Short communication: Prevalence of digital dermatitis in Canadian dairy cattle classified as high, average, or low antibody- and cell-mediated immune responders

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ABSTRACT

Lameness is a major animal welfare issue affecting Canadian dairy producers, and it can lead to production, reproduction, and health problems in dairy cattle herds. Although several different lesions affect dairy cattle hooves, studies show that digital dermatitis is the most common lesion identified in Canadian dairy herds. It has also been shown that dairy cattle classified as having high immune response (IR) have lower incidence of disease compared with those animals with average and low IR; therefore, it has been hypothesized that IR plays a role in preventing infectious hoof lesions. The objective of this study was to compare the prevalence of digital dermatitis in Canadian dairy cattle that were classified for antibody-mediated (AMIR) and cell-mediated (CMIR) immune response. Cattle (n = 329) from 5 commercial dairy farms in Ontario were evaluated for IR using a patented test protocol that captures both AMIR and CMIR. Individuals were classified as high, average, or low responders based on standardized residuals for AMIR and CMIR. Residuals were calculated using a general linear model that included the effects of herd, parity, stage of lactation, and stage of pregnancy. Hoof health data were collected from 2011 to 2013 by the farm's hoof trimmer using Hoof Supervisor software (KS Dairy Consulting Inc., Dresser, WI). All trim events were included for each animal, and lesions were assessed as a binary trait at each trim event. Hoof health data were analyzed using a mixed model that included the effects of herd, stage of lactation (at trim date), parity (at trim date), IR category (high, average, and low), and the random effect of animal. All data were presented as prevalence within IR category. Results showed that cows with high AMIR had significantly lower prevalence of digital dermatitis than cattle with average and low AMIR. No significant difference in prevalence of digital dermatitis was observed between high, average, and low CMIR cows. These results indicate that having more robust AMIR is associated with lower prevalence of digital dermatitis hoof lesions.
Key words: digital dermatitis, immune response, dairy cattle

Short Communication

Lameness is one of the most costly and serious animal welfare issues affecting the Canadian dairy industry today (O'Callaghan et al., 2003; Solano et al., 2015). The reason for this is that lameness has been associated with many issues, including reduced milk yield (Amory et al., 2008) and lower fertility (Hernandez et al., 2001; Buch et al., 2011), likely due to the fact that cattle are experiencing pain and discomfort and thus spending more time lying down and less time eating (Espejo et al., 2006). Studies have estimated the prevalence of lameness in Canadian dairy cattle to range between 20 and 35%, with the most common lesion being digital dermatitis at an incidence rate of 15% of all recorded lesions (Solano et al., 2016). Digital dermatitis is an infectious hoof lesion that typically affects the skin at the base of the hoof heel. It is highly contagious and is caused by bacterial pathogens that thrive in damp and dirty conditions (Clegg et al., 2016), making it difficult to prevent the occurrence of these lesions. Efforts have been made through foot bathing and treating the lesions with antibiotics during trimming; however, the prevalence of digital dermatitis continues to remain high.

Several studies have been done in different species showing that animals with a more robust or high IR have lower occurrence of disease (Covelli et al., 1989; Raymond et al., 1998; Wagter et al., 2000). Specifi-

cally in dairy cattle, studies have shown that cattle classified as high immune responders not only have a lower incidence of mastitis but also reduced incidence of other common diseases, including metritis, ketosis, displaced abomasum, and retained fetal membrane (Thompson-Crispi et al., 2012b, 2013). This is because the immune system controls an animal's ability to respond to invading pathogens. It has been shown that the cell-mediated immune response (**CMIR**; also known as type 1 response) predominates in protection against intracellular pathogens (such as viruses or *Mycobacterium paratuberculosis*), whereas the antibody-mediated immune response (**AMIR**; known as the type 2 response depending on the antibody isotype involved) predominates in protection against extracellular pathogens such as bacteria (Wikenheiser and Stumhofer, 2016). In studies where animals have been selected or identified for high or enhanced immune response, it has been shown that these animals have a balance between type 1 and type 2 responses (Crawley et al., 2005; Heriazon et al., 2011), suggesting they would have overall broad-based resistance to disease, which is important for defense against the wide array of pathogens that exist. (Stear et al., 2001, 2016; Mallard et al., 2015). Selection for resistance against particular pathogens may also improve disease resistance (Meijerink et al., 2000). Although this holds true for the particular pathogen being selected against, it is also limiting as it generally only confers resistance to one pathogen. Certain components of the immune system can be negatively genetically correlated, indicating that selecting for resistance against one pathogen may cause susceptibility to others (Nino-Soto et al., 2008; Thompson-Crispi et al., 2012a). Therefore, because high immune responders demonstrate a greater ability to respond to a wide array of pathogens and resist several different diseases, we hypothesized that cattle classified as high immune responders are able to resist pathogens that cause infectious hoof lesions, specifically digital dermatitis. To date, the prevalence of digital dermatitis has yet to be evaluated in animals ranked based on IR, leading to the objective of this study, which was to evaluate the prevalence of digital dermatitis in Canadian dairy cattle classified as high, average, and low immune responders.

Cattle (n = 329) from 5 different commercial dairy farms in Ontario were evaluated for immune response (**IR**) using a patented test protocol that was adapted from previously described protocols (Wagter et al., 2000; Hernandez et al., 2005). Briefly, blood samples were taken on d 0 from the tail vein and animals were immunized intramuscularly with known type 1 and type 2 antigens (Heriazon et al., 2013). Fourteen days later, animals were bled again from the tail vein and a delayed-type hypersensitivity test initiated in the tail folds. Triplicate measurements were taken on either side of the tail fold using Harpenden skin calipers (Creative Health Products Inc., Ann Arbor, MI) and intradermal injections of control (PBS) and type 1 antigen were given on either side of the tail folds (Hernandez et al., 2005). On d 15, final skin-fold measurements are taken in triplicate as an indicator of CMIR. The blood samples taken on d 0 and 14 were centrifuged (700 × *g* for 15 min), and serum was obtained and aliquoted for storage at −20°C until time of analysis. Antibody activity to the type 2 antigen was evaluated by indirect ELISA (Heriazon et al., 2013). Briefly, 96-well plates were coated with the type 2 antigen dissolved in carbonate buffer and incubated for 24 h at 4°C. The following day, plates were blocked with 3% Tween 20 (Sigma-Aldrich, Oakville, ON, Canada), 1.5% BSA (Sigma-Aldrich), and 1.5% fetal calf serum (Sigma-Aldrich) in PBS. Sera samples were diluted to 1:50 and 1:200 and controls diluted to 1:200 and 1:400. Positive controls were obtained by pooling d-14 sera from the top 10 cows for IgG antibody activity to the type 2 antigen, and negative controls were obtained by pooling d-0 sera from 10 cows. All controls and test sera were added to the plate in quadruplicate. Monoclonal mouse anti-bovine IgG conjugated to alkaline phosphatase (Sigma-Aldrich) was diluted 1:8,000 in Tris buffer and added to the plate. Plates were read at wavelengths of 405 and 630 nm, with the 630 nm results being subtracted from the 405 nm results. Plates were read until positive controls from this result reached an optical density (OD) ≥0.999. The positive controls were corrected back to an OD of 1 with all samples being corrected back to the positive control to compare samples across different plates and run on different days as described previously (Heriazon et al., 2013). To minimize error, if controls or samples had a coefficient of variation greater than 10%, the sample or plate (in the case of controls) was repeated.

All IR data were log-transformed to normalize the distribution, and CMIR and AMIR were analyzed using a general linear model in SAS (SAS Institute Inc., Cary, NC) as follows:

$$y_{ijkl} = \mu + d_i + l_j + p_k + h_l + e_{ijkl},$$

where y_{ijkl} = AMIR or CMIR, μ = overall mean, d_i = d 0 data for AMIR or control site for CMIR, l_j = parity effect (parity 0, 1, 2, 3, and ≥4), p_k = pregnancy status effect (not pregnant, 1–100, or 101–200, >200 d in calf), h_l = herd effect (1, 2, 3, 4, and 5), and e_{ijkl} = residual error. Residuals were obtained from the model, with the PROC UNIVARIATE procedure in SAS used to test the residuals for normality. Residuals were then

converted to standardized residuals with a mean of zero. Cattle were ranked as high or low if standardized residuals were >1 standard deviation (**SD**) above the mean or <1 SD below the mean response for each trait, respectively. Average AMIR and CMIR cows had standardized residuals within 1 SD of the mean (Thompson-Crispi et al., 2013).

Hoof health data were collected by the farms' hoof trimmer, using Hoof Supervisor software (KS Dairy Consulting Inc., Dresser, WI), from December 2011 to November 2013. Five trimmers were involved in the study, with each trimmer only being involved on a single farm. All trim events were included for each animal during this period. Digital dermatitis was assessed as a binary trait at each event (1 = presence of digital dermatitis, 0 = no digital dermatitis detected). To ensure that consistency of defining lesions was maintained between each hoof trimmer, all were trained by the same person and provided with visual documents created by Alberta hoof trimmers before commencing the study (Alberta Dairy Hoof Health Project, 2014). All hoof health data were analyzed using the following SAS mixed model:

$$y_{ijklm} = \mu + p_i + i_j + h_k + l_l + a_m + e_{ijklm},$$

where y_{ijklm} = digital dermatitis; μ = overall mean; p_i = parity at trim date (i = 0, 1, 2, 3, and ≥4); i_j = immune response ranking (j = high, average and low); h_k = herd (1, 2, 3, 4, and 5); l_l = stage of lactation at trim date (not lactating, 1–99 d, 100–199 d, and 200–500 d), a_m = random effect of animal, and e_{ijklm} = standard error. Significance is reported at $P < 0.05$. All graphs are presented as prevalence in percentages; however, significance is based on the differences between the least squared means calculated from the model.
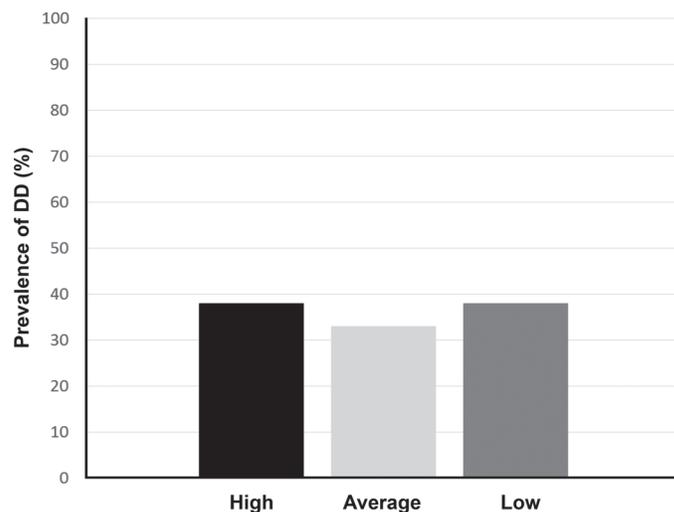
Overall prevalence of digital dermatitis in this study was 34%. Results for the prevalence of digital dermatitis in animals ranked as high, average, and low for AMIR are presented in Figure 1. Figure 1 shows that the high (20% of high responders infected with digital dermatitis) antibody responders had significantly lower ($P < 0.05$) prevalence of digital dermatitis compared with average (35% of average responders infected with digital dermatitis) and low (40% of low responders infected with digital dermatitis) antibody responders.

Figure 2 shows the results for prevalence of digital dermatitis in animals ranked as high, average, and low for CMIR. No significant difference was observed between high (38% of high responders infected with digital dermatitis), average (33% of average responders infected with digital dermatitis), and low (38% of low responders infected with digital dermatitis) CMIR for prevalence of digital dermatitis.
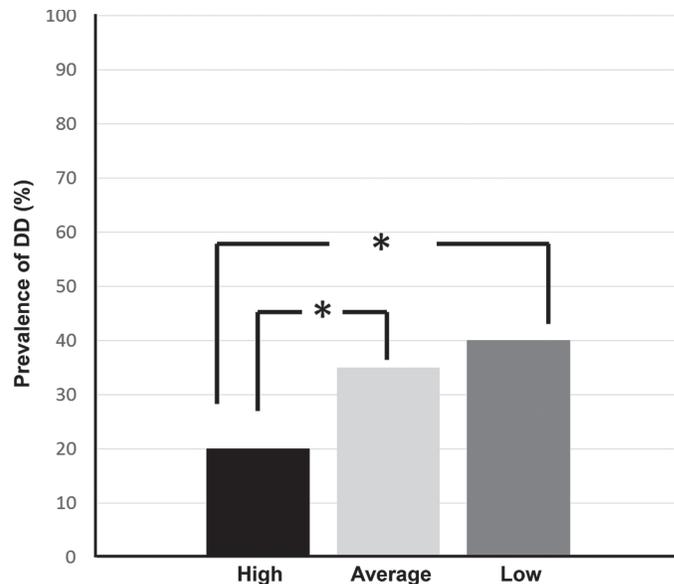
Figure 1. Prevalence of digital dermatitis (DD) in cattle ranked as high, average, and low for antibody-mediated immune response. *Significant differences reported at $P < 0.05$.

This study found that cows classified as high antibody responders had significantly lower prevalence of digital dermatitis compared with average and low responders. Digital dermatitis is an infectious disease that commonly affects hooves of cattle and can be caused by multiple bacterial pathogens (Yano et al., 2010). Because antibody responses are typically associated with defense against extracellular pathogens (Estes and Brown, 2002), including bacteria, it makes sense that antibody responses would play a major role

Figure 2. Prevalence of digital dermatitis (DD) in cattle ranked as high, average, and low for cell-mediated immune response.

in defense against the bacterial pathogens that cause digital dermatitis. These results are similar to those seen in a previous study examining mastitis, which is another disease typically caused by extracellular bacterial pathogens. In the previous study, cattle classified as high antibody responders had fewer cases of mastitis compared with average and low antibody responders, and cows with low AMIR tended to have the most severe cases of mastitis (Thompson-Crispi et al., 2013).

Results also indicated that no significant difference was observed between high, average, and low CMIR cows for prevalence of digital dermatitis. Because CMIR is primarily involved in defense against intracellular pathogens (Wikenheiser and Stumhofer 2016) and it has been shown that the main pathogen involved in digital dermatitis is extracellular (Clegg et al., 2016), it makes sense that a significant difference would not be observed for CMIR cows. Previous research has shown cows classified as both high AMIR and high CMIR have lower incidence of several different diseases (Thompson-Crispi et al., 2012b). Recent studies in commercial Holsteins bred using semen from high IR sires have also indicated that these offspring have less disease than females bred to other sires (Larmer and Mallard, 2016). Also, noteworthy is the fact that the cells mainly associated with the cell-mediated immune system, such as T-helper lymphocytes, secrete cytokines that help in initiation of a protective antibody response (Alberts et al., 2002). Therefore, because AMIR and CMIR often work together to control infectious disease and no detrimental effect was observed with high CMIR cows, these results suggest that having high CMIR in conjunction with high AMIR is beneficial in reducing the prevalence of infectious hoof lesions. Collectively, these studies support the idea that animals with more robust immune responses are better able to respond to invading pathogens, including the bacteria that may result in digital dermatitis, and to clear them before disease occurs.

In conclusion, this study showed that cows classified as high AMIR had significantly reduced prevalence of digital dermatitis lesions. We observed no significant difference between high, average, and low CMIR cows, indicating no detrimental effects of a high CMIR on the prevalence of digital dermatitis. Therefore, having high IR cattle in a herd or breeding for high IR will not only potentially improve disease occurrence but may also decrease the prevalence of infectious hoof lesions, thus improving both overall welfare and economic gains.

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