Intramammary Infections in Primiparous Holsteins: Heritabilities and Comparisons of Bovine Leukocyte Adhesion Deficiency Carriers and Noncarriers

ABSTRACT

The objective of this study was to determine the impact of bovine leukocyte adhesion deficiency on intramammary infection (IMI) in Holstein cows at first calving. Quarter milk samples were collected between 3 d prepartum and 4 d postpartum from 756 Holstein cows in first lactation. These samples were frozen and subsequently cultured using National Mastitis Council recommendations. Sixty-eight carriers of bovine leukocyte adhesion deficiency were identified (9.0% of cows) from an additional milk sampling collected in early lactation. Binary variables (infected or uninfected) for each quarter were defined as dependent variables to evaluate IMI incidence from all bacterial species and major species groups: coliforms, coagulase-negative staphylococci, and streptococci other than *Streptococcus agalactiae*. The model included herd-season of calving, days in milk when samples were collected, age at calving, quarter, cow (random effect), and bovine leukocyte adhesion deficiency. Sire was included as a random effect (instead of cow), and bovine leukocyte adhesion deficiency was dropped from the model to estimate heritabilities. Heritabilities for IMI incidence from the various groups of organisms ranged from 0.02 to 0.66 (0.21 from all bacterial species).

No differences were observed between carriers of bovine leukocyte adhesion deficiency and homozygous normal noncarriers for IMI from coliform, coagulase-negative staphylococci, and streptococci other than *Streptococcus agalactiae*, or all bacterial species combined. (Key words: bovine leukocyte adhesion deficiency, intramammary infection)

Abbreviation key: BLAD = bovine leukocyte adhesion deficiency, CNS = coagulase-negative staphylococci, SNA = streptococci other than *Streptococcus agalactiae*.

INTRODUCTION

Bovine leukocyte adhesion deficiency (BLAD) is a genetic disorder affecting Holstein cattle. The autosomal recessive condition is caused by a mutation of the β-subunit (CD18) of a glycoprotein family, resulting in limited expression of β2-integrin adhesion molecules on the surface of leukocytes (5, 14, 17). The lack of the adhesion molecules hinders leukocyte adherence and passage from the vascular system to the site of infection (5). Cattle that are homozygous for the recessive (D128G) allele for CD18 also have impaired random migration and phagocytosis of leukocytes, making them more susceptible to bacterial infections that result in premature death (9, 15).

Pedigree analysis and DNA testing have determined that the genetic defect likely originated from a single ancestor (14). Extensive use of several elite AI sires of the Holstein breed now known to be heterozygous carriers was responsible for the proliferation of BLAD. Powell and Norman (12) reported that 24% of the sires entering US AI organizations in 1988 were BLAD carriers. The possibility that the BLAD allele is linked to important production, type, or health traits continues to be researched. Recent studies (1, 7, 12) suggest little influence of BLAD on production or type traits in heterozygous cows. However, the effects of this allele on health traits have not been studied extensively. Kelm et al. (6) observed that, although not significant, BLAD carriers had higher EBV for IMI scores but lower EBV for SCS and clinical mastitis than did noncarriers.

The costly impact of mastitis on the dairy industry (2) warrants further investigation of the effect of...
BLAD on IMI and mastitis. The objective of this research was to determine the impact on IMI near parturition of the D128G allele in heterozygous carriers versus homozygous normal Holstein cows in first lactation.

MATERIALS AND METHODS

Herdspersons in eight cooperating Pennsylvania herds collected milk samples from each secreting quarter of 756 first lactation Holstein cows. The largest herd contributed samples from 470 cows, and each of the remaining seven herds contributed samples from 28 to 70 cows. The milk samples were aseptically collected in sterile 7-ml capacity polypropylene test tubes. Cow identification, quarter sample date, and quarter sample source were recorded. Samples were frozen soon after collection and transported within a week to the Animal Diagnostic Laboratory at The Pennsylvania State University for culturing. An additional composite milk sample was collected during early lactation for each cow in 125-ml capacity polyethylene bottles, frozen, and sent to the National Animal Disease Center (Ames, IA) to provide DNA for determination of BLAD status.

Herd 7 was the only herd that treated heifers with an intramammary antibiotic infusion in each quarter 30 d prior to the expected calving date.

Milk Culture

Blood agar plates were inoculated using a spread-plating technique with approximately 0.1 ml of milk that had been vortexed to disperse the fat layer; plates were incubated aerobically at 37°C (approximately 30% humidity) for 18 to 24 h. Bacterial growth was identified and recorded after 24, 48, and, in some cases, 72 h. The hemolytic pattern and positive coagulase test identified Staphylococcus aureus. Streptococci other than Streptococcus agalactiae (SNA) were differentiated from Strep. agalactiae by colony morphology, hemolytic pattern of colonies on blood agar, CAMP-esculin test, hippurate hydrolysis, growth in 6.5% NaCl, and fermentation of inulin, mannitol, and sorbitol. Coliform species were identified by morphologic characteristics of colonies on MacConkey agar, triple sugar iron agar, lysine iron agar, ornithine decarboxylase motility, citrate utilization, and urease activity. Other Gram-negative organisms were identified by means of the API 20 E micro ID system (BioMérieux, Hazelwood, MO). Actinomyces pyogenes were speciated by colony morphology and hemolysis on blood agar, Gram morphology, catalase, casein hydrolysis and acid in litmus milk, and gelatinase reactions. Corynebacteria were identified based on morphology, catalase urease, and carbohydrate utilization. A list of the pathogens that were identified during culture is found in Table 1. Samples showing no significant growth with fewer than three particular colonies of any colony morphology were considered negative. Quarter samples showing growth from two pathogens were reported as an infected quarter for each organism. If three organisms were cultured from a sample, the two organisms showing the most colony-forming units were recorded in the data set. Samples were coded as contaminated if more than three organisms were cultured.

BLAD Testing

Genomic DNA isolated from milk was used to determine the presence of the mutation at nucleotide 383 in bovine CD18 that is responsible for the BLAD condition. Milk samples were centrifuged at 10,000 × g for 1 min, the supernatant was removed, and milk fat was cleaned from the test tube with a sterile cotton swab. Milk leukocytes were washed three times in 1 µl of phosphate-buffered saline (10 mM sodium phosphate, pH 7.2, 3 mM KCl, and 140 mM NaCl). The resulting pellet was resuspended in 90 µl of 10 mM Tris, pH 7.5, 1 mM EDTA, 100 mM NaCl, and 10 µl of 1 M NaOH and then was heated at 95°C for 5 min.

Deoxyribonucleic acid (0.1 to 0.3 µl) was amplified for 35 cycles (94°C for 15 s, followed by 69°C for 20 s) in a 20-µl polymerase chain reaction containing 1 × PCR buffer, 0.2 mM deoxynucleotides, 0.5 units of AmpliTaq polymerase (Perkin-Elmer Cetus, Norwalk, CT), and 4 pmol of sense primer (5′-CCGAGGGCAAGGGCTA-3′) and antisense primer (5′-GAGTGGAGGTCCATCGGGTAG-3′) (14). The reaction tubes and contents were kept on ice until placed directly into the hot thermal cycler block. Ten-microliter aliquots of the amplification product were subjected to restriction endonuclease digestion separately by direct addition of 4 units of TaqI or HaeIII followed by incubation for 1.5 h at 65° or 37°C, respectively. The digested product was analyzed by 4% agarose gel electrophoresis and ethidium bromide staining and interpreted as described by Shuster et al. (14).

Variable Definitions

Binary variables were defined for each quarter (0 = quarter not infected, and 1 = infected quarter) and were constructed separately for IMI from all bacterial
TABLE 1. Organisms cultured and the number of cows and quarters infected by all species and major species groups.

<table>
<thead>
<tr>
<th>Species group</th>
<th>Cows infected</th>
<th>Quarters infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>All species</td>
<td>367</td>
<td>678</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>297</td>
<td>494</td>
</tr>
<tr>
<td>Streptococci other than Strep. agalactiae</td>
<td>86</td>
<td>110</td>
</tr>
<tr>
<td>Coliforms</td>
<td>54</td>
<td>62</td>
</tr>
<tr>
<td>Gram-positive bacillus</td>
<td>23</td>
<td>31</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Yeast</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pasteurella sp.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Actinomyces pyogenes</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yeast</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pasteurella sp.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Actinomyces pyogenes</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1One-hundred fourteen of 367 infected cows were infected in more than one quarter, or were infected with multiple organisms in a quarter, or both.

2Forty-one of 678 infected quarters were infected with multiple organisms.

species, coliforms, coagulase-negative staphylococci (CNS), and SNA. These binary variables were used as dependent variables in the analyses.

Herd-Season of Calving

The trial started in July 1991, and the last primiparous IMI samples were collected by February 1993. The first season of calving included all cows that freshened from the commencement of the project until the end of October 1991. Thereafter, four additional herd-seasons of calving were defined by 4-mo periods (November through February, March through June, and July through October).

Sire

One hundred thirty-six sires were represented. All sires had identification codes assigned by the National Association of Animal Breeders (Columbia, MO). The mean number of daughters per sire was 5.6, and the range was from 1 to 130 daughters. The mode was 1 daughter per sire; however, 2 sires had more than 100 daughters. The highest bull had 130 daughters (17.2% of the cows on project), and the second highest bull had 118 daughters (15.6% of the cows on project). All other sires had fewer than 49 daughters on the project.

Age at Calving

The mean age at calving was 24.8 mo and ranged from 22 to 33 mo; 11 cows outside this range were removed from the data set. The median and mode were both 24 mo.

DIM at Quarter Sampling

The mean DIM of sample collection was 1.8 d. The median and mode were both 2 d. Samples were collected prior to calving for 11 cows that were premilked. Twenty-two cows with samples that were taken more than 3 d prior to calving or more than 4 d postcalving were removed from the data set. Milk samples were collected at parturition, a period known for immunosuppression and increased IMI (4), to amplify potential differences in mammary gland immunity between BLAD carriers and noncarriers.

Statistical Analysis

The following mixed model was constructed using the MIXED procedure of SAS (13) to evaluate the impact of BLAD on IMI for the 756 cows that passed all edits:

\[ Y_{ijklmn} = \mu + H_i + L_j + Q_k + b_1D_l + b_2A_m + C_n + e_{ijklmn} \]

where

\[ Y_{ijklmn} = \text{binomial variable associated with infection status of a quarter}, \]
\[ \mu = \text{overall mean}, \]
\[ H_i = \text{effect of herd-season } i, \]
\[ L_j = \text{effect of BLAD status } j, \]
\[ Q_k = \text{effect of quarter } k, \]
\[ D_l = \text{days in milk } l, \]
\[ A_m = \text{age at calving } m, \]
\[ b_1, b_2 = \text{regression coefficients}, \]
\[ C_n = \text{random effect of cow } n, \]
\[ e_{ijklmn} = \text{unexplained residual effects assumed distributed as } N(0, \sigma_e^2). \]
TABLE 2. Number of cows sampled for IMI and number of cows infected by all pathogens and main pathogen groups by herds.

<table>
<thead>
<tr>
<th>Herd</th>
<th>All pathogens Uninfected</th>
<th>All pathogens Infected</th>
<th>Coliforms Uninfected</th>
<th>Coliforms Infected</th>
<th>Streptococci other than Strep. agalactiae Uninfected</th>
<th>Streptococci other than Strep. agalactiae Infected</th>
<th>Coagulate-negative staphylococci Uninfected</th>
<th>Coagulate-negative staphylococci Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>21</td>
<td>30</td>
<td>5</td>
<td>32</td>
<td>3</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>40</td>
<td>35</td>
<td>11</td>
<td>34</td>
<td>12</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>34</td>
<td>34</td>
<td>10</td>
<td>34</td>
<td>10</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>25</td>
<td>27</td>
<td>2</td>
<td>16</td>
<td>13</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>24</td>
<td>31</td>
<td>3</td>
<td>33</td>
<td>1</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>47</td>
<td>63</td>
<td>7</td>
<td>56</td>
<td>14</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td>7</td>
<td>356</td>
<td>154</td>
<td>458</td>
<td>12</td>
<td>441</td>
<td>29</td>
<td>345</td>
<td>125</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>22</td>
<td>24</td>
<td>4</td>
<td>24</td>
<td>4</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>389</td>
<td>367</td>
<td>702</td>
<td>54</td>
<td>670</td>
<td>86</td>
<td>459</td>
<td>297</td>
</tr>
<tr>
<td>Percentage</td>
<td>51.5</td>
<td>48.5</td>
<td>92.9</td>
<td>7.1</td>
<td>88.6</td>
<td>11.4</td>
<td>60.7</td>
<td>39.3</td>
</tr>
</tbody>
</table>

Cow was treated as a random effect, and quarter was modeled as repeated observations. An unstructured covariance matrix was assumed for the repeated measures (quarters). Cows were assumed to be unrelated. For estimation of heritabilities, a random sire effect was included in the model instead of cow, and the effect of BLAD status was removed. Only daughters of sires with three or more progeny in these data were used for estimating heritabilities.

RESULTS

Three hundred sixty-seven of the 756 cows (48.5%) had at least one infected quarter (Table 1). There were 249 contaminated or missing samples (8.2% of all quarters). Of the 2775 quarters that were successfully sampled and cultured, 678 were positive for one or more organisms (24.4%). The group of CNS was the most prevalent pathogen group that was isolated. Four hundred ninety-four quarters (17.8% of the 2775 usable quarters) were infected with CNS. One hundred ten (4% of the 2775 usable quarters) were positive for SNA. Coliform IMI were limited to 62 quarters (2.2% of 2775 usable quarters). All other pathogens combined infected fewer than 2% of the usable quarters. Eight quarters of 8 cows were colonized with contagious Staph. aureus. Contagious Strep. agalactiae was not isolated in any sample. Notably, herd 7 had the fewest cows infected (33%) for all possible organisms (Table 2) and noticeably fewer cows infected with CNS (27%) compared with the other herds (51 to 76%).

Herd-Season of Calving

Herd-season of calving was an important and highly significant ($P < 0.001$) explanatory variable for IMI incidence for all pathogens and all species groups. Clearly, herd and season of calving can be an important contributor to IMI at first calving.

DIM

The incidence of IMI caused by all organisms and by coliforms decreased ($P < 0.10$) as DIM increased (Table 3). The incidence of IMI from SNA and CNS also tended to decrease as DIM increased. This trend might not have been observed if all cows could have been sampled before the first milking.

Age at Calving

Age at calving influenced ($P < 0.05$) IMI by all organisms and IMI by CNS. Positive linear $\beta$-coefficients (Table 3) indicated that older heifers at calving had more frequent IMI at calving. Perhaps older heifers have more IMI at calving because they had longer exposure to bacteria. If substantiated in other studies, this finding could be another reason for heifers to calve at a relatively young age.

Sire

Sire influenced ($P < 0.10$) IMI incidence by all species combined, IMI incidence by coliforms, and IMI incidence by CNS. Sire was not significantly related

TABLE 3. Regression of IMI incidence on DIM at sample collection and age at calving.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Linear $b$-value</th>
<th>DIM at Sampling</th>
<th>Age at calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>$-0.025^{*}$</td>
<td>0.0223***</td>
<td></td>
</tr>
<tr>
<td>Coliform</td>
<td>$-0.009^{**}$</td>
<td>0.0018</td>
<td></td>
</tr>
<tr>
<td>Streptococci other than Strep. agalactiae</td>
<td>$-0.006$</td>
<td>0.0012</td>
<td></td>
</tr>
<tr>
<td>Coagulate-negative staphylococci</td>
<td>$-0.015$</td>
<td>0.0180***</td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.10$.
** $P < 0.05$. 
Heritability estimates for IMI incidence (unadjusted for binomial frequency) are shown in Table 4. Heritabilities ranged from 0.02 to 0.66 for IMI incidence. The heritability for IMI from all species was 0.21 and near the heritabilities for mastitis that were reported by Miller (8). The incidence of IMI by SNA was the least heritable. The heritability of incidence of IMI from coliforms was higher than most estimates (8). The resistance to IMI from coliform bacteria may be under more genetic control than IMI from other bacterial species.

Quarter

Quarter effect was modeled as a repeated measure using an unstructured covariance matrix among the repeated measures. The correlations among the quarters estimated from the analyses using IMI incidence from all species are in Table 5. Correlations among IMI between quarters on the same side of the mammary gland tended to be higher than correlations from the right side to the left side. The results indicate that it is important to consider the correlation between IMI on quarters of the same cow when the dependent variable is measured on each quarter.

BLAD

Sixty-eight (9.0%) of the 756 project cows were identified as BLAD carriers. The herd with the most carriers had 36 (7.7% of the 470 project cows in this herd). The remaining herds had between 1 and 9 carriers. The BLAD locus had no impact on the incidence of IMI from all species, from coliforms, from SNA, or from CNS (Table 6).

DISCUSSION

This study demonstrated a high number of heifers exhibiting evidence of IMI (by positive culture results) at first parturition. The prevalence of IMI was similar or slightly higher than previously reported in the literature (3).

The significant effect of herd-season on IMI prevalence in this study is attributed to differences in herd management and season, both of which have been implicated previously as influential in mammary gland health (3, 16). Furthermore, it was a common management practice in herd 7 to treat all heifers with an intramammary infusion of antibiotic in each quarter 30 d prior to the expected calving date. This practice reduced IMI at calving in several research trials (11, 18) and helped explain the lower IMI incidence exhibited by first-calf heifers in herd 7 (Table 2).

Smith et al. (16) reported that many IMI detected at calving were eliminated spontaneously during the first few days of lactation, which helps to explain the significant decrease in IMI as DIM at sample collection increased (maximum of 4 d). Also, samples collected prior to calving (−3 to −1 d) had a higher prevalence of IMI.

Older age at calving was associated with increased IMI. Longer exposure to the heifer environment associated with older age at calving might have been responsible for increased IMI.

The influence of sire on mammary gland health was previously reported. Miller (8) found that herita-
bility estimates of IMI ranged from 0 to as high as 0.20, and most estimates were less than or equal to 0.05. The heritability estimate for the incidence of bacterial status calculated by Weller et al. (20) was 0.045. More recently, Nash et al. (10) estimated the heritabilities of the ratio of infected to total usable quarters for species groups based on 921 first lactation cows (including some cows in this study). Their estimates, using Henderson’s method 3, ranged from 0.48 for CNS to 1.0 for coliforms.

For this study, heritability estimates of IMI (Table 4) varied greatly, depending on the pathogen. All estimates are associated with a large standard error from small sample size. The heritability estimate of IMI incidence from SNA was around zero, which indicated that the genetic impact on IMI incidence from SNA might be limited compared with the impact of environmental effects.

The frequency of cows that were heterozygous for the D128G allele (9.0%) was higher than earlier estimates of 6% (15). This higher frequency is not unreasonable given the high proportion of project animals sired by prominent AI sires and the relatively high frequency of BLAD carriers in AI sires of the late 1980s (12, 15). Another study involving 137 periparturient cows (6) reported carrier frequency of 11.3% for the BLAD allele.

Kelm et al. (6) calculated EBV for SCS, scores for clinical mastitis, and IMI scores for major (all streptococi, Staph. aureus, coagulase-positive SNA, and coliforms) and minor pathogens (CNS and C. bovis). They (6) observed no significant effect of BLAD genotype on IMI scores (taken at 35 d prepartum, at parturition, and at 35 d postpartum) from either pathogen group. In our study, there was also no difference in IMI incidence from all pathogens between BLAD carriers and noncarriers. These results appear logical given the protective role of mammary leukocytes and immunologic tests in previous research that showed no functional abnormalities for neutrophils and macrophages from heterozygotes compared with controls (5, 9).

The impact of BLAD on IMI by the individual species groups of coliforms, SNA, or CNS was also evaluated in this study (Table 6). No difference was observed between carriers and noncarriers in susceptibility to IMI caused by all species, SNA, CNS, or coliforms.

The CNS, as the most commonly cultured group of bacteria in this study, were most commonly isolated from milk samples in herds with effective mastitis control procedures and from heifer milk samples (18). The CNS are readily capable of colonization of the streak canal and teat canal (21). Subsequent incidence of clinical mastitis from CNS in first lactation (19) showed that cows that are heterozygous for BLAD do not differ in clinical mastitis caused by CNS from homozygous normal cows. It is not clear whether IMI from CNS in this study were CNS isolated in the streak canal or actual IMI.

**CONCLUSIONS**

The heritabilities of IMI at first calving appear to be moderate, especially when all bacterial species or coliform species are considered. Variation among families for IMI incidence is substantial.

The BLAD allele did not affect IMI incidence in Holstein cows. The frequency of IMI from all bacterial species, from coliforms, from SNA, and from CNS was similar between BLAD carriers and noncarriers. Overall, Holsteins that are heterozygous for BLAD do not appear to be more or less resistant to IMI than homozygous normal cows.

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**REFERENCES**


