Prevalence of Digital Dermatitis in Canadian Dairy Cattle classified as High, Average of Low Antibody and Cell-Mediated Immune Responders

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ABSTRACT

Lameness is a major animal welfare issue affecting Canadian dairy producers, it can lead to production, reproduction and health problems. Although there are a number of different lesions that affect dairy cattle hooves, studies show that digital dermatitis is the most common lesion identified on Canadian dairy herds. It has also been shown that dairy cattle classified as having high immune response (IR) have lower incidence of disease compared to 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 (AMIR) and cell-mediated immune response (CMIR). Cattle (n = 341) from five 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 and 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 was collected from 2011 to 2013 by the farm's hoof trimmer using Hoof Supervisor software. All trim events were included for each animal, but lesions were assessed as a binary trait at each trim event. Trimmers scored each lesion for severity with 1 = least, 2 = moderate, 3 = most. Hoof health data was 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 (P = 0.0089) and low (P = 0.0011) AMIR. No significant difference in prevalence of digital dermatitis was observed between high, average and low CMIR cows. Similarly cows with high AMIR also had significantly lower prevalence of severity 1 lesions compared to average (P = 0.03) cows and significantly lower prevalence of severity 2 lesions compared to average (P = 0.04) and low (P = 0.02) AMIR cows. These results indicate that having more robust AMIR is associated with lower prevalence of digital dermatitis hoof lesions.

INTRODUCTION

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., 2015b). The reason for this is that lameness has been associated with many issues including reduced milk yield (Amory et al., 2008) and lower fertility (Buch et al., 2011; Hernandez et al., 2001), likely due to the fact that cattle are experiencing pain and discomfort therefore 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 20-35%, with the most common lesion being reported as

digital dermatitis at an incidence rate of 15% of all recorded lesions (Solano et al., 2015a). 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.

There have been a number of studies done in different species showing 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). Specifically, if we look at dairy cattle, studies have shown that not only do cattle classified as high immune responders have 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; Thompson-Crispi et al., 2013). The reason for this is that the immune system is what controls an animal's ability to respond to invading pathogens. It has been shown that the cell-mediated response (also known as type 1 response) predominates in protection against intra-cellular pathogens (such as viruses or *microbacterium paratuberculosis*), whereas antibody mediated response (known as type 2 response depending on the antibody isotype involved) predominates in the protection against extra-cellular pathogens such as bacteria (Wikenheiser and Stumhofer, 2016). In studies where animals have been selected or identified for high/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; Mallard et al., 2015; Stear et al., 2016). It has been suggested that selection for resistance against particular pathogens may also improve disease resistance (Meijerink et al., 2000). While this does hold 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 since high immune responders demonstrate a greater ability to respond to a wide array of pathogens and resist a number of different diseases it was 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.

MATERIALS AND METHODS

Cattle (n=341) from five different commercial dairy farms in Ontario were evaluated for 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 day 0 from the tail vein and animals were immunized intra-muscularly 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 are taken on either side of the tail fold using Harpenden skin calipers (Creative Health Products Inc. Ann

Arbor, MI, USA) and intra-dermal injections of control (PBS) and type 1 antigen are given on either side of the tail folds (Hernandez et al., 2005). On day 15 final skin-fold measurements are taken in triplicate as an indicator of CMIR. The blood samples taken on days 0 and 14 are centrifuged (700 x g for 15 minutes) and sera is obtained and aliquoted for storage at -20°C until time of analysis. Antibody activity to the type 2 antigen was evaluated by indirect enzyme linked immunosorbant assay (Heriazon et al., 2013). Briefly 96-well plates were coated with the type 2 antigen dissolved in carbonate buffer and incubated for 24 hours at 4°C. The following day, plates were blocked with 3% Tween 20 (Sigma-Aldrich, Oakville, Ontario), 1.5% bovine serum albumin (Sigma-Aldrich Oakville, Ontario) and 1.5% foetal calf serum (Sigma-Aldrich, Oakville, Ontario) 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 day 14 sera from the top 10 cows for IgG antibody activity to the type 2 antigen and negative controls were obtained by pooling day 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, Oakville, Ontario) was diluted 1:8000 in tris buffer and added to the plate. Plates were read at wavelengths of 405 nm 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 OD $\geq$0.999. The positive controls were corrected back to an OD of 1 with all samples being corrected back to the positive control in order to compare samples across different plates and run on different days as described previously (Heriazon et al., 2013). In order to minimize error, if controls or samples had a coefficient of variation greater than 10% then the sample or plate (in the case of controls) were repeated.

All immune response data was log transformed, in order to normalize the distribution and CMIR and AMIR were analyzed using a SAS (SAS Institute Inc., Cary, NC) general linear model 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 = day 0 data for AMIR or control site for CMIR, l_j= parity effect (parity 0, 1, 2, 3 and $\geq$4), p_k = pregnancy status effect (not pregnant, 1-100, or 101-200, >200 days 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 greater than one standard deviation (**SD**) above the mean or less than one SD below the mean response for each trait, respectively. Average AMIR and CMIR cows had standardized residuals within one SD of the mean (Thompson-Crispi et al., 2013).

Hoof health data was collected by the farms' hoof trimmer, using hoof supervisor software (KS Dairy Consulting, Inc. Dresser, Wisconsin), from December 2011 to November 2013. All trim event were included for each animal during this time period, however digital dermatitis was assessed as a binary trait at each event (1 = presence of digital dermatitis, 0 = no digital dermatitis detected). Digital dermatitis was also scored for severity by the hoof trimmer as standardized for this study, with 1 = least severe, 2 = average severity and 3 = most severe. In order to make sure consistency of defining severity was maintained between each hoof trimmer, they were all trained by the same person and provided with visual documents prior to commencing study on how to define the severity of each lesion based on its morphology. These trait were also assessed as binary. All hoof health data including severity was analyzed using the following SAS (SAS Institute Inc., Cary, NC) mixed model (Chapinal et al., 2013):

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

where y_{ijklm} = digital dermatitis; 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 days, 100-199 days and 200-305), 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.

RESULTS AND DISSCUSSION

Overall prevalence of digital dermatitis in this study was 34%. The overall prevalence of severity 1 lesions was 12%, severity 2 lesions was 18% and severity 3 lesions was 4%.

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 the high (20% of highs) antibody responders had significantly lower prevalence of digital dermatitis compared to average (35% of averages) and low (40% of lows) antibody responders. Similarly high responders had significantly lower prevalence of severity 1 lesions compared to average and significantly lower prevalence of severity 2 lesions compared to average and low antibody responders (Table 1).

Figure 2 shows the results for prevalence of digital dermatitis in animals ranked as high, average and low for CMIR. Results indicated that no significant difference as observed between high (38% of highs), average (33% of averages) and low (38% of lows) CMIR for prevalence of digital dermatitis. Similarly no significant difference was observed between high, average and low CMIR cows for the prevalence of severity 1, 2 and 3 lesions. (Table 1)

This study found that cows classified as high antibody responders had significantly lower prevalence of digital dermatitis compared to average and low responders. Digital dermatitis is an infectious disease that commonly affects hooves of cattle and can be caused by multiple bacterial pathogens (Takahisa et al., 2010). Since antibody responses are typically associated with defense against extra-cellular pathogens (Estes and Brown, 2002), which includes bacteria, it makes sense the antibody responses would play a major role in defense against the bacterial pathogens which cause digital dermatitis. These results are similar to those seen in a previous study looking at mastitis, which is another disease typically caused by extracellular bacterial pathogens. In this previous study cattle classified as high antibody responders had reduced cases of mastitis compared to average and low, as well as 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 and severity of digital dermatitis. Since CMIR is primarily involved in defense against intra-cellular 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 a number of different diseases (Thompson-Crispi et al., 2012). 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 the initiation of a protective antibody response (Alberts et al., 2002). Therefore, since AMIR and CMIR often work together to control infectious disease and no detrimental effect was observed with high CMIR cows these results

may also suggest that having high CMIR in conjunction with high AMIR is beneficial in reducing the prevalence of infectious hoof lesions.

The findings of this study also indicated that cattle with high AMIR had significantly lower prevalence of severity 1 and 2 lesions compared to average and average low antibody mediated responders respectively. Previous studies in pigs selectively bred for immune response have shown that those bred for high immune response have less severe clinical signs in response to infection compared to those bred for low immune response (Magnusson et al., 1998). Recent studies in commercial Holsteins bred using semen from high immune responder sires indicates that these offspring have less disease than those females bred to other sires (Larmer and Mallard, 2016). 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 clear them before disease occurs.

In conclusion this study showed that cows classified as high AMIR, had significantly reduced prevalence and severity 1 and 2 digital dermatitis lesions. This study also observed no significant difference between high, average and low CMIR cows, indicating no detrimental effects of being a high CMIR on the prevalence and severity of digital dermatitis. Therefore, having high immune response cattle in a herd or breeding for high immune response not only will it 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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Table 1: Prevalence of severity of digital dermatitis in cattle ranked as high, average and low antibody (AMIR) and cell-mediated (CMIR) immune responders. Significance, at $P<0.05$, is denoted by different letters.

	AMIR Rank			**CMIR Rank**		
	High	Average	Low	High	Average	Low
Severity 1 (%)	6^{a}	13^{b}	12^{ab}	11^{a}	11^{a}	15^{a}
Severity 2 (%)	10^{a}	18^{b}	22^{b}	19^{a}	17^{a}	15^{a}
Severity 3 (%)	4^{a}	4^{a}	7^{a}	3^{a}	4^{a}	6^{a}

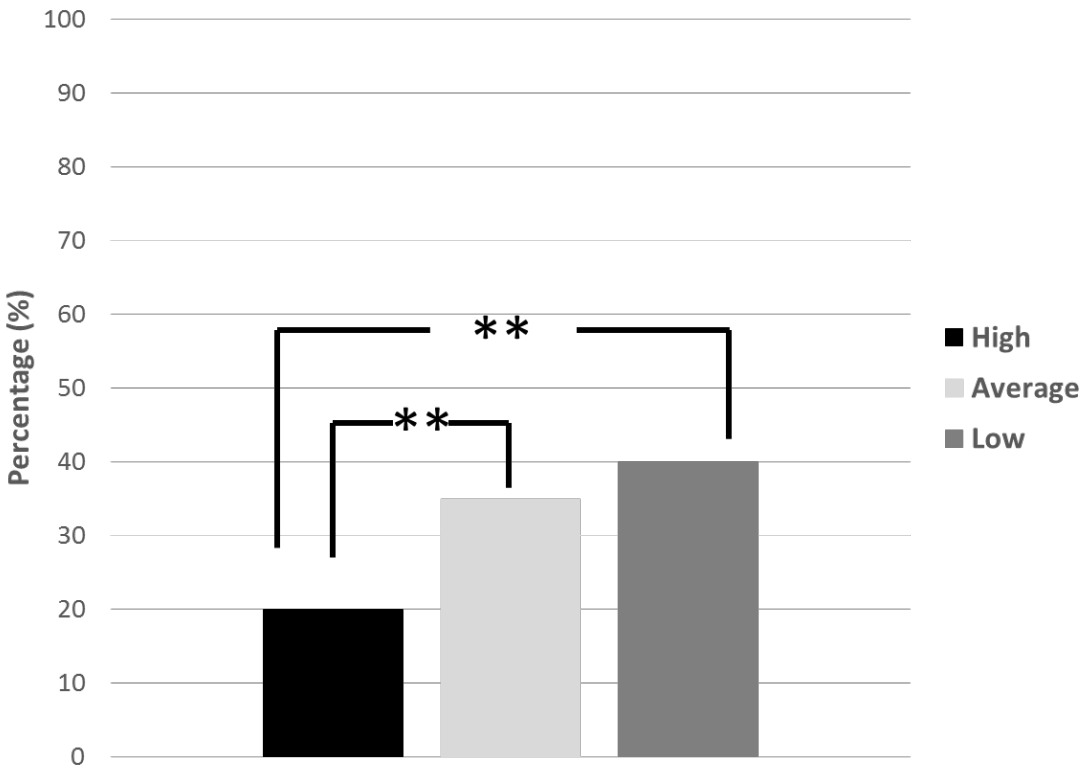

Figure 1: Prevalence of digital dermatitis in cattle ranked as high, average and low for antibody-mediated immune response. Significance reported at $P < 0.05$ (**).

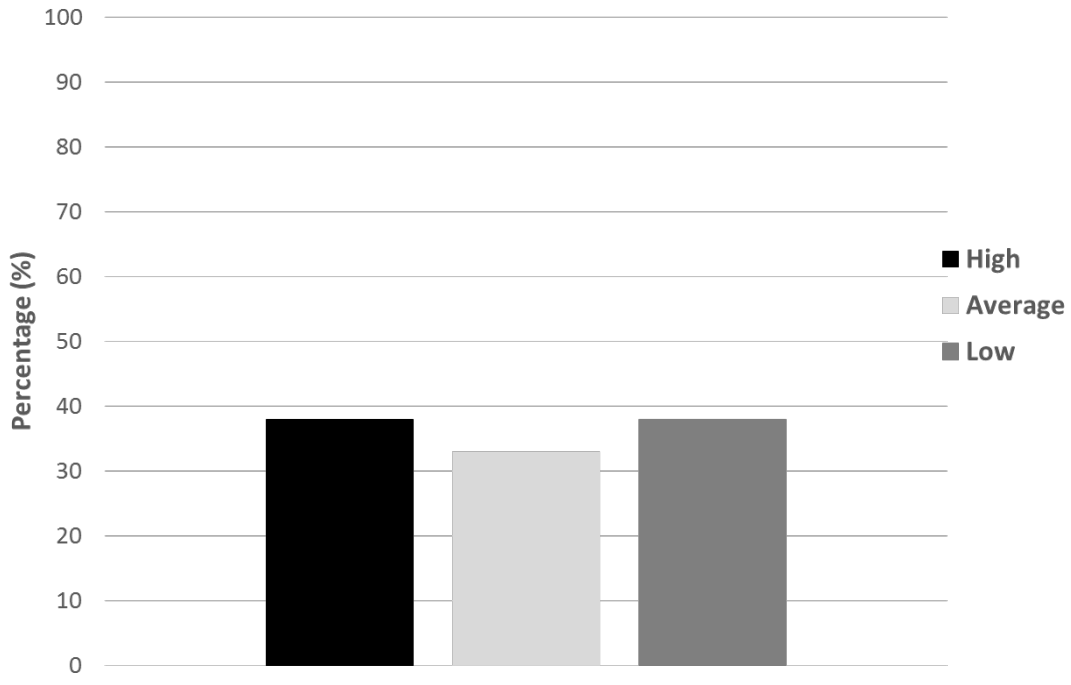

Figure 2: Prevalence of digital dermatitis in cattle ranked as high average and low for cell-mediated immune response.