0.001	1.03	0.03
August	−0.02	0.03	0.51	1.11	0.04
September	−0.08	0.03	0.02	1.06	0.04
October	−0.09	0.03	0.007	1.04	0.04
BRD ² between 2 and 5 wk of age					
No	Reference	—	—	1.03	0.03
Yes	−0.05	0.02	0.009	0.99	0.04
BRD between 5 wk and 3 mo					
No	Reference	—	—	1.06	0.03
Yes	−0.09	0.02	<0.001	0.96	0.03
Weight at 2 wk of age (kg)	0.007	0.008	<0.001		

¹Failure of passive transfer with serum total protein below 5.2 g/dL.²Bovine respiratory disease.

In the present study, the incidence of BRD did not differ by vaccination status among calves with FPT or among calves from herds with high incidence of FPT. The incidence of FPT was lower than previously reported in commercial dairy calves (Trotz-Williams et al., 2008). Serum total protein provides information about the amount of immunoglobulins absorbed by the calf but not about the antigenic specificity of maternal antibodies. Calves with STP concentrations above cut-points associated with the risk of disease or death may, or may not, have absorbed sufficient quantities of the specific antibodies to interfere with response to vaccination against a particular virus. Furthermore, the titers of maternal antibody that may interfere with vaccination are uncertain and may vary by pathogen (Fulton et al. 2004; Kirkpatrick et al. 2008). These factors make it difficult to predict the best time to vaccinate calves at the herd level (Chase et al., 2008). Vaccination at 2 or 5 wk or both in this population with low levels of FPT did not reduce the incidence of BRD.

The neonatal immune system presents several challenges, particularly for the induction of specific immune responses through vaccination and are well reviewed elsewhere (Firth et al., 2005; Chase et al., 2008; Cortese, 2009). Although all the components of a mature

immune system are present at birth, many components are present in relatively low concentrations or with reduced function, which may affect the ability to mount an immune response to either vaccination or pathogen exposure. Cellular components, such as phagocytes and lymphocytes, are functionally deficient or naïve in the neonate and, therefore, it takes longer to mount an immune response (Firth et al., 2005), and this response is generally weaker than expected in adult animals (Cortese, 2009). The unique characteristics of the neonatal immune system result in an increased susceptibility to pathogen challenge, and different responses to vaccination compared with older individuals. In the present study, vaccination group was not associated with BRD morbidity, perhaps because the calves' response to vaccination at 2 or 5 wk of age (or both) was insufficient to protect against disease.

The incidence risk of BRD of 22% and median age at onset of 30 d were similar to those of other studies (Waltner-Toews et al., 1986; Virtala et al., 1996). Although some reports indicate that BRD in dairy calves usually occurs around 1 mo of age, BRD is often considered a postweaning disease and is the cause of the greatest proportion of deaths (46%) in postweaned heifers in the United States (NAHMS, 2007). A large

proportion of BRD cases (44%) developed before the completion of the vaccination schedule at 5 wk of age and, therefore, could not have been prevented by vaccination. A single vaccination at 2 wk of age, before the majority of cases of BRD, did not prevent the occurrence of BRD between 2 and 5 wk. Studies of children less than 1 yr of age have demonstrated that repeated vaccination during early life is necessary to induce active immunity in the face of maternal antibodies (Siegrist, 2007). Although calves were only vaccinated once before peak onset of disease in the present study, multiple vaccinations before 30 d of age might conceivably have reduced the incidence of BRD. On the other hand, induction of protective active immunity in neonatal calves is inherently challenging (Cortese, 2009), and nutrition, housing, and hygiene remain important determinants of health. As new vaccine technologies become available, further research should be conducted in a field setting to evaluate if multiple vaccinations before 1 mo of age would be more effective than the 2 and 5 wk of age used in the present study.

The multivalent, MLV vaccine used in this study contained antigens of BVD-1 and 2, BRSV, BHV-1, and PI-3. These pathogens have all been associated with BRD (Radostits et al., 2007). Some descriptive studies have reported the association of viruses with BRD in dairy herds; however, study design and statistical analysis make it difficult to extrapolate these results to clinical disease in calves (Verhoeff and van Nieuwstadt, 1984; Hägglund et al., 2006). The prevalence of exposure to wild-type BVD, BRSV, BHV-1, and PI-3 within North American dairy herds remains uncertain, as is their association with BRD in young dairy calves. Although studies of vaccination against BRD using viral vaccines have shown mixed results, vaccination of calves on arrival at the feedlot with a multivalent vaccine is generally considered effective in reducing morbidity and mortality losses (Martin, 1983; Perino and Hunsaker, 1997). The vaccination programs evaluated in this study may not have reduced the incidence risk of BRD if the antigens in the vaccine were not the primary causative agent(s) in these herds.

The herd-level incidence of BRD ranged from 0 to 44%, which is similar to the range in other reports (Waltner-Toews et al., 1986; Lundborg et al., 2005). Herd immunity refers to the phenomenon of the decreased overall risk of infectious disease in a population (vaccinated and unvaccinated individuals) associated with decreasing the proportion of susceptible individuals (Halloran et al., 1991). It is mediated by direct effects on the vaccinated individual and indirect effects of decreasing the probability of transmission by reducing the prevalence of the pathogen. In a study investigating

the effects of vaccination on the transmission of bovine viral diarrhea virus in a drylot dairy in California, the incidence of BRD was similar in vaccinated and unvaccinated calves but transmission of the virus was reduced by 48% overall (Thurmond et al., 2001). If vaccination of three-quarters (72%) of the calves in the present study was effective in reducing viral shedding, as has been shown previously (Peters et al., 2004), it may have reduced pathogen load within the herd and, thus, reduced infection pressure in both the vaccinated and unvaccinated calves. To test this hypothesis, it would be necessary to design a group-level study in which otherwise similar groups were housed without contact with each other and some groups were designated to have a proportion of calves vaccinated and other groups would have no vaccinated calves (Halloran et al., 1997). The incidence of BRD could then be assessed to determine if BRD was lower in unvaccinated calves in groups that vaccinated a portion of the calves compared with the control groups.

The overall mortality of 3.5% in this study was slightly lower than in reports of dairy calves of similar age in other parts of North America and in Europe (Donovan et al., 1998; Gulliksen et al., 2009a), but similar to those in a previous study in Canada (Waltner-Toews et al., 1986). In 3 studies, multivalent viral vaccination reduced (Kirkpatrick et al., 2008), increased (Martin et al., 1982), or had no effect (Schunicht et al., 2003) on BRD mortality in feedlot cattle. Vaccination studies of feedlots and calf ranches differ from smaller dairy populations. Infection pressure is likely to be much greater in the feedlots and calf ranches, because calves are being commingled after transportation from numerous sources. Furthermore, feedlot calves differ in that calves are typically 6 to 8 mo old at vaccination so maternal antibodies are unlikely to interfere with vaccine response. In the present study, vaccination did not affect the survival of dairy calves under 3 mo of age. Because the median age at death was 19 d, and vaccination protocols were not complete until 35 to 42 d of age, it is not surprising that vaccination did not influence this outcome.

No published field studies exist comparing growth rates of commercial dairy heifer calves given a viral vaccine. Dairy calves vaccinated at 0 and 14 d of age with a *M. haemolytica* and *P. multocida* vaccine had the same average daily gain as unvaccinated controls (Aubry et al., 2001). However, a study of commercial beef calves in a feedlot compared a multivalent viral vaccine against BHV-1, BVD, BRSV, and PI-3 with a univalent vaccine against BHV-1, and found significantly greater ADG in cattle vaccinated with the multivalent vaccine (Schunicht et al., 2003). In a study that inves-

tigated vaccination against BHV-1, BVD, and BRSV in veal calves, a reduction in ADG was associated with BRD, but vaccination was not associated with growth rate (Frankena et al., 1994). The effects of multivalent, MLV vaccination on growth in young calves remain to be fully understood.

CONCLUSIONS

In this randomized clinical trial in commercial herds with diverse management practices but overall low incidence of FPT, vaccination of home-raised calves with a multivalent, MLV vaccine at 2 or 5 wk of age or both was not associated with a decreased risk of BRD or mortality, or with growth until 3 mo of age. Further research is necessary to explore appropriate strategies for preweaning vaccination for the reduction of BRD in dairy herds.

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