

Sire effects on antibodies to nematode parasites in grazing dairy cows

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Abstract A study was carried out on New Zealand dairy cows at pasture, to test for evidence of genetic differences in immunological response to nematode parasites. Nine widely used Holstein-Friesian artificial insemination bulls, with daughters in many herds, were evaluated for nematode antibodies by sampling milk of their daughters in mid lactation in each of 20 North Island herds. One milk sample was taken from each cow (ranging from 4 to 11 years of age), during a routine herd test in the period from mid November 2000 to early February 2001. Assays were undertaken subsequently on pooled samples of skim milk (0.2 ml per cow; up to 20 cows per sire × herd) to assess antibodies to both the infective third larval (L3) and adult parasitic stages of *Cooperia oncophora* and *Ostertagia ostertagi*. Sire effects were significant for all four antibody types ($P < 0.001$). For *O. ostertagi* L3, there was a 1.20-fold range in the mean antibody levels of the sire groups; corresponding proportional ranges for the other three antibody types were from 1.22 to 1.23. The correlations of sire means among the four antibody types were high, averaging 0.81 (range 0.65–0.99). These results show that significant sire effects on

anti-parasite antibody levels were present in mature lactating dairy cows at pasture in New Zealand, with repeatabilities of sire means across herds of 0.29–0.39, and that sire effects on antibody levels to two different nematode parasite species were highly correlated (for antibodies to either the L3 or the adult stages).

Keywords antibody; nematode; genetics; cattle

INTRODUCTION

Controlling nematode parasites can lead to improved milk production in New Zealand dairy cows (Bisset 1994). The obvious cost of nematode parasitism is in the anthelmintic treatments used (c. NZ\$14 million annually; Nielsen 1997), but the real cost also includes the lost production from subclinical parasitism, as quantified in milking cows by Bisset et al. (1987a,b). In calves the effects have been quantified in terms of growth rates (Bisset 1994), but the most important effects in calves are likely to be seen in terms of their subsequent milk production, either directly or as a result of failing to conceive.

Since 1980, when the first case of anthelmintic resistance on a New Zealand sheep farm was reported (Vlassoff & Kettle 1980), the prevalence of drench resistance has increased dramatically and now “anthelmintic resistance in New Zealand [sheep farms] is common throughout the country” (McKenna et al. 1995). Fortunately, this situation has not progressed as far on New Zealand dairy or beef farms (Vermunt et al. 1995; McKenna 1996), but there is the potential for drench resistance in cattle, with the expansion of dairy bull beef production (Bisset 1994; McKenna 1996), and with farmers being encouraged to drench in-calf or lactating dairy cows through access to anthelmintic products with nil withholding time.

Alternative methods of dealing with parasitism would be required if drench resistance became widespread on individual farms or across a district. One such method is the genetic approach. In sheep,

flocks have been genetically selected for resistance to nematode parasite infection. This is measured by faecal worm egg count (FEC), which is a heritable trait in lambs (Morris et al. 1995) and in peri-parturient ewes (Morris et al. 1998). For example, selection responses have been achieved in New Zealand in an experimental Romney flock (Morris et al. 2000) and in an experimental Perendale flock (Morris et al. 1997), where the predominant nematode genera in both cases were gastro-intestinal *Trichostrongylus* and *Ostertagia*. Responses have also been achieved in Australia in an experimental Merino flock (Woolaston & Piper 1996), where the predominant nematode species was the blood-sucking parasite *Haemonchus contortus*. Subsequent studies were carried out on the Romney flock, mentioned above, to measure anti-parasite antibodies in lambs and to relate the levels to their FEC data; antibody levels were heritable (Douch et al. 1995a,b), differences in antibody levels were demonstrated between FEC selection flocks grazed together, genetic correlations of -0.41 and -0.48 were found between antibody and FEC (Douch et al. 1995a), and rank correlations between antibody level (infective third larval (L3) stage) and total worm count (-0.56) and between adult antibody level and total worm count (-0.63) were found (Bisset et al. 1996).

Although there have been dairy-cattle host studies of nematode parasitism under temperate pasture-based farming systems (e.g., Agneessens et al. 2000), these have generally not concentrated on host genetic factors. We believe that three genetic components are relevant and need to be studied: (1) host resistance to nematode infection; (2) genetic differences in the host's ability to mount an immune response to nematodes in calves or cows; and (3) genetic differences in the host's ability to show resilience or production under challenge.

The objective of the present paper is to report an exploratory study of (2) above, by investigating genetic differences among over 2000 mature, mid-lactation cows. The present data set arose as a spin-off from a larger study which involved milk sampling c. 4100 cows.

MATERIALS AND METHODS

Trial design

The study was carried out on 20 large dairy herds in the upper North Island of New Zealand. Nine widely used Holstein-Friesian artificial-insemin-

ation bulls (part of the Livestock Improvement Corporation's team of Premier Sires bulls (proven sires), with daughters in many herds), were evaluated by sampling up to 20 of these daughters in each herd (average = 13.8 daughters per sire \times herd, minimum = 4 per sire \times herd). Over all herds, 115–333 daughters per sire were sampled for the study. Over all sires, 21–168 daughters per herd were sampled, with an average of 104 per herd; only 4 herds were represented by fewer than 70 daughters each. In all, 2072 samples were collected. There were 150 of the potential 180 sire \times herd pooled samples from the 9 sires and 20 herds. The remaining 30 sire \times herd combinations represented missing subclasses of daughters across the herds under study. The nine bulls in the study were themselves the sons of six sires.

The herds were spring calving, grazing mainly pasture, year-round; cows were in mid-lactation, averaging about 4 months post-calving. The average size of the herds in the study was c. 750 cows, over 3 times the national average, and a restricted number of cows from within each herd was included here from 9 pre-selected sire groups. A single sample of milk was taken from each selected cow during a routine herd test in the period from 17 November 2000 to 6 February 2001. Milk samples for this trial were only from mature cows ranging from 4 to 11 years of age. Over all herds, percentages of cows in each age group were: 4-year-olds 21%, 5-year-olds 20%, 6-year-olds 11%, 7-year-olds 21%, 8-year-olds 19%, 9-year-olds 6%, 10- and 11-year-olds 2%; one 2-year-old was sampled in error. The unusual age distribution (e.g., few 6-year-olds) reflected the particular choice of the nine sires for the study and the years in which they were heavily used after selection as proven sires.

Processing of milk samples

Milk samples were left to stand in 50 ml tubes at 4°C overnight for the cream to separate. Samples were then centrifuged and the cream was collected from the top of each tube for other studies, leaving skim milk below. A sample of 0.2 ml of skim milk per tube was taken from the cows in each pre-selected sire group, up to a maximum of 20 cows' samples per sire group per herd. These samples were pooled, giving a maximum of 4.0 ml of skim milk per sire. The process was repeated in each herd.

Antibody concentrations were analysed using the method described by Douch et al. (1994), with modifications. Briefly, ELISA plates were coated

with L3 excretory/secretory and adult (Ad) somatic antigens of *Cooperia oncophora* (*Co*) or *Ostertagia ostertagi* (*Oo*) (i.e., four combinations of nematode species and L3/Ad stages). Milk samples (diluted 1:40) were added to the wells, and horse-radish peroxidase conjugated Sigma rabbit anti-bovine immunoglobulins (Cat. no. A-5295) at 1:4000 dilution were used with 3, 3', 5, 5'-tetramethylbenzidine and H₂O₂ to detect the bound antibody. Absorbance of the resultant colour reaction was determined at 450 and 630 nm using a dual wavelength plate reader. Each serum sample was assayed in triplicate and results expressed as mean absorbance in optical density units. Standard serum samples were included in each ELISA plate and results were corrected multiplicatively to each standard, both between plates within any one assay run, and between assay runs.

Data analyses

Data on each of the four antibodies (*Co* L3, *Oo* L3, *Co* Ad, and *Oo* Ad) were tested for normality. The two sets of L3 antibodies each showed a normal distribution. However, the two sets of adult antibodies showed distributions with significant tails to the right, so they were transformed to natural logarithms for analysis. The data (150 pooled samples representing sire \times herd combinations, comprising 9 sires, 20 herds, and 30 empty subclasses) were analysed using the JMP package from SAS (1995), fitting fixed effects for sire and herd. Herd means were calculated which were adjusted for sire, and vice versa. An assumption in the analyses was that cows, and therefore also their sires, could be compared genetically for antibodies to parasites on the basis of antibody concentrations per ml of milk; differences in milk yield were not accounted for.

Because of the pooled samples, the error term and the standard deviations (SDs) were obtained from the sire \times herd level in the analysis. For reporting the SDs for the adult-antibody traits (which had been analysed on a log scale), they were converted to the original scale of measurement using the product of the SD on the log_e scale (equivalent to a coefficient of variation) and the overall mean on the original scale. On the assumption of heritabilities of 0.2, 0.3, or 0.4 for each of the four antibody traits, it would be necessary to multiply the pooled-sample SDs by 2.91, 2.66, or 2.47 to be equivalent to phenotypic SDs for individual-cow records (where sire \times herd means consisted of 14 progeny each).

Correlations were calculated between adjusted herd means for each antibody concentration and adjusted herd means for test-day milk yield. Correlations were also calculated between adjusted sire means for all six pairwise combinations of antibody concentrations, both on a weighted and unweighted basis. Unadjusted sire \times herd means for antibody levels to *Co* were plotted against corresponding values for *Oo*. This was done for antibodies to the L3 and the Ad stages.

A repeated-record restricted maximum likelihood (REML) analysis was used (Gilmour 1997) to estimate the repeatability of sire means across herds. The model included herd as a fixed effect, sire as a random effect, with ancestors in a relationship matrix.

RESULTS

Herd means

Herd effects were significant for all four antibody types ($P < 0.001$). There was a range of herd means (adjusted for sire) for each of the four antibody traits (Table 1). For the antibodies to *Co* L3 and to *Oo* L3, there was a 27% difference between the highest and lowest herd means. The correlation between herd means for antibodies to the L3 types was 0.94, and the correlation between the two adult parasite antibodies was 0.70, probably indicating that herds showed consistently high or low levels of antibody to these parasitic challenges. From herd means for test-day milk yield, plotted against herd means for antibodies, negative correlations were found in all four cases: *Co* L3 -0.25, *Oo* L3 -0.37, *Co* Ad -0.29, and *Oo* Ad -0.36.

SDs at the bottom of Table 1 are shown for pooled samples per sire \times herd. On the assumption of heritabilities of 0.2, 0.3, or 0.4 for each trait, coefficients of variation of individual cows' values were 0.18–0.24 for the antibodies to L3 parasites, and 0.20–0.33 for the antibodies to Ad stages.

Sire means

Sire effects were significant for all four antibody types ($P < 0.001$). There was a range of sire means (adjusted for herd) for each of the four antibody traits (Table 1). For the antibody to *Oo* L3, there was a 20% difference between the highest and lowest sire means, and corresponding ranges of sire means for the other three antibody types were 22–23%.

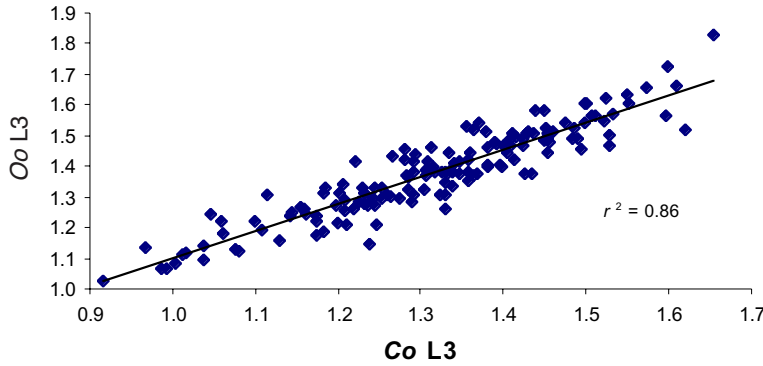


Fig. 1 Unadjusted sire \times herd means for antibody levels to *Cooperia oncophora* (Co) and *Ostertagia ostertagi* (Oo) infective third larval (L3) stages.

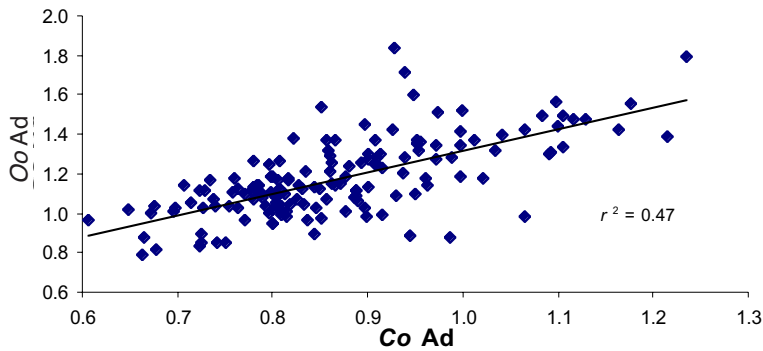


Fig. 2 Unadjusted sire \times herd means for antibody levels to *Cooperia oncophora* (Co) and *Ostertagia ostertagi* (Oo) adult worm stages.

Correlations among the nine adjusted sire-group means were high for each of the four antibody types studied (Table 2), ranging from 0.65 to 0.99, with an average of 0.81 from the weighted analyses. Repeatability estimates for sires were 0.35 ± 0.14 for Co L3, 0.39 ± 0.15 for Oo L3, not estimable for Co Ad, and 0.29 ± 0.13 for Oo Ad. Results from all 150 raw measurements for Oo L3, plotted against those for Co L3 (Fig. 1), gave an r^2 value of 0.86; the corresponding r^2 value for antibodies to the two adult worm stages was 0.47 (Fig. 2).

DISCUSSION

Herd means

Large, significant herd differences were found in this study, which were consistent across the sires used to generate daughters within each herd. At a bulk-tank level, Guitian et al. (2000) found similar results in that their herd-to-herd differences for Oo antibodies were consistent across sampling months. “Herd” effects could include effects of herd management, district, nutrition, culling, and time,

Table 1 Antibody results (optical density units) by herd and sire for four anti-parasite types, comprising *Cooperia oncophora* (Co) and *Ostertagia ostertagi* (Oo) infective third larval (L3) and adult (Ad) types; Ad data were back-transformed from \log_e values.

Parameter	CoL3	OoL3	CoAd	OoAd
Overall mean	1.314	1.381	0.861	1.165
Ratio, highest:lowest herd means	1.270	1.270	1.480	1.620
Best sire	1.431	1.493	0.974	1.330
Ratio, highest:lowest sire means	1.220	1.200	1.230	1.230
Standard deviation of pooled samples	0.107	0.099	0.071	0.132

as well as consistent animal differences among herds. There was potentially an inequality of variances across sire \times herd subclasses, because of uneven numbers of daughters' milk samples represented in each pooled sample. However, comparison of sire \times herd means on a weighted and unweighted basis showed little effect of inequality on the correlations observed (Table 2).

A 12-month sample of digestive tracts from adult dairy cows processed through a Belgian abattoir after a pre-slaughter grazing period showed a close relationship between antibodies to adult *Cooperia* and *Ostertagia* species (reported as $r^2 = 0.52$ ($r = 0.72$) Agneessens et al. 2000). Results in the present study were quite similar, with a correlation of 0.70 among herd means for adult *Cooperia* and *Ostertagia* species. This probably reflects herd-to-herd differences in the parasitic challenge from pasture or the herds' history of exposure, and hence different burdens of adult parasites within the cows. Bisset et al. (1987b) showed that parasitic management had significant effects on milk production, particularly where cows had been overwintered on pastures where calves had grazed previously. The study of Agneessens et al. (2000) also showed that 65% of cows had nil or low *Ostertagia* worm burdens in the abomasum, 20% had moderate burdens (5000–10 000 worms), and 15% had high burdens.

The high correlation between the antibody responses to *Cooperia* and *Ostertagia* L3 antigens reflects that a continuing larval challenge is present to stimulate immunity. The correlation between the antibody responses to *Cooperia* and *Ostertagia* adult antigens are a reflection of the worm burden carried. It has been shown in New Zealand (Bisset & Marshall 1987) and in Europe (Agneessens et al. 2000; Borgsteede et al. 2000) that the burden of *Ostertagia* is more consistent than *Cooperia*.

The coefficients of variation for the L3 stages (0.18–0.24 in these data) can be compared with values of 0.30–0.38 obtained from antibodies to the

L3 stages of four nematode parasite species in sheep (Douch et al. 1995b).

Sire means

Large, significant, adjusted sire-group differences were found, with the highest versus lowest sire group representing a difference in mean antibody level of at least 20% of the mean. These reflected important genetic differences, especially since they were consistent for the sires from herd to herd, and also across antibody types. In sheep, Douch et al. (1995a,b) found that sire effects on antibody levels to nematodes showed significant heritabilities, and Douch et al. (1995b) observed high genetic correlations (averaging 0.84) among the host levels of antibodies to four different nematode species in sheep. In beef calves, Gasbarre et al. (1990) also found that host differences in FEC were reflected in worm-count differences in five different nematode species.

Gasbarre et al. (1993) reported heritability estimates of 0.7–0.8 for anti-parasite antibody levels, based on the progeny of 21 United States Angus sires (an average of 9 calves per sire), and in purebred Friesian calves in Holland, significant sire effects were found for antibody levels following an artificial challenge with *Cooperia* spp. larvae (Kloosterman et al. 1978). All these results are consistent in showing significant sire variation in anti-parasite antibody levels. Most of these references were in young stock, but our data show that significant host genetic variation in antibody level (sire-group effects) was also present in daughters when they were mature dairy cows in mid lactation at pasture (animals generally considered to be relatively refractory to gastro-intestinal parasites).

Esdale et al. (1986) demonstrated that it is possible (at least in beef cattle) to select to change FEC (as has been described earlier for sheep), and Gasbarre et al. (1990) have shown sire effects on both FEC and antibody levels in calves.

In conclusion, it seems that genetic selection for lower FEC and/or higher antibody levels is feasible in cattle, if it were considered necessary. The expected direct responses to selection are still likely to persist in adult cows at pasture, but correlated responses in a wide range of production and disease traits also need to be evaluated.

Table 2 Correlations among adjusted sire means (nine sires) for antibody traits; unweighted values are in parentheses if different from the weighted values.

Antibody	CoL3	OoL3	CoAd	OoAd
CoL3	1			
OoL3	0.99 (0.98)	1		
CoAd	0.65 (0.70)	0.66	1	
OoAd	0.88 (0.84)	0.91 (0.86)	0.76	1

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