Influence of maternal and sire effects on antibody response following clostridial vaccination of Friesian-Jersey crossbred calves

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ABSTRACT

As part of an ongoing project to identify quantitative trait loci (QTL) for economically important traits in New Zealand dairy cows, a vaccine trial was conducted to measure the antibody response of 10 week old Friesian-Jersey crossbred calves to two antigenic compounds of a clostridial vaccine (*Clostridium novyi* B and *Clostridium tetani* toxoids). Blood samples were collected from 469 calves prior to vaccination at 10 weeks of age, prior to re-vaccination at 13 weeks of age and at 16 and 22 weeks of age. ELISA assays using conjugated rabbit anti-bovine IgG were conducted for both toxoids. Correlations and explanatory variables were calculated using correlation and general linear model (GLM) procedures in SAS. Antibody response to both toxins were relatively low after the first vaccination (week 13), but were boosted to high levels after revaccination (week 16). GLM analysis revealed a moderate sire effect contributing to the variability seen in the 13 week test against *C. tetani* (P=0.001) and the 16 week test against *C. novyi* (P=0.01). This variation in individual antibody response will be used in future research to identify genetic factors controlling the acquisition of immunity.

Keywords: antibody response; vaccine; sire effect, immune function, dairy cattle.

INTRODUCTION

Immune function is a trait of primary importance to the dairy industry as infectious diseases, including mastitis and laminitis, result in considerable condition and production losses in dairy cattle. Vaccination of calves with clostridial vaccines like Ultravac™ 5 in 1[1] (CSL Ltd., Parkville, Australia) at 10-15 weeks of age is routine practice on New Zealand dairy farms. Treatment of calves with this product with a follow-up booster shot will confer resistance to tetanus, black leg, black disease, enterotoxaemia (pulpy kidney) and malignant oedema. Although effective in most cases the complex biology involved in the acquisition of resistance means results can be variable. Vaccination of stock is one method of mitigating the impact of infectious diseases. For genetic selection of dairy cattle, it is important to consider both enhanced resistance to disease and responsiveness to vaccination.

Genetic selection of dairy cows in New Zealand has focused predominantly on productivity traits, leading to large increases in milk production and improvements to milk solids composition (Lopez-Villalobos *et al*. 2000). In the United Kingdom such reliance on selection for increased milk production has resulted in an increase in the incidence of clinical mastitis and other health related conditions (Pryce *et al*. 1998). The production of antibodies through up-regulation of the humoral immune system is a primary mechanism for development of resistance to bacterial pathogens. Wagter *et al.* (2000) found that dairy cattle with the lowest antibody response to an antigen had the highest incidence of mastitis. More recently the antibody response to ovalbumin has been used as an indictor of immune function in Holstein dairy cows and heifers (Wagter *et al*. 2003). Furthermore, studies showing heritability estimates of greater than 0.5 for disease resistance traits (Wagter *et al.* 2000) are stimulating significant interest in the use of information from immune function studies in genetic evaluations (Kadarmideen & Pryce 2001). Nielsen *et al*. (2002) demonstrated sire effects of 6.3% in a study of antibody response in a population of 1106 mixed breed dairy cows. Sire effects have also been demonstrated for serum IgM concentrations in foetal bovine from cows mated to Simmental bulls compared to cows mated to bulls of other breeds (Norman *et al.* 1981). Sire effects such as this are evidence that the innate resistance to disease in dairy cows is at least partially influenced by genetics. Exploitation of this genetic variation will be the basis for further investigation of this trial herd.

The aim of this experiment was to evaluate the antibody response of 469 crossbred heifers to two constituents of a vaccination programme using a '5 in 1' clostridial vaccine. The long-term objective of this research programme is to identify genes responsible for the regulation of the antibody mediated immune response in cattle.

[1] Ultravac™ 5 in 1 clostridial vaccine (CSL Ltd) contains: *Clostridium perfringens* D, *C. tetani*, *C. novyi* B and *C. septicum* toxoids and *C. chauvoei* inactivated cells and toxoid.

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MATERIALS AND METHODS

A herd of second cross (F_2) Friesian-Jersey crossbred cows were generated by selective contract mating in commercial New Zealand dairy herds. Amongst the 469 heifers used in this trial, six first cross (F_1) Friesian-Jersey crossbred sires are represented. The 469 trial heifers were produced by contract mating these six F_1 bulls to moderate BW F_1 Friesian-Jersey crossbred cows in commercial New Zealand dairy herds during the spring of 2000. The trial design is documented by Spelman *et al.* (2001). Of the 469 calves, each sire was represented by between 67 and 98 calves. The trial heifers were collected from these commercial farms between 4-10 days of age and reared at a research facility (Tokanui Research Farm, AgResearch) in the southern Waikato from July 2001. The heifers were reared on unprocessed whole milk before being weaned onto grain meal and ryegrass/white clover pasture at six weeks of age. All trial heifers were genotyped to confirm parentage, and any animals that failed parentage checks were excluded from the current trial.

At 10 weeks of age a total of 469 Friesian-Jersey crossbred heifers were vaccinated with a '5 in 1' clostridial vaccine (Ultravac^{TM} 5 in 1, CSL Ltd), followed by a second vaccination administered three weeks later. Ten milliliters of non-heparinised blood was collected from each animal at 10 weeks (prior to vaccination), at 13 weeks (prior to revaccination), and at 16 and 22 weeks of age. Sera were separated from the blood samples and stored at –86°C until analysis.

Concentrated *C. tetani* toxoid and concentrated, ultrafiltered *C. novyi* B toxoid were obtained from CSL Ltd, Melbourne, Australia to develop an enzyme linked immunosorbent assay (ELISA) for characterizing the level of immunity acquired by individual animals in the trial. These antigens are the same as two of the antigen constituents present in the '5 in 1' clostridial vaccine. Preliminary studies were carried out to determine the optimum concentrations of antigens and antibodies, and development times for each assay. Two components that differ in molecular weight were chosen, to determine whether immune response was similar at different molecular weight antigens.

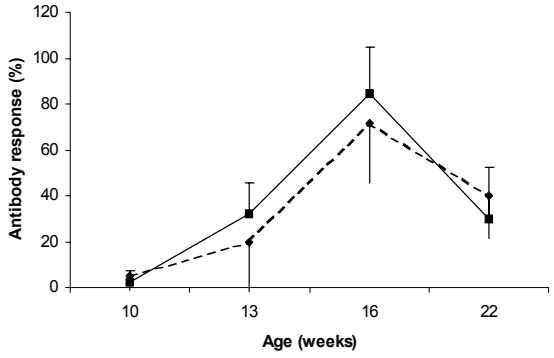
Individual wells in flat-bottom microtitre plates (Nunc, Maxisorb, Denmark) were coated with 50 µl of 1/5000 dilution of tetanus toxoid, or 1/2000 dilution of C. *novyi* B toxoid in a 0.05 M carbonate buffer (Ph 9.6) and left overnight at 4°C. Plates were washed three times with phosphate buffer saline containing 0.05% Tween 20 (PBST) and 50 µl of a 1/100 dilution of serum in PBST containing 1% casein and 1/5000 thiomersal was added to each well. For each antigen a duplicate sample was set up. The plates were incubated at room temperature for an hour, and washed as described above. A 50 µl volume containing 1/6000 dilution of rabbit anti-bovine IgG (H and L chains) conjugated to horse radish peroxidase (Jackson ImmunoResearch Laboratories Inc., West Grove, USA) prepared in PBST containing 1% casein was added to each well and plates incubated for an hour at room temperature. The plates were washed again, and 50 µl of substrate (3, 3', 5, 5' tetra methyl benzidine) was added for 10 minutes for tetanus toxoid antigen, and for 12 minutes for C. *novyi* B toxoid antigen. Colour development was stopped with the addition of 25 µl of 0.5 M H_2SO_4 and plates read at 450 nm on a plate reader. The mean of all the negative control duplicates from each plate was subtracted from the mean of all duplicate test and positive control serum samples from the same plate. Results were expressed as a percentage of the response of the positive control serum for each plate. Test samples recorded as less than 1% of the positive control serum were considered to be zero.

Statistical analysis was conducted in SAS version 8 (SAS institute, New York). Correlations were determined using the correlation procedure of SAS and statistical models were fitted using the general linear model (GLM) procedure in SAS. All explanatory variables, except sire, were fitted as covariates. Models were constructed with the result at 13, 16 and 22 weeks for each assay as the dependent variable. Explanatory variables included in the model were the response at 10, 13 and 16 weeks (for the appropriate assay) as well as live weight, sire and age at the time of sample collection.

RESULTS

The mean antibody response for both assays peaked between 13 and 22 weeks, and was declining by week 22 (Figure 1). The response variance between animals was greatest at weeks 13 and 22. Although direct comparison of the assay methods is strictly not valid, on average the pattern of immune response was similar between the two assays. The responses to *C. novyi* were generally lower than those for *C. tetani*.

FIGURE 1: Mean antibody response (standard deviation) to *C. novyi* B (-----) and *C. tetani* (—) toxoids in heifers before a primary and second vaccination with a '5 in 1' clostridial vaccine at 10 weeks and 13 weeks respectively and at 16 and 22 weeks of age.

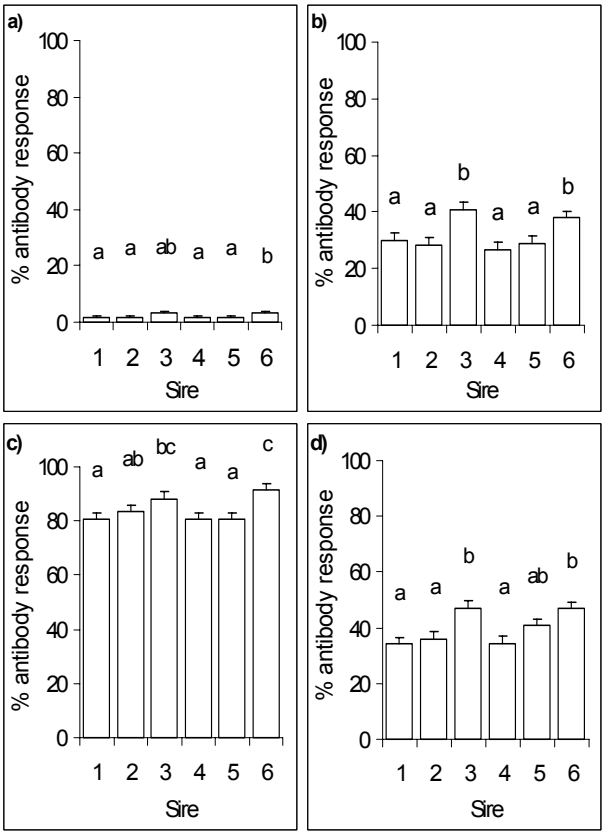


Significant correlations ($p<0.05$) between assays were identified for all weeks (wk 10, r=0.34; wk 13, r=0.66; wk 16, r=0.65; and wk 22, r=0.66). The within assay correlation between weeks was also determined and the results are displayed in Table 1.

TABLE 1: Within assay correlations (R value) between weeks for response to *C. novyi* B and *C. tetani* toxoids.

		13 wks	**16 wks**	**22 wks**
C. novyi	10 wks	0.16	0.01	0.001
	13 wks		0.32	0.40
	16 wks			0.66
C. tetani	10 wks	0. 10	0.08	0.19
	13 wks		0.40	0.44
	16 wks			0.60

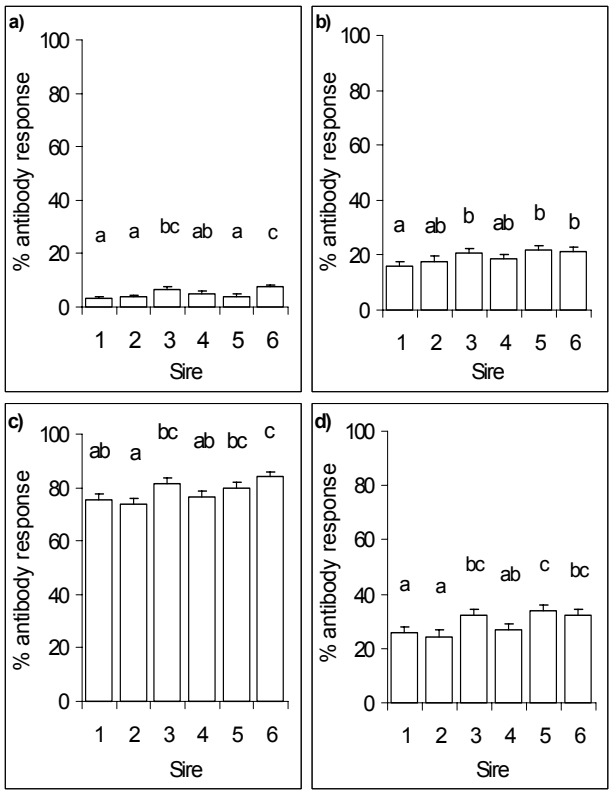
FIGURE 2: Percentage antibody (AB) response to C. *tetani*, by sire at a) 10 weeks, b) 13 weeks, c) 16 weeks and d) 22 weeks of age (n=469). Letter superscripts for individual columns which do not correspond to letters for other columns indicate a significant difference ($P<0.05$).



A number of the heifers showed relatively high antibody response prior to primary vaccination (10 week), followed by only a small increase at 13 weeks and there was a reduction in variability between week 10 and 13. In order to identify the effect of this change on the trial data, the dataset for each antigen was split into two categories; animals that were elevated (>10% antibody response compared to the control) at 10 weeks of age and animals that were not elevated (<10% antibody response compared to the control) at 10 weeks of age. The significant difference (elevated 83% greater then the non-elevated, P=0.001) between the means for the >10% and the <10% subgroup at 10 weeks of age, reduced to and remained non-significant for the rest of the trial period for *C. novyi* (16%, 3% and 0.1% for 13, 16, and 22 weeks respectively). *C. tetani* on the other hand retained a significant difference ($P<0.001$) between the elevated and low groups for the duration of the trial (94%, 35%, 11%, and 32% for 10, 13, 16, and 22 weeks respectively).

Analysis of the antibody response of the six sires revealed a significant difference in the response of two of the sires (3 and 6) compared to the remaining four sires at all time points for *C. tetani* (Figure 2) and at 10 and 16 weeks for *C. novyi* (Figure 3). Offspring of sires 3 and 6 in particular, stood out as exhibiting desirable immune characteristics in this population.

FIGURE 3: Percentage antibody (AB) response to C. *novyi*, by sire at a) 10 weeks, b) 13 weeks, c) 16 weeks and d) 22 weeks of age (n=469). Letter superscripts for individual columns which do not correspond to letters for other columns indicate a significant difference ($P<0.05$).



A general linear model (SAS) was fitted to the data to identify the variables contributing the majority of the variation in antibody response and more clearly define the sire effect. Significantly, sire (P=0.001) accounted for most of the variation at 13 weeks for *C. tetani*, while at weeks 16 and 22 nearly all variation was accounted for by the antibody response in the preceding weeks

TABLE 2: Explanatory variables for the variation seen in the antibody response at 13, 16 and 22 weeks with 10, 13, 16 weeks and age fitted as covariates and sire as a dependent variables. T represents response to *C. tetani* toxoid and N represents response to *C. novyi* B toxoid.

Dependant		**Explanatory variables – P values**				
variable	R^2	Sire	10 wks	13 wks	16 wks	Age
13 wks T	0.02	0.0019	0.12			0.92
16 wks T	0.18	0.13	0.61	<0.0001		0.52
22 wks T	0.44	0.1	0.0008	<0.0001	<0.0001	0.19
13 wks N	0.03	0.07	0.0034			0.1
16 wks N	0.13	0.01	0.07	<0.0001		0.79
22 wks N	0.12	0.23	0.5	<0.0001	<0.0001	0.02

(P<0.001). Age was not a significant factor (Table 2). The model predicted a similar trend in *C. novyi*, however, the sire effect was evident at 16 weeks (P=0.01). The proceeding weeks accounted for the majority of the variation at 13, 16 and 22 weeks for *C. novyi* (P<0.003). There was a small but significant effect of age on the response at 22 weeks (P=0.02).

DISCUSSION

The overall antibody response to both the toxoids followed classical models of humoral immune response. Although, the difference between the mean responses to the two toxoids was not significant, many of the correlations between different time points and sire effects were different. However, there were a number of individual animals (~20) with dramatically different response profiles from the mean. The existence of these extreme animals, the large variation observed among animals that fit the classic response profile, combined with the highly controlled experimental conditions, suggests that factors other than environment are playing a role in the up-regulation of the antibody response.

Correlation analyses between weeks showed a weak correlation between the antibody response at 10 weeks. Correlations became stronger towards the end of the trial period, which reflected the acquisition of immunity to the antigens at 13 and 16 weeks of age influencing subsequent antibody responses.

Some animals were found to have an elevated antibody response to *C. tetani* or *C. noyvi* at 10 weeks of age, prior to vaccination. The maternal transfer of antibodies in colostrum has long been demonstrated in sheep (Oxer *et al.* 1971). The vaccination status of the dams of the trial animals was not ascertained, so the antibody response that was present in some animals in the current trial prior to vaccination may have been due to previous maternal response to these commonly used clostridial antigens or natural exposure. Heifers that showed a relatively high antibody response to both toxoids prior to primary vaccination (10 week), but then exhibit a significant reduction in variability between week 10 and 13, most likely reflects an inhibitory effect of maternal antibodies. The presence of maternal antibodies in juvenile stock has been shown to block the induction of an antibody response in vaccinated calves (Ellis *et al.* 2001, Lemaire *et al.* 2001). The clear differentiation between animals exposed to the antigens prior to the trial and those naive to the antigens, makes fitting a model to remove maternal effects on the primary response relatively easy, however estimating the effect of maternal antibodies on the secondary antibody response is not so clear. By splitting the data sets into elevated (>10%) and low (<10%) categories it was possible to differentiate the effect of this early elevation across the trial period. For *C. novyi* the difference between the elevated and low groups reduces between 10 and 13 weeks and is not significant at 16 and 22 weeks indicating that the maternal effect may have blocked the primary antibody response but did not affect the secondary response. This is borne out by a seven fold increase in the antibody response in the <10% group between week 10 and 13 for *C. novyi*, while only a two fold increase is seen in the antibody response for the >10% group over the same period. For *C. tetani*, however the difference between the elevated and low groups remains significant throughout the trial period, which indicates that a slightly different mechanism for T-cell activation or antibody production may be operating in response to *C. tetani*. This suggests that some calves may have been actively immunized by natural exposure to tetanus antigens prior to vaccination (10 weeks of age) rather than acquiring antibody solely by maternal transfer. The variability in the distributions between the six sires at 22 weeks was similar between the two assays suggesting that despite the maternal effects observed between weeks 10 and 16, it would not impact significantly on the effectiveness of the vaccine.

Despite the previous observation there is strong evidence to suggest that some animals simply fail to up-regulate any significant response to vaccination. In fact, at 22 weeks, 15 heifers failed to stimulate an antibody response above 5% of the positive control for each antigen and of those heifers, five failed to respond to both antigens. A further nine heifers failed to respond above the 10% level against both the antigens at the

same age. Alternatively, the vaccination technique may have been faulty for these calves or they had missed a vaccination.

Analysis of the antibody response of the six sires revealed a moderate sire effect on the magnitude of the response to both antigens. The effect ranges between 2-5% from the group mean, which is in line with recent research on other pathogens (Nielsen *et al*. 2002). The result, although significant may not be purely a function of the magnitude of the immune response in these animals. An alternative explanation for this variability is that the between animal variations in the speed of up-regulation of the antibody pathway could result in a phase shift in the response profile. Further detailed investigation will be required to determine this.

Data on the incidence of infectious disease in this herd is currently being collected. This data will be used to determine if the observed animal differences in antibody response correspond to the incidence of clinical diseases such as mastitis. The long term aim of this research is to identify QTL and eventually genes for increased resistance to disease. The results of this trial clearly demonstrate a high level of variability around the antibody response of dairy heifers before and after a clostridial vaccination programme. Results such as those by Wagter *et al*. (2003) indicate that selection for such traits may not conflict with current milk yield breeding objectives and in younger stock may even enhance milk solids production.

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