Variation in Immune Response between Canadian Purebred Holstein and Crossbred Norwegian Red Calves and First Calf Heifers

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Introduction

Inbreeding in the Canadian Holstein population has increased over the years and has resulted in an inbreeding level of 5.75% being reported for 2007 and an average annual increase in inbreeding of 0.08% for the period 2000-2007 (Canadian Dairy Network (2008)). Studies have associated increases in inbreeding with increased risk of disease occurrence. Miglior et al. (1995) found lactation somatic cell score increased by 0.012 per 1% increase in inbreeding. This suggests that inbred animals may have increased risk of diseases, such as mastitis.

Problems associated with inbreeding may be resolved by crossbreeding with more robust breeds. The Norwegian-Red (NR) dairy breed has been selected over many generations for resistance to disease and studies have shown this has resulted in genetic improvement for disease resistance (Heringstad et al. (2007)). The possibility that disease resistance in the NR breed is related, at least in part, to enhanced immune responsiveness was recently investigated in Canadian Norwegian-Red X Holstein (NRFX) calves. Results of this preliminary study found crossbred calves displayed significantly greater primary IgG antibody response to a type 2 antigen compared to purebred Holstein (HO) calves (Begley et al. (2009 a)).

The objectives of this study were to extend the study previously done by Begley et al. (2009 a) and evaluate a larger sample size of purebred HO and crossbred NRFX calves for both antibody (AMIR) and cell-mediated immune response (CMIR). As well a subset of purebred HO and crossbred NRFX first calf heifers, previously immunized as calves, were evaluated for a variety of immune response traits, including both AMIR and CMIR.

Material and methods

Animals and immunization protocol. Purebred HO (n = 140) and crossbred NRFX (n = 142) 2-6 month old calves, from 25 commercial farms in Southern Ontario, were evaluated

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for AMIR and CMIR. Calves were immunized using a previously described protocol (Wagter et al. (2000); Hernandez et al. (2005); Begley et al. (2009 a)) with both type 1 and type 2 antigens in order to induce both AMIR and CMIR. A subset of these calves, from 18 of the 25 commercial farms, (HO n = 69 and NRFX n = 81) were then re-immunized 6-9 days post calving, using a similar protocol as was used for the calves, to assess these traits in first calf heifers. Briefly, day 0 (6-9 days post calving) heifers were bled and immunized intramuscularly with type 1 and type 2 antigens. Day 7 post immunization heifers were again bled.

Evaluation of AMIR. Sera were obtained from all blood samples collected from both calves and heifers in order to assess AMIR by enzyme linked immunosorbant assay (ELISA). ELISAs were preformed as previously described (Wagter et al. (2000); Begley et al. (2009 a)) in order to determine anti-bovine IgG to type 2 antigen for both calves and heifers. Fetal calf serum was used as a negative control in ELISAs preformed on heifers, but all other controls remained the same as previously described (Begley et al. (2009 a)).

Evaluation of CMIR. On day 21 (for calves) or day 7 (for heifers) of the immunization protocol background skin-fold measurements of each side of the tail were taken in triplicate. Calves and heifers were injected intradermally with a control on the left side tail fold and type 1 antigen, as a test, on the right side tail fold. On day 23 (for calves) or day 9 (for heifers) skin-fold measurements were again taken in triplicate at both control and test sites in order to assess delayed type hypersensitivity (DTH) as an indicator of CMIR. For purposes of analysis the triplicate measurements, taken at 0 and 48 hours for test and control sites, were averaged.

Statistical analyses. A SAS general linear model was used for analysis of all data and to determine statistical significance between breeds. A p-value ≥ 0.05 was considered statistically significant. All data for both heifers and calves were log transformed to ensure normalization. Secondary antibody response and DTH response for calves were both initially analyzed using the model: $Y_{ijklm} = year_i + farm*breed_j + breed*gender_k + breed*age_l + breed*time0_m + e_{ijklm}$, where Y = immune response trait, year = the year the calf was tested, farm = the herd of origin, breed = the fixed effect of breed (HO or NRFX), gender = gender of calf (male or female), age = age of calf at first test date, time0 = baseline measurement of immune response trait and e = residual error term. Primary antibody response for calves was analyzed using the same model as above however the model did not include the variable time0, since the baseline line OD values are similar to OD values seen with just wash buffer. Therefore the log of baseline OD values was subtracted from the log of day 14 OD values before primary antibody response data were analyzed. Preliminary antibody and DTH response for heifers were initially analyzed with the model: $Y_{ijkl} = season_i + farm*breed_j + breed*age_k + breed*time0_l + e_{ijkl}$, where Y = immune response trait, season = season and year of testing, farm = herd of origin, breed = fixed effect of breed (HO or NRFX), age = age of heifer at calving, time0 = baseline measurement of immune response trait and e = residual error term. Interaction terms remained in both of the above models if they had a p-value ≤ 0.05. Otherwise these terms were dropped out of the model and replaced with the single variables within the interaction term. All single variables remained in both models whether significant or not.

Results and discussion

Results for antibody and DTH responses for both calves and heifers are shown in figures 1-4. Figure 1, which reports least squares means (LSM) on primary IgG antibody response for calves, shows crossbreds had significantly greater primary IgG antibody response compared to purebreds ($p = 0.019$). These results are similar to results reported in previous studies on crossbred calves in that Begley et al. (2009 a) also found NRFX calves had significantly greater primary IgG antibody response to test antigen. Similarly, a study on Jersey X HO calves showed crossbred calves had significantly greater concentrations of serum IgG and lower incidence of scours compared to purebreds (Maltecca et al. (2006)). These results may suggest crossbreds have an enhanced ability compared to purebreds to defend against extracellular pathogens.

Figure 2, which depicts secondary IgG antibody response in calves, shows the interaction term farm*breed was significant ($p = 0.002$) and therefore the effect of breed depends on the farm of origin. Similar results were found for tertiary IgG antibody response in heifers, which are shown in figure 3 ($p = 0.027$). Both figures 2 and 3 display only those farms which showed significant differences between breeds ($n = 3/25$ for calves and $n = 3/18$ for heifers). Figure 2 reports that across all farms shown crossbred calves had significantly greater secondary IgG antibody response compared to purebreds (farm A $p > 0.001$, farm B $p > 0.001$, farm C $p = 0.001$). Preliminary results in figure 3 show that on 2 farms (farm C $p = 0.002$ and farm D $p = 0.022$) crossbred heifers had significantly greater tertiary IgG antibody response and on farm E ($p = 0.009$) purebred HO heifers had significantly greater tertiary IgG antibody response. The results depicted in figures 2 and 3 are different to those previously reported in other studies. Begley et al. (2009 a) found no significant difference between purebred and crossbred calves for secondary IgG antibody response. In another study, Begley et al. (2009 b) found no significant difference between purebred and crossbred second lactation heifers for secondary IgG antibody response. The differences seen may be attributed to the fact that in the previous studies interaction terms were not included in the models used to analyze data.

Finally figure 4 shows results for both DTH response in calves and preliminary results for DTH response in heifers. Figure 4 shows no significant difference in mm increase of skin fold thickness at 48 hours between breeds of calves ($p = 0.401$). These results are similar to the results previously shown by Begley et al. (2009 a) who also found no significant difference between purebred and crossbred calves for DTH. Preliminary results also depicted in figure 4 show crossbred heifers had significantly greater mm increase in skin fold thickness at 48 hours compared to purebreds ($p = 0.025$). These results are different to those shown in a previous study by Begley et al. (2009 b) who found no significant difference in DTH response between breeds. One reason for the observed differences may be that heifers in this study were tested soon after calving whereas cows in the study by Begley et al. (2009 b) were tested 158 days after calving. Therefore these results may indicate that during stressful times, such as the peripartum period, crossbreds may have enhanced defense against intracellular pathogens.

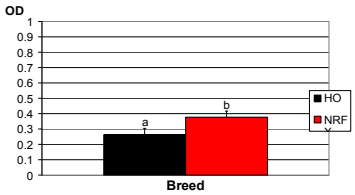

Figure 1: Calves LSM primary IgG antibody response to type 2 antigen

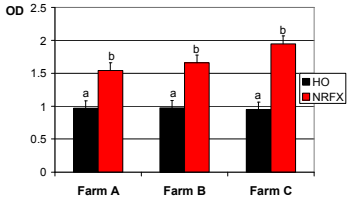

Figure 2: Calves LSM secondary IgG antibody response to type 2 antigen

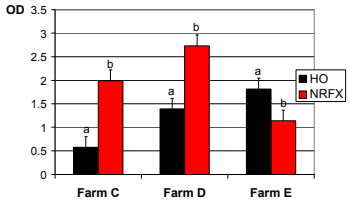

Figure 3: Heifer LSM tertiary IgG antibody response to type 2 antigen

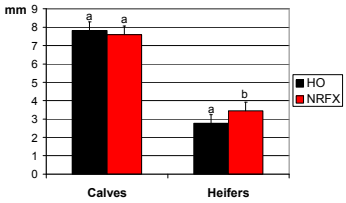

Figure 4: Heifer and calves LSM DTH response at 48 hours to type 1 antigen

Conclusion

These results show that crossbred calves had both significantly greater primary and secondary IgG antibody responses compared to purebreds and therefore may have enhanced ability to defend against extracellular pathogens. Crossbred heifers had significantly greater DTH response at 48 hours and on 2 farms out of 3 farms had significantly greater tertiary IgG antibody response compared to purebreds. Therefore, in general, crossbreeds compared to purebreds may have a greater ability to defend against both intracellular and extracellular pathogens and may be more resistant to the diseases caused by these pathogens.

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