A Quantitative Approach to Classifying Holstein Cows Based on Antibody Responsiveness and Its Relationship to Peripartum Mastitis Occurrence

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ABSTRACT

A quantitative approach was developed to classify Holstein cows and heifers based on phenotypic variation of serum antibody response and to determine associations with peripartum mastitis. Using an index, 136 cows and heifers were classified into high (Group 1), average (Group 2), or low (Group 3) antibody groups following immunization with ovalbumin at wk –8, –3, and 0 relative to parturition. The ranking of groups based on the quantitative index of serum antibody response to ovalbumin were similar for sera and whey antibody such that Group 1 > Group 2 > Group 3. Animals were also vaccinated with *Escherichia coli* J5 (Rhône Mérieux, Lenexa, KS) at wk –8 and –3 relative to parturition. The ranking of groups for *E. coli* J5 was similar to that observed for serum and whey antibody to ovalbumin. Serum and whey IgG_1 and IgG_2 concentrations were measured at wk 0, 3, and 6 but differences between groups were not significant. There was no occurrence of mastitis for Group 1 animals in two of the herds. In contrast, Group 1 animals from the third herd had the highest occurrence of mastitis; however, these cases all occurred in first-parity heifers. According to pooled data across all herds, Group 3 animals had the highest occurrence of mastitis. Heritability estimates of serum antibody response to ovalbumin varied between 0.32 to 0.64 depending on week relative to parturition. Heritability estimates of serum antibody response to *E. coli* J5 also varied between 0.13 to 0.88 depending upon week relative to parturition. These results indicate that high peripartum antibody may be beneficial in some herds.

(**Key words:** antibody, variation, mastitis, peripartum period)

Abbreviation key: OD = optical density, **OR** = odds ratio, **OVA** = ovalbumin.

INTRODUCTION

Gestation, parturition, and lactation affect nonspecific and specific host defense mechanisms of periparturient-dairy cows (4, 20, 21). Prevalence of disease in the periparturient period is high compared with other stages of lactation, coincident with impaired immune and innate host resistance mechanisms either before or immediately after parturition. In the pre- or postpartum periods, neutrophil function (2, 4, 5, 11), complement activity (4), conglutinin concentration (4), and milk SCC (23) suggest impaired innate resistance in dairy cows. Indicators of acquired immunity such as antibody dependent cell cytotoxicity (10), number of antibody plaque forming cells (20), and lymphocyte proliferation to antigen and mitogen (9, 10, 21) have also been reported to be low in peripartum cows.

Altered innate and immune responsiveness during the periparturient period has been associated with susceptibility to disease, particularly mastitis (2, 7). Cai et al. (2) indicated that periparturient cows with mastitis had lower neutrophil chemotaxis and periparturient cows with metritis had lower neutrophil superoxide anion production than did healthy controls. As selection for high and low immune response has been reported to affect indicators of health and production in other species (12, 15, 16), it may also be possible to select for high and low immune response in dairy cattle. A previous study of 32 cows in one herd indicated that there may be sufficient phenotypic variation in peripartum serum antibody production to rank cows based on this trait and that there may be associated health benefits (14). Classification of animals was based on a biological, yet subjective, assessment of individual antibody patterns following immunization. Such a classification method is not practical when classifying cows across a number of herds or within a large population.

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Not all dairy animals will be easily classified into three subjective groups because antibody response is a variable that can range from high to low on a continuum; therefore, a more objective method for classification of animals is needed.

The objectives of this study were to test for the existence of high and low peripartum serum and whey antibody responses among animals from three herds, to estimate heritability of peripartum antibody response, and to devise a method for quantitatively and objectively classifying cows into groups based on antibody response following a standardized immunization protocol. Relationships were evaluated between antibody group classification and serum and whey antiody concentration, serum and milk immunoglobulin concentration, milk SCS, and mastitis occurrence.

MATERIALS AND METHODS

Cows and Treatments

Antibody response was evaluated in 136 Holstein dairy cows and heifers from two research herds (Herd 1, n = 32, six heifers and 26 cows; Herd 2, n = 67, 34 heifers and 33 cows) and one commercial herd (Herd 3, n = 37, eight heifers and 29 cows) from 8 wk prepartum (wk –8) based on predicted parturition dates, to 6 wk postpartum (wk 6). Forty-eight animals were primiparous heifers, 47 animals were in their second lactation, and 41 were multiparous cows (>two lactations). Briefly, animals received an i.m. injection of ovalbumin (OVA; Type VII, Sigma Chemical Co., St. Louis, MO) dissolved in an *Escherichia coli* J5 mastitis endotoxemia preventive vaccine (Rhône Mérieux, Lenexa, KS) with the manufacturer's adjuvant, at wk –8 (4 mg) and –3 (2 mg). At parturition (wk 0), animals received an additional immunization of OVA in PBS (–0.1 *M*, pH 7.4) (2 mg, i.m.). Ovalbumin was chosen as an inert test antigen to which these animals had not likely been previously exposed, and *E. coli* J5 was used as a complex antigen having possible biological relevance to mastitis. It was not an objective of this study to evaluate the effect of the *E. coli* J5 vaccine on mastitis occurrence.

Blood and Milk Sampling

Blood was collected by caudal venipuncture at wk –8, –3, 0, 3, and 6 relative to parturition. Serum was used to monitor IgG_1 and IgG_2 concentration and antibody to OVA and *E. coli* J5. Colostrum and milk samples were collected at wk 0, 2, 3, 4, and 6 relative to parturition to determine whey IgG_1 and IgG_2 concentration and antibody to OVA. Whey was collected to observe whether immunoglobulin transport would account for antibody variation, and also to observe antibody response in a biologically relevant compartment with respect to mastitis. Colostrum was collected at the first postpartum milking. Milk was stripped from all quarters approximately 2 to 4 h after morning milking. Colostrum and milk were stored frozen without preservative at –20°C until whey was separated.

Detection of Antibody to OVA in Serum and Whey

Antibody to OVA was measured by ELISA, using a previously described method (1, 14). Briefly, sera (wk –8, –3, 0, 3, and 6) were diluted 1/50 and 1/200 in wash buffer (PBS + 0.05% Tween 20; Fisher Scientific, Don Mills, ON) and were assayed in duplicate. Whey (wk 0, 2, 3, 4, and 6) were diluted 1/10, 1/100, and 1/400 and left undiluted for assay in quadruplicate. Negative and positive controls for both assays were diluted 1/50 and 1/200 and were assayed in duplicate. Negative and positive controls included a pooled sample of preimmunization sera and a pooled sample of sera from animals 14-d postsecondary immunization, respectively. Antibody concentration was measured with an automatic ELISA plate spectrophotometer reader (BIO-TEK Instruments, Highland Park, VT) set to an absorbance of 405 and 630 nm, and was expressed in optical density units (**OD**). The mean of the positive control was corrected to an OD = 1.0. All test values were corrected by multiplying by the inverse of the mean of the positive control. Corrected means for each test serum dilution were then added together to give an additive OD value.

Detection of Antibody to *E. coli* J5 in Serum

Antibody response to *E. coli* J5 was measured by ELISA using a heat-killed whole-cell preparation of *E. coli* J5 antigen as previously described (14). Sera (wk –8, –3, 0, 3, and 6) were diluted 1/1000 and were assayed in quadruplicate. The same pooled positive used in the OVA ELISA was used in this assay. It was tested to ensure a differentiation between preimmune negative sera and postsecondary immunization sera for *E. coli* J5 antibody. Negative control sera in this assay included fetal bovine serum (Bockneck Laboratories, Can Sera, Rexdale, ON) and *E. coli* J5-absorbed pooled nonvaccinated sera. Negative and positive controls for both assays were diluted 1/50 and 1/200 and were assayed in duplicate. Antibody concentration was measured with an automatic ELISA plate spectrophotometer reader (BIO-TEK Instruments, Highland Park, VT) set to an absorbance of 540 nm, and was expressed in OD units. The mean of the positive control was corrected to an OD = 1. All test values were corrected by multiplying by the inverse of the mean of the positive control.

Immunoglobulin G Concentration

Radial immunodiffusion was used according to the method described by Mallard et al. (13) to determine the concentration of serum IgG_1 and IgG_2 at wk 0, 3, and 6; whey IgG_1 at wk 0, 3, and 6; and whey IgG_2 at wk 0 and 3.

Quantitative Classification of Animals Based on Antibody to OVA

Individual profiles of antibody response to immunization with OVA were analyzed using intervals from wk –8 to 6 relative to parturition (wk 0). The first interval (I1) defined the change in the concentration of antibody to OVA from wk –8 to –3 relative to parturition following primary immunization at wk –8 (where I1 = OD value at wk –3 minus OD value at wk –8). The second interval (I2) defined the change in the concentration of antibody to OVA from wk –3 to parturition following secondary immunization at wk –3 (where I2 = OD value at wk 0 minus OD value at wk –3). The third interval (I3) defined the change in the concentration of antibody to OVA from parturition to wk 3 following tertiary immunization at parturition (where I3 = OD at wk 3 minus OD at wk 0). The fourth interval (I4) defined the change in the concentration of antibody to OVA from wk 3 to 6 (where I4 = OD value at wk 6 minus OD value at wk 3). The fourth interval was included to observe the change in antibody between the end of the immediate postpartum period (wk 3) and peak lactation. These response intervals were added together to give a total index of antibody to OVA between wk –8 and +6 relative to parturition as follows:

$$y_{total} = I1 + b_1I2 + b_2I3 + I4$$

where

$$y = \text{total antibody; and,}$$

I1, I2, I3, and I4 are as previously defined; and b_1 and $b_2 = 1.5$ if negative and are otherwise equal to 1.0. The second and third intervals were weighted this way to emphasize negative results during the peripartum period when lowered host resistance mechanisms are thought to contribute to increased occurrence of disease. Several iterations were run to determine coefficients for I2 and I3 that reflected the initial biological classification of cows previously reported (14).

The index was then used to classify animals into antibody groups. The mean of y_{total} was determined and animals with index values greater than one standard deviation above the mean were classified into the high antibody group (Group 1; n = 18). Animals with index values less than one standard deviation below the mean were classified into the low antibody group (Group 3; n = 23). Animals with index values that ranged between one standard deviation below and above the mean were classified into the average antibody group (Group 2; n = 95).

Heritability Estimates

Concentrations of antibody to OVA or *E. coli* J5 at wk –3, 0, 3, and 6 relative to parturition were each considered to be a distinct genetic trait. Sire and error variance components of serum antibody to OVA were estimated by multiple trait REML using variance component estimation (VCE) software (6). Herd, parity, and sire (n = 63) were included in the estimation of heritability. Approximate standard errors were computed from the variance covariance matrix of sire and error variance component estimates.

Milk Somatic Cell Count

Milk (a.m./p.m. composite sample) was collected weekly to determine SCC, an indicator of subclinical mastitis. The SCC were transformed to SCS for analysis. The SCS is the natural logarithm of SCC in cells/ml and is calculated as follows:

$$SCS = [\log_e(SCC/100) \div \log_e(2)] + 3 \quad (22)$$

Mastitis Occurrence

Clinical mastitis events were recorded throughout the study period by experienced herd managers. Two or more events of mastitis were recorded as one event for the study period (17). Mastitis occurrence was calculated by dividing the number of animals within an antibody group that had at least one mastitis event by all the animals in that antibody group, and multiplying this number by 100. Mastitis occurrence was evaluated for associations with antibody group within each herd, using odds ratio (**OR**) (17). Odds ratios in this study were calculated on a within-herd basis, as the ratio between mastitis occurrence in one antibody group versus mastitis occurrence in the rest of the herd (i.e., the other two groups). Odds ratio is the approximate relative risk when the rate of disease in the population is relatively infrequent (≤5%) (17).

Statistical Methods

Type III least squares ANOVA and corrected means (least square means; LS means) were calculated using

the general linear models procedure of SAS (8) to evaluate the effects of herd, season-year, cow, antibody group, parity, week, and their interactions on antibody to OVA and *E. coli* J5, and immunoglobulin concentration (Table 1). Antibody group was included in the model to determine if the classification of animals based on OVA has implications with respect to other immune response traits. An animal term (cow) was included in the model to account for repeated observations on the same individual. Main effects were tested against the mean square for cow because the animal term was nested, and the data followed a split plot design. Sources of variation that were not significant were removed from the model for calculation of LS means. Data that were not normally distributed (*E. coli* J5 antibody, serum IgG_2, and whey IgG_2) as indicated by the univariate procedure of SAS (8), were transformed to natural logarithms. Least squares means were converted back to original units from $\log_e$ transformed data. Consequently, standard errors of means are not reported. The Proc CORR procedure of SAS was used to generate Pearson product moment correlation coefficients between immune response parameters. Results were considered to be statistically significant if the P value was ≤ 0.05 and trends were reported at the P value ≤ 0.10. Odds ratio values were tested for significance using the chi-square test (17).

RESULTS

Serum Antibody to OVA

Cow, antibody group, week, and the interaction between antibody group and week significantly contributed to the variation ($P \leq 0.0001$) in serum antibody to OVA (Table 1). Neither herd nor parity significantly contributed to the variation in serum antibody to OVA. The rank of antibody to OVA was Group 1 > Group

Table 1. Analysis of variance of antibody to ovalbumin (OVA) and *Escherichia coli* J5, the concentration of IG_1 and IgG_2 in serum and whey, and somatic cell score (SCS) in Holstein cows and heifers. This table represents the significant contribution of each independent variable on the dependent variable (P values).

				Source of variation								
Dependent	Variable	R^{2a} (%)	CV[b] (%)	Herd	Season-yr[c]	Cow[d]	Group[e]	Parity	Group * parity	Wk	Group * wk	Parity * wk
Serum anti-OVA		79.35	27.48	. . .[f]	. . .	0.0001	0.0001	. . .	NS[g]	0.0001	0.0001	. . .
Whey anti-OVA		73.25	32.21	0.007	. . .	0.0001	0.0007	NS	NS	0.0001	NS	. . .
	Herd 1	73.81	38.91	. . .	. . .	0.004	0.005	. . .	. . .	0.0001	NS	. . .
	Herd 2	69.02	33.96	. . .	. . .	0.003	0.02	. . .	. . .	0.0001	NS	. . .
	Herd 3	83.90	23.90	. . .	. . .	0.0001	0.0001	. . .	. . .	0.0001	NS	. . .
Serum anti-*E. coli*		74.77	–43.34[h]	0.02	. . .	0.0001	NS	0.0001	0.0001	0.0001	0.0001	0.0001
	Herd 1	78.63	–54.79	. . .	. . .	0.0001	NS	. . .	. . .	0.0001	0.06	. . .
	Herd 2	76.84	–45.20	. . .	. . .	0.0001	NS	0.0001	0.0001	0.0001	NS	. . .
	Herd 3	70.48	–31.56	. . .	. . .	0.0001	NS	. . .	. . .	0.0001	0.002	. . .
Immunoglobulin concentration												
Serum IgG_1		52.67	7.37	. . .	. . .	NS	0.08	NS	NS	0.0001	NS	0.008
Serum IgG_2		63.26	4.33	0.0001	. . .	0.0001	NS	0.04	NS	0.07	NS	NS
	Herd 1	59.97	4.67	. . .	. . .	0.02	NS	. . .	. . .	0.0015	NS	. . .
	Herd 2	48.49	4.34	. . .	. . .	0.02	0.08	NS	0.06	NS	NS	NS
	Herd 3	52.47	3.83	. . .	. . .	0.02	. . .	0.05	. . .	0.095	. . .	NS
Whey IgG_1		90.66	14.74	. . .	. . .	NS	NS	0.007	NS	0.0001	NS	0.07
Whey IgG_2		96.63	13.22	0.03	. . .	NS	NS	0.06	NS	0.0001	NS	NS
	Herd 1	94.85	14.96	. . .	. . .	NS	NS	. . .	. . .	0.0009	NS	. . .
	Herd 2	98.94	8.36	. . .	. . .	NS	NS	. . .	NS	0.003	NS	. . .
	Herd 3	97.44	12.63	. . .	. . .	NS	0.07	. . .	. . .	0.0001	NS	. . .
Somatic cell score												
SCS (Herd 1)		73.07	52.96	. . .	. . .	0.0001	NS	. . .	. . .	0.0009	NS	. . .
SCS (Herd 2)		65.16	58.83	. . .	. . .	0.0001	NS	. . .	. . .	0.0001	NS	. . .
SCS (Herd 3)		67.67	31.88	. . .	. . .	0.0001	NS	. . .	. . .	0.07	NS	. . .

[a]R^2 = coefficient of determination.

[b]CV = coefficient of variation.

[c]Season-Year = season and year of calving.

[d]Cow nested or 'grouped' within the interaction between antibody group and parity. i.e. Cow(group*parity). If parity is not significant, parity is removed and the model becomes cow nested within antibody group only.

[e]Group = variation due to antibody group of animals classified with high, average or low antibody to OVA.

[f]. . . not significant, therefore removed and no longer relevant to that dependent variable.

[g]NS = not significant.

[h]Coefficient of variation is negative due to analysis of variance of natural logarithm transformed data.

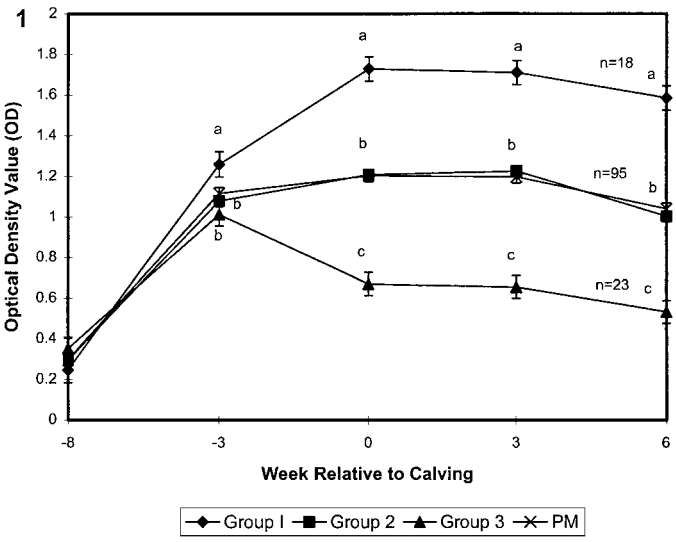

Figure 1. Least squares means of serum antibody to ovalbumin (OVA) by antibody group. Group 1 = high antibody (◆), Group 2 = average antibody (■), and Group 3 = low antibody (▲) based on described index, and Population mean (PM; ×). Significant differences between groups are indicated with lower case letters ($P \leq 0.05$).

2 > Group 3, except at wk –8 prior to immunization. Significant differences were noted between all groups at wk –3, 0, 3, and 6. Least square means significantly ($P \leq 0.0001$) increased from wk –8 (pre-immunization) to wk –3 (post primary immunization) in all antibody groups; however, significant increases in antibody after wk –3 occurred only in Group 1 animals (Figure 1).

Heritability Estimates of Antibody to OVA & *E. coli* J5

Heritability estimates of antibody to OVA at wk –3, 0, 3, and 6 relative to parturition were 0.62, 0.32, 0.50, and 0.58, respectively. Heritability estimates of antibody to *E. coli* J5 at wk –3, 0, 3, and 6 relative to parturition were 0.13, 0.88, 0.32, 0.5, and 0.88, respectively. Due to the small sample size, standard errors of heritability estimates could not be calculated for either antibody response.

Whey Antibody to OVA

Herd contributed significantly ($P \leq 0.007$) to variation in antibody to OVA and therefore, herds were further analyzed separately. Cow, antibody group, and week all contributed significantly to the variation in antibody to OVA in whey ($P \leq 0.0007$); however, the interaction between antibody group and week was not significant. For all herds, antibody to OVA in whey ranked similarly to the antibody observed for serum, such that Group 1 > Group 2 > Group 3. This was consistent for colostral and milk whey from parturition until wk 6 of lactation (Figure 2A, B, and C). Least square means of antibody to OVA in whey in all herds declined significantly from parturition to peak lactation. Correlation analysis between antibody to OVA in sera with antibody to OVA in whey, indicated a positive and significant relationship within Herd 1 (r = 0.45; $P \leq 0.0001$), Herd 2 (r = 0.28; $P \leq 0.001$), and Herd 3 (r = 0.44; $P \leq 0.001$), respectively.

Antibody to *E. coli* J5 in Sera

Herd contributed significantly ($P \leq 0.02$) to variation in antibody to *E. coli* J5 and therefore, herds were further analyzed separately (Table 1). In Herd 1, cow and week each contributed significantly ($P \leq 0.0001$) and the interaction between antibody group and week tended ($P \leq 0.06$) to contribute to the variation in antibody to *E. coli* J5. The rank of LS means of antibody to *E. coli* J5 by antibody group was Group 1 > Group 2 > Group 3 (Figure 3A). Least squares means of antibody to *E. coli* J5 varied during the peripartum period (wk –3 to +3) and up to peak lactation (wk +6) and were significantly higher ($P \leq 0.0001$) than preimmunization antibody at wk –8 for all animals, regardless of group (herd OD value = 0.275) (Figure 3A). Correlation analysis between antibody to *E. coli* J5 with antibody to OVA in sera for all groups, indicated a positive and significant relationship (r = 0.56; $P \leq 0.0001$). The correlation between serum *E. coli* J5 and OVA antibody within Groups 1, 2, and 3 were 0.66 ($P \leq 0.001$), 0.59 ($P \leq 0.0001$), and 0.38 ($P \leq 0.06$), respectively. In Herd 2, cow, parity, antibody group by parity, and week significantly contributed to the variation in antibody to *E. coli* J5 ($P \leq 0.0001$). The LS means of antibody for Group 3 animals at wk –8 was significantly higher (OD value = 0.386) than for animals of Group 1 (OD value = 0.257; $P \leq 0.005$) and Group 2 (OD value = 0.292; $P \leq 0.05$). Antibody to *E. coli* J5 was not significantly different between antibody groups from wk –3 to 6; however, animals in Group 3 were consistently, but not significantly, lower than the population mean (Figure 3B). Least squares means of antibody to *E. coli* J5 at wk –3, 0, 3, and 6 were significantly higher ($P \leq 0.0001$) than preimmunization antibody at wk –8 regardless of group (herd OD value = 0.307). Correlation analysis between serum antibody to *E. coli* J5 and serum antibody to OVA for all groups indicated a positive and significant relationship (r = 0.49; $P \leq 0.0001$). The correlation between antibody to *E. coli* J5 and antibody to OVA within Groups 1, 2, and 3 were 0.64 ($P \leq 0.0001$), 0.55 ($P \leq 0.0001$), and 0.31 ($P \leq 0.08$), respectively. In Herd 3, cow, week, and the interaction between antibody group and week significantly contributed to variation in anti-

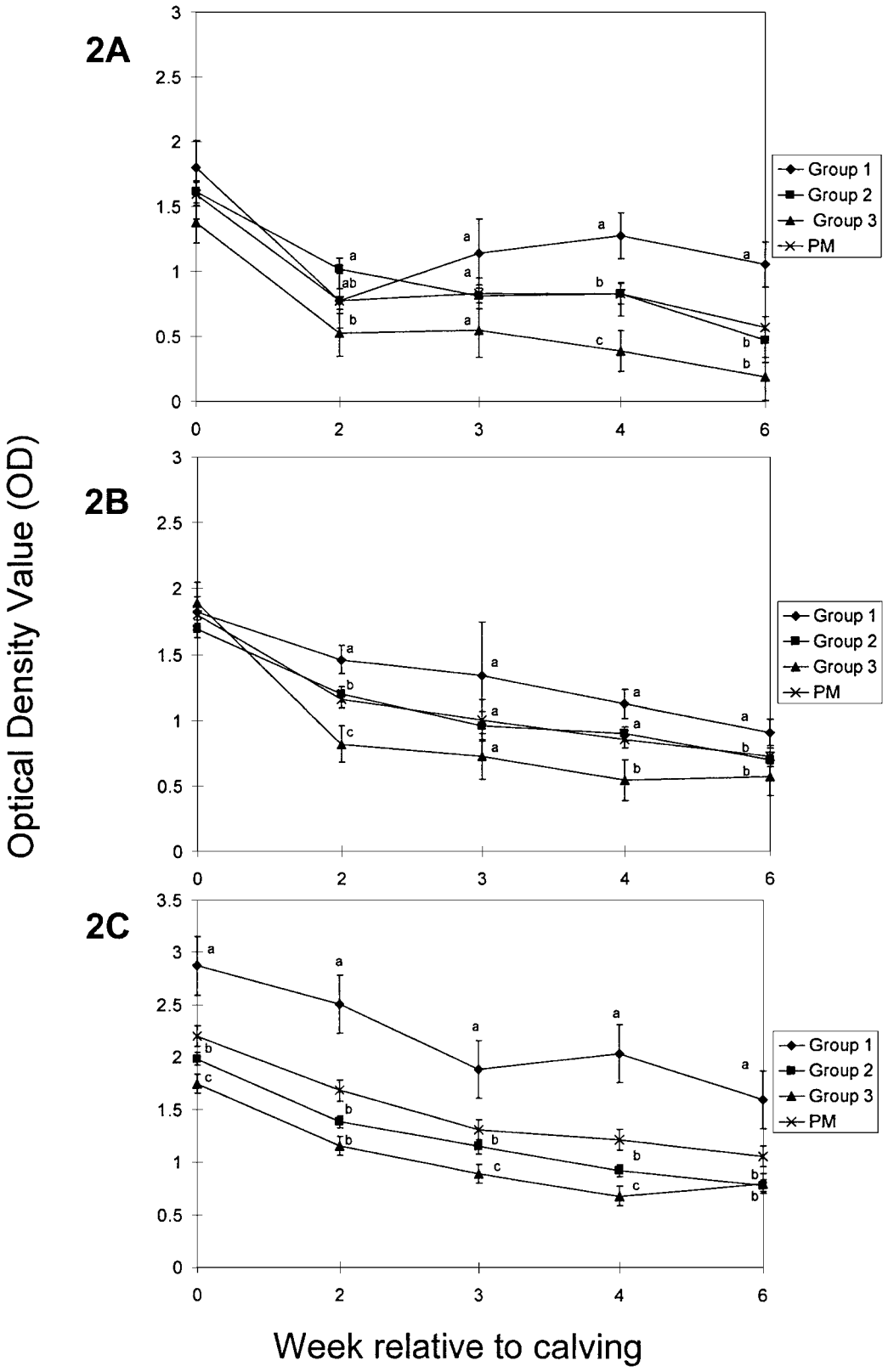

Figure 2. Least squares means of whey antibody to ovalbumin (OVA) by antibody group for A) Herd 1, B) Herd 2, and C) Herd 3. Group 1 = high antibody (◆), Group 2 = average antibody (■), and Group 3 = low antibody (▲) based on described index, and Population mean (PM; ×). Significant differences between groups are indicated with lower case letters ($P \leq 0.05$).

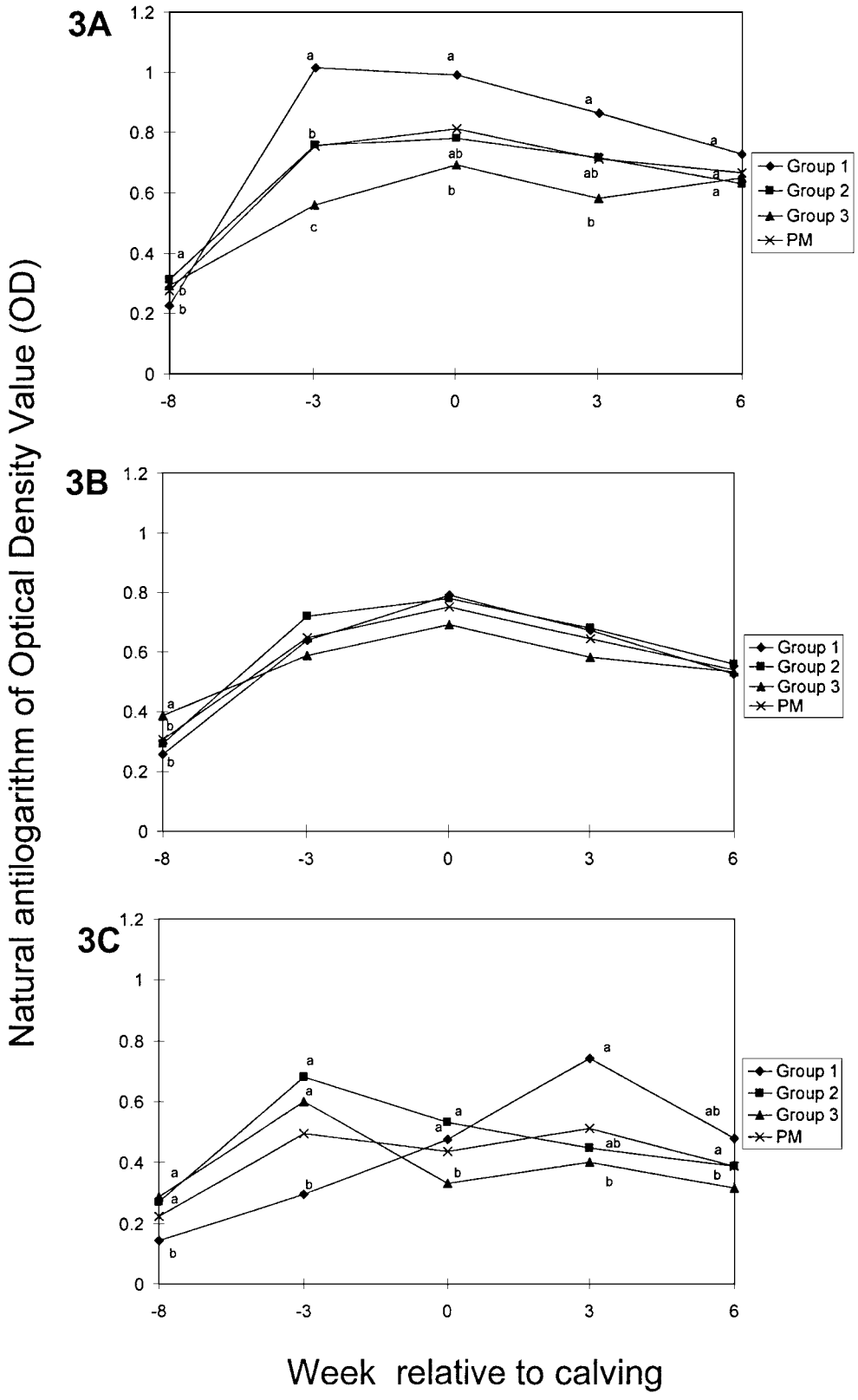

Figure 3. Least squares means of serum antibody to *Escherichia coli* J5 by antibody group for A) Herd 1, B) Herd 2, and C) Herd 3. Group 1 = high antibody (◆), Group 2 = average antibody (■), and Group 3 = low antibody (▲) based on described index, and Population mean (PM; ×). Significant differences between groups are indicated with different lower case letters ($P \leq 0.05$).

body to *E. coli* J5 ($P \leq 0.002$). Antibody for Group 1 animals was lower at wk −8 and −3 compared with Group 2 and 3 animals. At parturition, Group 1 and 2 animals had higher antibody to *E. coli* J5 than did Group 3 animals. At wk 3 and 6, however, the rank of antibody group for antibody to *E. coli* J5 was similar to the other herds, such that Group 1 > Group 2 > Group 3 (Figure 3C). Least squares means of antibody to *E. coli* J5 at wk −3, 0, 3, and 6 were significantly different across time and were significantly higher ($P \leq 0.0001$) than pre-immunization antibody regardless of group (herd OD value = 0.224) (Figure 3C). Correlation analysis between serum antibody to *E. coli* J5 and serum antibody to OVA for all groups indicated a positive and significant relationship ($r = 0.48$; $P \leq 0.0001$). The correlation between serum antibody to *E. coli* J5 and antibody to OVA within Groups 1, 2, and 3 were 0.93 ($P \leq 0.007$), 0.48 ($P \leq 0.0001$), and 0.36 ($P \leq 0.005$), respectively.

Immunoglobulin Concentration

The ANOVA of serum and whey IgG_1 and IgG_2 are presented in Table 1. Serum IgG_1 did not differ significantly between antibody groups at wk 0 and 6. However, at wk 3, Group 1 animals had lower serum IgG_1 compared with animals of Group 2 ($P \leq 0.07$) and Group 3 ($P \leq 0.03$). Whey IgG_1 concentration was not significantly different between antibody groups. Herd contributed significantly to the variation in serum and whey IgG_2 concentration ($P \leq 0.03$). Serum and whey IgG_2 concentrations, however, did not differ between antibody groups within herds.

Milk Somatic Cell Score

For Herds 1 and 2, cow within antibody group and week contributed significantly ($P \leq 0.0001$) to the variation in SCS (Table 1). In Herd 3, only the effect of cow within antibody group accounted for the variation in SCS. Least square means of SCS in Herd 1 at wk 3 and 4 were significantly lower ($P \leq 0.05$) for the high antibody group compared with the low antibody group. Though not significantly different, LS means of SCS for Herd 1 at wk 5 and 6 tended also to be lower for the high antibody group compared with the low antibody group. The LS means of SCS were not different between antibody groups within Herds 2 and 3.

Mastitis Occurrence

Mastitis occurrence varied between groups and between herds. Occurrence of clinical mastitis is presented in Table 2. Though groups were small, mastitis did not occur in Group 1 of either Herds 1 or 3. Mastitis occurrence in Herd 1 was 22.7 and 33.3% for Groups 2 and 3, respectively. Mastitis occurrence in Herd 3 was 11.5 and 10% for Groups 2 and 3, respectively. However, in Herd 2, Group 1 animals had the highest occurrence of mastitis (15.4%). Animals with mastitis in Herds 1 and 3 were in their second or greater parity. Animals with mastitis in Herd 2 were all heifers. Using pooled data across all herds, animals in Group 3 were found to have the highest mastitis occurrence (13.0%) (Table 2). Mastitis occurrence was not observed to differ between herds. Within herd, OR calculations comparing animals of one antibody group with the other two groups indicated that only heifers in Group 1 of Herd 2 had a significantly higher OR of having mastitis (by 7.57 times) compared with the animals in the rest of the herd. Though not significant, the risk of mastitis occurrence within Group 3 of Herds 1 and 3 was 2.16 and 1.8 times greater (respectively) than for Groups 1 and 2 within those herds.

DISCUSSION

A previous study of one herd indicated that animals could be classified according to the amplitude and direction of their individual OVA antibody profiles and that this ranking had some association with mastitis occurrence (14). The objectives of the current study were to test for the existence of high and low peripartum antibody response among animals across three herds, to estimate heritability of peripartum antibody response, and to devise a method for quantitatively and objectively classifying cows into groups based on antibody response following a standardized immunization protocol.

The results indicated substantial variation in antibody response to OVA from the peripartum period to peak lactation and that animals could be ranked using a quantitative index. Animals that ranked high, average, or low for serum antibody response to OVA, also ranked similarly for whey antibody to OVA. It has been reported that low serum immunoglobulin in cattle at parturition may be due to sequestration of immunoglobulin into the mammary gland; however, the results presented here would suggest that lower antibody response in serum is not a consequence of immunoglobulin transport. For instance, Group 1 animals, with the highest serum antibody response, also had a high concentration of whey antibody to OVA postpartum compared with animals of Groups 2 and 3. These data suggest that animals with high serum antibody response also supply higher concentrations of antibody to the mammary gland. More study is required to evaluate the consis-

Table 2. Percent occurrence (%) of clinical mastitis by antibody group within herd.

	Number of animals (n_1) in each antibody group			Number of animals in each herd	Animals having at least one mastitis event by antibody group within herd n_2 (% = n_2/n_1 * 100)			Occurrence of mastitis (%) by herd
	Group 1	Group 2	Group 3		Group 1	Group 2	Group 3	
Herd 1	4	22	6	**32**	0 (0)	5 (22.7)[1]	2 (33.3)	**7 (21.9)**
Herd 2	13	47	7	**67**	2 (15.4)	1 (2.1)	0 (0)	**3 (4.5)**
Herd 3	1	26	10	**37**	0 (0)	3 (11.5)	1 (10)	**4 (10.8)**
All herds	**18**	**95**	**23**	**136**	**2 (11.1)**[2]	**9 (9.5)**	**3 (13.0)**	**14 (10.3)**

[1]Within each herd, mastitis occurrence was calculated by dividing the number of animals within an antibody group that had at least one mastitis event by all the animals in that antibody group, and multiplying this number by 100.

[2]Based on all herds, mastitis occurrence was calculated by dividing the number of animals within an antibody group that had at least one mastitis event by all the animals in that antibody group, and multiplying this number by 100.

tency and breadth of antibody response before and after parturition and in subsequent lactations; however, this study has demonstrated significant individual variation during the peripartum period, and that not all animals experience peripartum depression of antibody.

The ranking for antibody to *E. coli* J5 was similar to the ranking for antibody to OVA, particularly at wk 0, 3, and 6 after parturition. Nonetheless, ranking based on antibody response to OVA was less ambiguous compared with *E. coli* J5, because OVA is not normally encountered in the dairy cow's environment. Thus, the confounding effect of preexisting antibody to *E. coli* J5 is not encountered when using OVA. Further, antibody to *E. coli* J5, but not to OVA was significantly affected by herd, thus making comparisons of populations difficult.

A quantitative approach to assess variation in innate and immune host resistance mechanisms during the peripartum period developed by Detilleux et al. (3) used a fitted polynomial model to assess hyporesponsiveness during the peripartum period. Results were utilized in an animal model to detect variation between daughters of various sire groups. This method of assessment was not suitable for the current study because it requires many data points across time. In the current study, variation in antibody to OVA was partitioned using a simpler model, wherein animals that had low antibody to OVA in the immediate peripartum period were ranked lower compared with animals that responded consistently and positively to OVA immunization.

Antibody response to OVA in dairy calves has been reported by Burton et al. (1) to be heritable ($h^2 = 0.48$). Although standard errors of sire and error variances could not be calculated, heritiability estimates of serum antibody to OVA were high ($h^2 > 0.50$) at time points before and after parturition. That the heritability estimate of antibody to OVA at parturition ($h^2 = 0.32$) was lower than at other time points evaluated may be explained by the complex interactions that occur between hormones and the immune system during the immediate pre- and postpartum period. Taken together, these results indicate that bovine antibody response to OVA is heritable, although heritability may be lower at times when the dairy cow is subject to the physical and metabolic stresses of parturition and early lactation. Heritability of antibody to OVA needs to be tested on a larger population but could suggest that genetic selection for increasing antibody responsiveness is possible in the peripartum cow, if deemed significant and beneficial. Antibody to *E. coli* J5 was also found to be heritable. The evaluation of heritability to *E. coli* J5 is less relevant to selection for enhanced immune responsiveness as antibody to *E. coli* is more influenced by the environment than antibody to OVA; however, heritability of *E. coli* J5 may be relevant for vaccine development. The heritiability estimates determined in this research may be useful to vaccine manufacturers who might consider designing a vaccine with the genetic component of antibody response in mind. If we know the trait is heritable and that genes such as BoLA genes influence this heritability, then vaccines can be designed to maximize response for a particular genotype.

The occurence of mastitis by antibody group was not similar between herds. In Herd 1 and Herd 3, the occurrence of mastitis was greatest for animals with low antibody (Group 3). All mastitic animals in these herds were in their second or later parity. Though not significant, OR in these herds indicated that there was a 2.16 and a 1.80 times greater chance of mastitis if animals were classified in Group 3 versus Groups 1 or 2. In contrast, animals from Herd 2 had a very different distribution of mastitis occurrence among groups. Group 1 animals were 7.57 ($P \leq 0.05$) times more likely to have mastitis than Group 2 or 3 animals. All cases in Herd 2 were first parity heifers. Differences in herd manage-

ment and the distribution of heifers and cows within each herd and antibody group may help explain the differences in distribution of mastitis occurrence between herds. Herd 1 (n = 6 heifers; n = 26 cows) and Herd 3 (n = 8 heifers; n = 29 cows) had a greater ratio of cows to heifers within each antibody group, while in Herd 2 (n = 34 heifers; n = 33 cows) heifers and cows were more evenly distributed among all antibody groups. Previous studies (18, 24) have acknowledged an increase in the occurrence of mastitis with advancing parity. This may explain the disparity among herds. It has been reported (19) that in well-managed herds with high milk production and low SCC, the rate of treatment of heifers for mastitis increased from 1.8 to 4.4% over an 8-yr period. In contrast to clinical mastitis observed in second parity and multiparous cows, that study further indicated that mastitis in heifers only resulted in small production losses and did not predispose heifers to more mastitis or other diseases later in lactation. In addition, the recovery rate from mastitis was high as indicated by a rapid decline in SCC following infection. This may indicate that mastitis in heifers and in cows should not be compared directly and that comparison of mastitis occurrence between herds with different distributions of parity groups may not be valid. That mastitis occurrence was not different between herds may be explained by a number of factors including the relatively small sample size evaluated, environmental (management) differences, distribution of heifers and multiparous cows, the type of mastitis (subclinical vs. clinical), and the pathogens involved in mastitis. One might argue that, in terms of immune response to mastitis, ability to produce average antibody is best because Group 2 had the lowest occurrence of mastitis when data were pooled across herds (9.5%). However, further research is warranted because in two of the three herds evaluated, cows with high antibody had no occurrence of mastitis.

CONCLUSIONS

Holstein cows and heifers could be readily classified quantitatively into high, average, and low antibody groups using an index based on measurement of antibody to OVA from wk –3 to 6 relative to parturition. Initial estimates suggest that serum antibody to OVA and *E. coli* J5 is heritable. Animals that were identified as high antibody responders to OVA also tended to have high antibody to the vaccine *E. coli* J5. Further investigations of larger populations with more disease data would be required to more accurately determine heritabilities of peripartum antibody response to OVA and *E. coli* J5, and to assess associations between antibody profiles and mastitis occurrence during the peripartum period. If variation of antibody response to OVA can be significantly correlated with mastitis, it may be useful as a selection tool for future breeding strategies to enhance immune responsiveness and improve disease resistance.

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