Comparison of Holstein and Jersey Innate Immune Responses to *Escherichia coli* Intramammary Infection

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ABSTRACT

Mastitis is one of the most prevalent diseases in cattle and remains among the most costly diseases to the dairy industry. Various surveys have indicated a greater prevalence of and risk for mastitis in Holstein cows than in Jersey cows. The innate immune system comprises the immediate host defense mechanisms that respond to infection, and differences in the magnitude and rapidity of this response are known to influence susceptibility to and clearance of infectious pathogens. The reported differences in the prevalence of mastitis between Holstein and Jersey cows may suggest the occurrence of breed-dependent differences in the innate immune response to intramammary infection. The objective of the current study was to compare the acute phase and cytokine responses of Holstein and Jersey cows following intramammary infection by the bacterial pathogen *Escherichia coli*, a leading cause of clinical mastitis. All cows in the study were in similar stages of lactation, of the same parity, subjected to the same housing and management conditions, and experimentally infected on the same day with the same inoculum preparation. Before and after infection, the following innate immune parameters were monitored: bacterial clearance; febrile response; induction of the acute phase proteins serum amyloid A and lipopolysaccharide-binding protein; alterations in total and differential white blood cell counts; changes in milk somatic cell counts and mammary vascular permeability; and induction of the cytokines IFN- γ , IL-1 β , IL-8, IL-12, and tumor necrosis factor- α . Overall innate immune responses were similar between the 2 breeds; however, temporal differences in the onset, cessation, and duration of several responses were detected. Despite these differences, intramammary clearance of *E. coli* was comparable be-

tween the breeds. Together, these data demonstrate a highly conserved innate immune response of Holstein and Jersey cows to *E. coli* intramammary infection.

Key words: breed, dairy cow, innate immunity, mastitis

INTRODUCTION

Bovine mastitis, which is an inflammation of the mammary gland that often develops following intramammary bacterial infection, is one of the most prevalent and economically costly diseases to the dairy industry (Seegers et al., 2003). Annual economic losses due to this disease approach \$2 billion in the United States alone (Wells et al., 1998) and worldwide losses are estimated to approach \$35 billion (Wellenberg et al., 2002). Mastitis remains among the most frequently cited reasons for culling cows (Esslemont and Kossaibati, 1997; Bascom and Young, 1998) and this is attributed, in part, to the poor cure rates of antimicrobial agents approved for the treatment of this disease.

Gram-negative bacteria are responsible for approximately one-third of all clinical cases of bovine mastitis, and nearly 25% of these cases result in culling or death of the animal (Eberhart, 1984; Ziv, 1992). Although adoption of certain management practices has been successful at reducing the number of IMI caused by contagious pathogens, varying reports indicate that the incidence of mastitis caused by gram-negative bacteria has either remained constant or increased (Hillerton et al., 1995; Bradley, 2002; Makovec and Ruegg, 2003). Because there is an inverse relationship between the incidence of clinical mastitis caused by gram-negative bacteria and milk SCC (Barkema et al., 1998), the incidence of these clinical cases may be expected to increase as producers strive for lower SCC.

Among the gram-negative bacteria, *Escherichia coli* remains the most prevalent cause of IMI and clinical mastitis in cows (Wilson et al., 1997; Barkema et al., 1998; Makovec and Ruegg, 2003). Relative to other pathogens that cause clinical mastitis, IMI caused by

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E. coli tend to be more severe and have a greater mortality rate (Hazlett et al., 1984; Wilesmith et al., 1986; Bradley and Green, 2001). Correspondingly, the costs incurred by producers as a result of clinical mastitis are greatest when the causative pathogen is *E. coli* (Miller et al., 1993). Existing vaccines fail to prevent *E. coli* IMI and there are conflicting reports about whether they are able to reduce the severity of these infections (Hill, 1991; Hogan et al., 1992a,b; Tomita et al., 2000). Complicating the management of clinical cases of mastitis caused by *E. coli*, current antibiotic therapy for the treatment of these infections remains suboptimal (Erskine et al., 1991).

Genetic differences between breeds of food-production animals are known to influence disease resistance, and several published studies have reported breed-dependent differences in the prevalence of mastitis (Kelm et al., 2001). In regards to the 2 most populous US dairy cow breeds, various small- and large-scale surveys have indicated a lower prevalence of and risk for mastitis in Jersey cows than in Holstein cows (Erb and Martin, 1978; Motie et al., 1985; Morse et al., 1987; Washburn et al., 2002; Dego and Tareke, 2003; Youngerman et al., 2004; Biffa et al., 2005; Berry et al., 2007). A summary of DHI data from US cows calving in 2004 showed that 4.3% (91,444 of 2,144,804) of Holstein cow lactations were assigned the termination code "cow sold due to mastitis or high somatic cells" in contrast to 3.6% (5,432 of 152,201) of Jersey cow lactations (H. D. Norman, Animal Improvement Programs Laboratory, Agricultural Research Service, USDA; personal communication). Because udder health and longevity are related traits, and mastitis is one of the most frequently cited reasons for culling, the reported lower prevalence of mastitis in Jersey cows may explain their improved functional longevity compared with Holstein cows (Caraviello et al., 2005; Garcia-Peniche et al., 2006).

Milk somatic cells within the healthy mammary gland confer protection against IMI through their ability to recognize pathogens and initiate a rapid inflammatory response (Kehrli and Shuster, 1994). This may account, in part, for the association between lower milk SCC and both increased risk for clinical mastitis (Barkema et al., 1998; Suriyasathaporn et al., 2000; Beaudeau et al., 2002) and increased severity of mastitis (Green et al., 1996; Tadich et al., 1998; Bradley and Green, 2001). Large-scale surveys have reported that Jersey populations in the United States and Canada have higher milk SCC than Holsteins (Sewalem et al., 2006; Paape et al., 2007). Whether these differences in SCC reflect a differential prevalence of underlying IMI between the breeds or influence the ability of these breeds to respond to an IMI remains unknown.

Establishment of infection is governed, in part, by the nature of the host innate immune response to the invading organism (Burvenich et al., 2003; Bannerman et al., 2004). We and others have established that the differential inflammatory response elicited by Holstein cows to *E. coli* vs. *Staphylococcus aureus* IMI corresponds to the outcome of infection (Riollet et al., 2000; Bannerman et al., 2004). This finding indicates that variations in the inflammatory response to different pathogens within a breed influence the ability of the host to clear IMI. Whether breed-dependent differences exist in the inflammatory responses elicited during IMI remains unknown. Therefore, the current study investigated the innate immune response of Holstein and Jersey cows to *E. coli* IMI.

MATERIALS AND METHODS

Animals

Clinically healthy, primiparous Holstein and Jersey cows were selected from the USDA National Animal Disease Center herd on the basis of milk SCC of <200,000 cells/mL and the absence of detectable bacterial growth in aseptically collected milk samples. All selected Holstein (184 ± 12 DIM) and Jersey (208 ± 10 DIM) cows were in mid lactation and there was no statistically relevant difference between the number of DIM of the 2 groups ($P = 0.1418$). None of the cows had been previously immunized with an *E. coli* J5 bacterin. The use and care of all animals in this study was approved by the USDA National Animal Disease Center's Animal Care and Use Committee.

Experimental IMI

Escherichia coli strain P4, which was originally isolated from a clinical case of mastitis (Bramley, 1976), was used to induce experimental infection. Preparation of the inoculum for intramammary infusion was performed as described previously (Bannerman et al., 2004). Immediately following the morning milking on experimental d 0, the left rear quarters of 10 Holstein and 10 Jersey cows were infused with 3 mL of the *E. coli* inoculum. Plating of the final inoculum on blood agar confirmed that cows received 188 cfu of *E. coli* in the infused quarters.

Determination of Intramammary *E. coli* Growth

Following challenge (time 0), milk samples were aseptically collected from all infused quarters at 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, 96, 120, 168, and 240 h after infection. The samples were serially diluted in sterile PBS and 100 μ L of the resulting dilutions spread

on blood agar plates. Following a 24-h incubation at 37°C, plates were examined for bacterial growth and colonies enumerated.

Determination of Milk Somatic Cell and Circulating White Blood Cell Counts

To quantitate somatic cells, milk samples were heated to 60°C for 15 min and subsequently maintained at 40°C until counted on an automated cell counter (Bentley Somacount 150, Bentley Instruments, Inc., Chaska, MN). For the determination of total and differential white blood cell (WBC) counts, blood was collected from the jugular veins into Vacutainer glass tubes containing EDTA (Becton Dickinson Corp., Franklin Lakes, NJ). The tubes were inverted 10 times to avoid coagulation, and analyzed using a Hemavet 1500 multi-species hematology system (CDC Technologies/Drew Scientific Inc., Oxford, CT).

Whey and Plasma Preparation

For the preparation of whey, milk samples were centrifuged at $44,000 \times g$ at 4°C for 30 min and the fat layer removed with a spatula. The skimmed milk was decanted into a clean tube and centrifuged again for 30 min as above and the translucent supernatant collected and stored at -70°C. For the preparation of plasma, jugular vein blood samples were collected as above, inverted 10 times, centrifuged at $1,500 \times g$ for 15 min, and the clear plasma supernatant was aliquoted and stored at -70°C.

ELISA

Enzyme-linked immunosorbent assays for serum amyloid A (SAA), LPS-binding protein (LBP), BSA, IL-8, IL-1 β , tumor necrosis factor (TNF)- α , IL-12, and IFN- γ were performed as described previously (Bannerman et al., 2004, 2006).

Statistical Methods

Repeated-measures ANOVA was performed using SAS PROC MIXED (SAS version 9.1.3., SAS Institute, Cary, NC) to compare the mean responses of variables to control (time 0) values and to compare breed-dependent differences for a given variable. Milk bacterial counts, SCC, and IFN- γ concentrations were transformed to log₁₀ values to satisfy distributional requirements of ANOVA. Correlations among repeated measurements across time within cows and between breeds were modeled using appropriate covariance structures (first-order ante-dependence, spatial exponential, or

Figure 1. Intramammary growth of *Escherichia coli* in Holstein and Jersey cows following experimental infection. Immediately before (time 0) and at various times following intramammary infusion of 188 cfu of *E. coli* into one mammary quarter of 10 Holstein and 10 Jersey cows, sterile milk samples were collected and plated. The percentage of quarters in which viable bacteria were recovered is indicated (A). In those quarters in which bacteria were recovered, the mean ($\pm$ SE) log₁₀ milk bacterial concentration (cfu/mL) is reported (B).

spatial anisotropic exponential) for each parameter analyzed. A *P*-value of < 0.05 was considered significant.

RESULTS

Intramammary Growth of *E. coli* Following Infusion

Six hours after infusion of 188 cfu of *E. coli* into one mammary quarter of 10 Holstein and 10 Jersey cows, viable *E. coli* were recovered from the milk of 90% of the infused Holstein and Jersey quarters (Figure 1A). From 12 to 42 h after experimental infection, *E. coli*

Figure 2. Effect of *Escherichia coli* IMI on Holstein and Jersey milk production. Total daily milk weights were recorded before (d -4 to 0) and following (d 1 to 11) experimental *E. coli* IMI of 10 Holstein and 10 Jersey cows. Mean ($\pm$ SE) daily milk production is reported in kilograms. *#Decreased ($P < 0.05$) compared with preinfection (d 0) production amounts of Holstein or Jersey cows, respectively; @differences ($P < 0.05$) in milk production between breeds at a given time point.

were recovered from all infused quarters of Holstein and Jersey cows. The percentage of quarters infected in both breeds decreased from 48 h until the end of the study. Ten days following experimental infection, all of the infused Holstein quarters had cleared the infection and only 1 of the 10 infused Jersey quarters remained infected. Throughout the study, bacterial concentrations in the milk of the infected quarters of Holstein cows were comparable ($P = 0.6401$) to those of Jersey cows (Figure 1B). Within 6 h of infusion, the mean ($\pm$ SE) *E. coli* concentrations in the milk of infected quarters of Holstein and Jersey cows were 3.468 ± 0.154 and $3.675 \pm 0.247 \log_{10}$ cfu/mL, respectively. Growth of *E. coli* within the infected glands of Holsteins and Jerseys reached comparable mean ($\pm$ SE) maximal concentrations of 5.277 ± 0.354 and $5.033 \pm 0.219 \log_{10}$ cfu/mL, respectively, at 18 and 12 h after infusion. On the final day of the study, the sole Jersey quarter that remained infected had a milk *E. coli* concentration of $1 \log_{10}$ cfu/mL (10 cfu/mL).

Changes in Milk Production Following Experimental *E. coli* IMI

Milk production was measured at both the morning and evening milkings for several days before and after intramammary infusion of *E. coli* (Figure 2). Because experimental infection was initiated just after the morning milking, total daily milk output was calculated by summing the morning and prior evening milk

weights. Overall milk production throughout the study was lower ($P = 0.0059$) in Jersey cows than in Holstein cows. Relative to mean ($\pm$ SE) preinfection milk production amounts of 25.72 ± 1.50 kg on d 0, decreased milk production was observed in Holstein cows for 3 d following *E. coli* infection. Milk production amounts 4 d after infection approached, but did not reach, a level that was statistically different from preinfection amounts ($P = 0.0528$). In contrast, milk production in Jersey cows, relative to mean ($\pm$ SE) d 0 preinfection amounts of 18.87 ± 1.10 kg, was decreased for 7 d following IMI. Milk production in Holstein and Jersey cows reached nadirs of 9.77 ± 2.26 and 6.93 ± 1.91 kg, respectively, 2 d after infection.

Acute Phase Systemic Response to *E. coli* IMI

To determine whether *E. coli* IMI could elicit comparable systemic acute phase responses in Holstein and Jersey cows, changes in body temperature, acute phase protein synthesis, and differential WBC counts were evaluated. Relative to basal body temperature measurements immediately before infection (time 0), elevated temperatures were observed from 12 to 24 h and 12 to 18 h after infection in Holstein and Jersey cows, respectively (Figure 3A). Maximal febrile responses were evident in both breeds 12 h postinfection when mean ($\pm$ SE) rectal temperatures reached $39.59 \pm 0.23^\circ\text{C}$ in Holstein cows and $39.57 \pm 0.27^\circ\text{C}$ in Jersey cows. The overall febrile responses were similar ($P = 0.3411$) between the 2 breeds.

Induction of acute phase protein synthesis of SAA and LBP was also used to evaluate the systemic response of the 2 breeds to *E. coli* IMI. Relative to preinfection (time 0) concentrations, increases in circulating concentrations of SAA were detected earlier and sustained for a greater period of time in Holstein cows than in Jersey cows (Figure 3B). Relative to the response of Holstein cows, initial increases in SAA were delayed by 12 h in Jersey cows. The duration of the increase in SAA in Jersey cows was 24 h less than that of Holstein cows. Maximal increases in SAA were observed in both breeds 42 h after infection when mean ($\pm$ SE) blood SAA concentrations reached 251.81 ± 38.79 and $232.35 \pm 46.76 \mu\text{g/mL}$, respectively, in Holstein and Jersey cows. The overall SAA response was comparable between the 2 breeds ($P = 0.6625$). In contrast to SAA, initial increases in blood LBP were detected at the same time (18 h postinfection) in both breeds and the duration of the increase was identical (Figure 3C). Similar to SAA, maximal concentrations of LBP were detected in blood 42 h after infection in both Holstein ($493.84 \pm 36.75 \mu\text{g/mL}$) and Jersey ($474.67 \pm 31.99 \mu\text{g/mL}$) cows, and the

Figure 3. Acute phase responses of Holstein and Jersey cows to intramammary *Escherichia coli* infection. Rectal temperatures (A) were measured and blood samples collected immediately before (time 0) and at various time points following experimental *E. coli* IMI of 10 Holstein and 10 Jersey cows. Plasma derived from the blood samples was assayed by ELISA for serum amyloid A (SAA; B) and LPS-binding protein (LBP; C). Mean ($\pm$ SE) rectal temperatures are reported in Celsius and mean ($\pm$ SE) concentrations of SAA and LBP are reported in micrograms per milliliter. * $\#$ Increased ($P < 0.05$) compared with preinfection (time 0) measurements in Holstein or Jersey cows, respectively.

overall LBP response was comparable between the 2 breeds ($P = 0.5295$).

Changes in the circulating numbers and types of leukocytes were evaluated as another parameter of the systemic response to *E. coli* IMI (Figure 4). For both breeds, a sustained leucopenia was observed within 12 h of infection (Figure 4A). Analysis of differential WBC counts revealed a temporally coincident decrease in the circulating concentrations of neutrophils (Figure 4B) and lymphocytes (Figure 4C). Circulating WBC ($2,468 \pm 615$ vs. $2,320 \pm 410$ cells/ μ L), neutrophil (800 ± 338 vs. 777 ± 219 cells/ μ L), and lymphocyte ($1,456 \pm 409$ vs. $1,439 \pm 193$ cells/ μ L) counts reached similar nadirs in both Holstein and Jersey cows 12 to 18 h after infection. Relative to preinfection (time 0) counts, decreases in total circulating WBC and neutrophils were sustained for a greater period in Jersey cows than in Holstein cows. The overall changes in total WBC ($P = 0.2571$), neutrophil ($P = 0.4555$), and lymphocyte ($P = 0.4944$) counts in response to IMI were comparable between the 2 breeds.

Changes in Milk SCC and the Blood-Milk Barrier During Experimental *E. coli* IMI

As a local sign of inflammation, SCC in milk were determined before and after infection (Figure 5A and 5B). Preinfection (time 0) milk SCC were greater ($P = 0.0345$) in Jersey cows ($50,450 \pm 15,699$ cells/mL) than in Holstein cows ($19,300 \pm 3,611$ cells/mL). Relative to preinfection concentrations, initial increases in milk SCC were evident within 6 and 12 h after infection in Holstein and Jersey cows, respectively. For both breeds, postinfection milk SCC remained higher than preinfection counts throughout the study. Overall SCC responses of the 2 breeds were similar ($P = 0.2235$), and maximal SCC, which were detected in Holstein ($89.82 \times 10^6 \pm 17.52 \times 10^6$ cells/mL) and Jersey cows ($118.19 \times 10^6 \pm 13.95 \times 10^6$ cells/mL) 30 h postinfection, did not differ statistically ($P = 0.1560$).

As an indicator of perturbation of mammary vascular barrier function, milk was assayed for increases in BSA (Figure 5C). Relative to preinfection (time 0) concentrations, increases in milk BSA were initially detected 12 h after *E. coli* IMI in both breeds. Increases in milk BSA concentrations were continuously sustained for 48 and 60 h, respectively, in Holstein and Jersey cows. Maximally detected milk BSA concentrations of 5.72 ± 0.89 mg/mL in Holstein cows and 5.47 ± 0.80 mg/mL in Jersey cows were comparable, as was the overall change in milk concentrations of BSA ($P = 0.4849$) between the 2 breeds.

Figure 4. Effect of *Escherichia coli* IMI on Holstein and Jersey circulating white blood cell (WBC) counts. Blood samples were collected immediately before (time 0) and at various time points following experimental *E. coli* IMI of 10 Holstein and 10 Jersey cows. Blood was analyzed for total (A) and differential WBC counts (B and C). Mean ($\pm$ SE) cell counts are reported in thousands per microliter. *,#Decreased ($P < 0.05$) compared with preinfection (time 0) cell counts of Holstein or Jersey cows, respectively.

Figure 5. Effect of *Escherichia coli* IMI on Holstein and Jersey milk SCC and BSA concentrations. Milk samples were collected immediately before (time 0) and at various time points following experimental *E. coli* IMI of 10 Holstein and 10 Jersey cows. Whole milk was analyzed for SCC (A and B) and milk whey assayed for BSA by ELISA (C). Mean ($\pm$ SE) milk SCC are reported in millions of cells per milliliter (A) and $\log_{10}$ cells per milliliter (B), and BSA concentrations indicated in milligrams per milliliter (C). *,#Increased ($P < 0.05$) compared with preinfection (time 0) concentrations in Holstein or Jersey cows, respectively; @differences ($P < 0.05$) in SCC between breeds at a given time point.

Proinflammatory Cytokine Response Following *E. coli* IMI

To evaluate whether *E. coli* IMI could elicit a similar proinflammatory response in Holstein and Jersey cows, IL-8, IL-1 β , and TNF- α concentrations were quantified in milk samples collected before (time 0) and following experimental infection (Figure 6). Within 12 h of infection, increased milk concentrations of IL-8 were detected in both Holstein and Jersey cows (Figure 6A). In contrast to later time points where comparable concentrations between breeds were detected, IL-8 concentrations at 12 h postinfection were greater ($P = 0.0048$) in Holstein cows (763.19 ± 110.53 pg/mL) than in Jersey cows (445.85 ± 129.22 pg/mL). In both breeds, concentrations of IL-8 reached a maximum 18 h after infection and remained augmented up to 42 h postinfection. The overall induction of IL-8 was comparable ($P = 0.6913$) between the 2 breeds.

Relative to preinfection (time 0) concentrations, increases in milk IL-1 β were evident earlier and sustained longer in Jersey cows than in Holstein cows (Figure 6B). Maximally detected IL-1 β concentrations were comparable between the 2 breeds as was the overall IL-1 β response ($P = 0.6351$). Similar to IL-8, increased concentrations of TNF- α in milk were detected in both breeds 12 h after infection and the concentrations of TNF- α at this time were greater ($P = 0.0071$) in Holstein cows than in Jersey cows (Figure 6C). Maximal concentrations of TNF- α , which were detected in both breeds 18 h after infection, were greater ($P = 0.0168$) in Holstein cows as well. At the 2 sampling times before the end of the response (42 and 48 h postinfection), milk concentrations of TNF- α were greater in Jersey cows than in Holstein cows. The overall duration (36 h) and magnitude ($P = 0.8293$) of the TNF- α response was similar between breeds.

Type 1 Helper T Cell Cytokine Response Following *E. coli* IMI

To determine whether Holstein and Jersey cows elicited a similar type 1 helper T cell (T_H1-type) cytokine response to *E. coli* IMI, concentrations of IL-12 and IFN- γ were quantified in milk samples collected before and following experimental infection (Figure 7). Relative to milk samples collected before infection (time 0), those collected from both breeds 18 to 72 h after infection contained increased concentrations of IL-12 (Figure 7A). Maximal concentrations of IL-12, which were detected in Holstein (443.08 ± 95.91 U/mL) and Jersey (482.19 ± 115.01 U/mL) cows 42 h postinfection, were comparable, as was the overall IL-12 response ($P = 0.5948$).

Figure 6. Proinflammatory cytokine responses of Holstein and Jersey cows to intramammary *Escherichia coli* infection. Milk samples were collected immediately before (time 0) and at various time points following experimental *E. coli* IMI of 10 Holstein and 10 Jersey cows. Milk whey was assayed by ELISA for IL-8 (A), IL-1 β (B), and tumor necrosis factor (TNF)- α (C). Mean ($\pm$ SE) concentrations of IL-8 and IL-1 β are expressed in picograms per milliliter and mean ($\pm$ SE) TNF- α concentrations reported in nanograms per milliliter. *, #Increased ($P < 0.05$) compared with preinfection (time 0) concentrations in Holstein or Jersey cows, respectively; @differences ($P < 0.05$) in concentrations between breeds at a given time point.

Figure 7. Effect of *Escherichia coli* IMI on Holstein and Jersey production of IL-12 and IFN- γ . Milk samples were collected immediately before (time 0) and at various time points following experimental *E. coli* IMI of 10 Holstein and 10 Jersey cows. The concentrations of IL-12 (A) and IFN- γ (B) in milk were determined by ELISA and expressed as mean ($\pm$ SE) units per milliliter and picograms per milliliter, respectively. *, #Increased ($P < 0.05$) compared with pre-infection (time 0) concentrations in Holstein or Jersey cows, respectively; @differences ($P < 0.05$) in concentrations between breeds at a given time point.

In contrast to IL-12, the duration of the IFN- γ response of Jersey cows exceeded that of Holstein cows (Figure 7B). Relative to preinfection (time 0) milk samples, those obtained from Holstein and Jersey cows contained increased concentrations of IFN- γ over a 36- and 66-h period, respectively. At 60 and 72 h postinfection, milk IFN- γ levels were greater in Jersey cows than in Holstein cows. Differences in the overall IFN- γ response approached but did not reach a statistically significant level ($P = 0.0816$).

DISCUSSION

The current study investigated the innate immune response of Holstein and Jersey cows during experimentally induced *E. coli* mastitis in a controlled setting. A previous study investigated immunization effects on the response of Holstein and Jersey cows to *E. coli* IMI, but breed differences were not examined (Todhunter et al., 1991). Because only 3 Jersey cows were included in that study, animal variation would have precluded the ability to draw statistically relevant conclusions from such a small sample size. Another study investigated the inflammatory response of 5 Holstein and 5 Jersey cows to experimental *Streptococcus uberis* IMI (Rambeaud et al., 2003). The investigators of that study reported no significant differences between the 2 breeds in the parameters evaluated. Because the main objective of that research did not focus on investigating breed-dependent differences, the investigators presented combined data for all cows and did not infect the Holstein and Jersey groups in a controlled manner. In their study, the amount of bacteria used to establish infection in the 2 breeds differed by approximately 60%. In addition, the 2 breeds were housed on different farms and subjected to different management practices. Finally, the 2 breeds were not infected at the same time nor was sampling and monitoring of the cows conducted in parallel. In contrast, all cows in the present study were housed in a single barn, subjected to the same management practices, infected in the same quarter with the same preparation and amount of bacterial challenge inoculum, and infected and monitored in parallel. A more robust sample size was used in the current study to investigate breed-dependent differences to IMI, and several additional innate immune response parameters were monitored, including acute phase protein synthesis, changes in total and circulating WBC, and induction of T_H1-type cytokine production. Because parity (Gilbert et al., 1993; Van Werven et al., 1997; Mehrzad et al., 2002) and lactational stage (Lescourret and Coulon, 1994; Shuster et al., 1996; Vangroenweghe et al., 2005) can influence immune responses to and severity of IMI, all cows in this study were of the same parity (i.e., primiparous) and in similar DIM.

In the current study, mastitis was experimentally induced by the intramammary infusion of equal numbers (cfu) of *E. coli* into the mammary glands of Holstein and Jersey cows. The objective of the study was to not only investigate whether overall differences in the magnitude of the responses differed between breeds, but also to assess whether the kinetics of the onset of the responses differed. Because the kinetics of the inflammatory response to intramammary *E. coli* infection is influenced by inoculum size (Vangroenweghe et al.,

2004), equal numbers of bacteria were infused into all glands despite breed-dependent differences in milk production and udder mass. Although breed-dependent differences in the size of the udders and teat ends may influence the inoculum size that initially infects the mammary gland under natural conditions, the scope of this study was limited to investigating postinfection differences in inflammatory responses.

The results of the current study demonstrated that overall cytokine production and induction of acute phase protein synthesis in response to *E. coli* IMI were comparable between Holstein and Jersey cows. There was a tendency ($P = 0.0816$), however, for Jersey cows to produce increased concentrations of IFN- γ , a cytokine that links the innate and adaptive immune systems by activating neutrophils and macrophages and promoting a Th1-type immune response (Trinchieri, 1997). Although overall production of inflammatory mediators was similar, the temporal induction of certain responses differed by breed. Relative to Jersey cows, earlier induction or increased induction at earlier time points of SAA, IL-8, and TNF- α was observed during the response of Holstein cows to *E. coli* IMI. Interestingly, IL-8 and TNF- α are involved in neutrophil recruitment (Ming et al., 1987; Caswell et al., 1999), and the heightened induction of these cytokines early in the response may have contributed to the earlier initial increase in SCC in Holstein cows.

Initial induction of IL-1 β and IFN- γ occurred earlier in Jersey cows than in Holstein cows. Both cytokines remained elevated, relative to baseline concentrations, for a longer duration in Jersey cows. The proinflammatory cytokine, TNF- α , was expressed at greater concentrations in Jersey cows at later time points. Interleukin-1 β , IFN- γ , and TNF- α are all known to evoke increased vascular permeability (Martin et al., 1988; Brett et al., 1989; Munro et al., 1989). Because increased concentrations of milk BSA reflect increased vascular permeability, the increased concentration of milk BSA at later time points in Jersey cows is consistent with the prolonged expression of these cytokines. Further, because these 3 cytokines induce profound metabolic changes that can culminate in cachexia (Bielefeldt Ohmann et al., 1989; Matthys and Billiau, 1997; Morley et al., 2006), their enhanced expression at later time points may explain the prolonged delay in resumption of milk production to basal (preinfection) amounts in the Jersey cows. Based on the percentage of quarters infected and milk bacterial counts, it does not appear that the heightened expression of these cytokines at later time points conferred an advantage to the Jerseys in controlling or clearing the IMI.

Consistent with national surveys (Sewalem et al., 2006; Paape et al., 2007), the Jersey cows (50,450 $\pm$

15,699 cells/mL) in this study had greater basal (preinfection) milk SCC than the Holstein cows (19,300 $\pm$ 3,611 cells/mL). In terms of the overall inflammatory response and bacterial clearance, the greater milk SCC before IMI did not confer a protective advantage to the Jersey cows. This is consistent with previous studies indicating that basal milk SCC $\geq 200,000$ to 300,000 cells/mL are required for a protective effect (Schalm et al., 1964a,b; Bramley, 1976). Following initial infection, rapid increases in milk SCC due to neutrophil recruitment is reported to enhance bacterial clearance (Vandeputte-Van Messom et al., 1993; Shuster et al., 1996). Relative to basal (preinfection) counts, milk SCC increased within 6 h of IMI to 78,300 $\pm$ 27,690 cells/mL in Holstein cows. In contrast, there was no increase in milk SCC in Jersey cows during this period. The rapid increase in SCC in Holstein cows, however, did not appear to confer a beneficial advantage presumably due to too minimal of an increase.

Increased milk production is associated with increased risk of clinical mastitis (Oltenu and Ekesbo, 1994; Waage et al., 1998; Fleischer et al., 2001). In the present study, basal (preinfection) milk production was greater in Holstein than in Jersey cows. However, all Holstein and Jersey cows infused with *E. coli* developed an IMI, which was characterized by the recovery of viable bacteria from infused quarters at several time points. All cows also developed clinical mastitis in response to the experimentally induced *E. coli* IMI. The finding that overall inflammatory responses were comparable between the 2 breeds despite the differences in milk production is compatible with a previous study reporting that within-breed differences in milk production do not affect the severity of *E. coli* mastitis (Kornalijnslipier et al., 2003).

The overall finding that the innate immune responses of the 2 breeds were highly conserved was based on data from a limited sample size of 10 cows per breed. Thus, one could postulate that with a larger sample size, and corresponding increased statistical power, that breed-dependent differences in innate immune responses might have been identified. The percentage difference in the overall magnitude of the responses for many of the variables, however, differed by less than 10% and the plotted mean responses at individual time points were often superimposable. Thus, although increasing the sample size would be expected to increase the statistical power to a point where significant differences could be detected, the biological implications of such small differences in mean responses would be questionable. Finally, one cannot summarily reject the possibility that this sample size was not large enough to identify any breed-dependent differences, because

overall milk production was demonstrated to be significantly greater in Holstein cows than in Jersey cows.

To our knowledge, the present report is the first to compare the innate immune responses of Holstein and Jersey cows to *E. coli* IMI in a controlled setting. Although overall innate immune responses were similar between the 2 breeds, differences in the temporal onset, cessation, and duration of several immune parameters were observed. These temporal differences, however, did not differentially affect clearance of *E. coli* IMI and resolution of inflammation. Together, these data demonstrate that despite genetic and phenotypic differences, the innate immune response of Holstein and Jersey cows to *E. coli* IMI remains highly conserved.

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