Short communication: Genetic and nongenetic factors influencing *Ostertagia ostertagi* antibodies in UK Holstein-Friesian cattle

C. Hayhurst,^{*1} A. Bradley,[†] A. B. Forbes,[‡] K. Hunter,^{*} and M. D. Royal^{*}

^{*}Department of Veterinary Clinical Sciences, University of Liverpool, Leahurst, Neston, South Wirral, CH64 7TE, United Kingdom

[†]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, United Kingdom; Quality Milk Management Services Ltd., Unit 1, Lodge Hill Industrial Park, Station Road, Westbury-sub-Mendip, Nr Wells, Somerset, BA5 1EY, United Kingdom

[‡]Meril SAS, 29 Avenue Tony Garnier, Lyon 69007, France

ABSTRACT

The purpose of this study was to estimate and discuss the genetic variation, heritability, and effects of nongenetic factors on the ability of Holstein-Friesian cows to produce an immune response by producing IgG antibodies to *Ostertagia ostertagi*. Total IgG (IgG₁ and IgG₂) antibody levels were determined using an ELISA and measured using optical density ratio ($ODR = \frac{OD_{\text{sample}} - OD_{\text{negative control}}}{OD_{\text{positive control}} - OD_{\text{negative control}}}$) from milk samples collected from 1,276 Holstein-Friesian cattle in 229 commercial dairy farms from 2002 to 2004 during their first (82%) and other (2 to 12) lactations. A sire ($n = 461$) model was fitted to the ODR data using ASREML software, and variance components were estimated. The ability to produce *O. ostertagi* antibodies as measured by ODR had a heritability of 0.13 ± 0.12 , and both season of sample and herd had a significant effect on total IgG levels. To conclude, this study has ascertained that genetic variation is present in the ability of dairy cows to mount an immune response to the parasite *O. ostertagi*. Inasmuch as evidence exists that IgG is linked to protective immunity against the parasite via a reduction in its reproductive ability, this trait may be of potential interest to genetic selection programs as an aid to reduce the effect of *O. ostertagi* in dairy herds.

Key words: heritability, *Ostertagia ostertagi*, antibody, susceptibility

Genetic selection in dairy cows has, in the past, focused largely on productivity and product quality, often at a cost to health and welfare (e.g., Royal et al., 2002; Miglior et al., 2005). Although today's selection indices are much broader, several areas of interest, specifically for health and welfare, still require investigation.

Subclinical gastrointestinal (GI) nematode infections are one such area of concern to the dairy industry, because they can result in biologically and economically significant losses in milk production (Gross et al., 1999; Sanchez et al., 2004a). The abomasal parasite *Ostertagia ostertagi* is one of the most widespread and important parasites of cattle in temperate regions. Infection by most GI nematodes eventually leads to a level of protective immunity after some months of grazing. However, immunity to *O. ostertagi* develops more slowly and 2 full grazing seasons are required before immunity is considered complete (Claerebout and Vercruysse, 2000). Nevertheless, this immunity is not sterile and adult cows continue to harbor burdens of this parasite (Agneessens et al., 2000).

The predominant immunoglobulin involved in the humoral immune response to GI nematodes is IgG (Canals and Gasbarre, 1990; Gasbarre et al., 1993; Sanchez et al., 2004b). Total IgG in serum has been related to acquired immunity to *O. ostertagi* in cattle (Kloosterman et al., 1984), and animals with a greater IgG titer have fewer and shorter worms with fewer ova per female, and more females with reduced vulval flaps (Kloosterman et al., 1984), suggesting a route for protective immunity via inhibition of parasite reproductive success. The role of IgA response in immunity to *O. ostertagi* in cattle is yet to be resolved.

The aim of this work was to estimate the genetic variation, heritability, and effects of nongenetic factors on *O. ostertagi* specific total IgG concentration in milk in the UK dairy cattle population. Additionally, the genetic correlations of *O. ostertagi* with fertility and production parameters were estimated.

Milk samples were collected between 2002 and 2004 from UK Holstein-Friesian dairy cows ($n = 1,276$) by National Milk Record technicians during the routine milk recording process (National Milk Record, Chippenham, UK; Table 1). Milk samples were preserved with bronopol and frozen (-20°C) until laboratory analysis. Animals were selected according to sire with the aim of achieving 10 to 20 daughters per sire. Sires

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¹Corresponding author: catherine.hayhurst@liverpool.ac.uk

Table 1. Number of samples collected and mean optical density ratio (ODR)¹ for each year and season in the full data set

Year	Season	Samples (n)	ODR (mean $\pm$ SD)
1	December to February	107	0.496 $\pm$ 0.251
	March to May	382	0.596 $\pm$ 0.272
	June to August	300	0.794 $\pm$ 0.270
	September to November	248	0.785 $\pm$ 0.227
2	December to February	170	0.576 $\pm$ 0.256
	March to May	63	0.666 $\pm$ 0.246
	September to November	1	0.343
3	December to February	4	0.675 $\pm$ 0.356
	March to May	1	0.530
Total		1,276	0.672 $\pm$ 0.279

$$^1\text{ODR} = (\text{OD}_{\text{sample}} - \text{OD}_{\text{negative control}}) / (\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}}).$$

were simply those that were widely in use at the time of the study, and a large proportion was undergoing progeny testing (thus helping increase half-sib family sizes). Animals were distributed over 229 commercial dairy herds in the UK, and between 2 and 23 samples were collected per herd (mean $\pm$ SD: 5.13 $\pm$ 4.14). To accurately account for the potential herd effect, extra sires with smaller half-sib family sizes were included as contemporaries in many herds. The majority of milk samples were collected from heifers during the first lactation ($n = 1,068$). Samples were taken up to 379 d postpartum (mean $\pm$ SD: 23.6 $\pm$ 24.8 d). All animals had been managed by grazing, and thus were assumed to have been naturally exposed to the parasite *O. ostertagi*.

Before analysis, milk samples were thawed and centrifuged (1,500 $\times g$, 3 min) and the whey was removed. Anti-*O. ostertagi* antibody level (total IgG₁ and G₂) was determined by using a solid-phase indirect ELISA developed by Svanova (Svanova Biotech AB, Uppsala, Sweden) in conjunction with Ghent University (Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University; Charlier et al., 2005). The results were displayed as the optical density ratio (ODR) calculated as follows:

$$\text{ODR} = \frac{\text{OD}_{\text{sample or control}} - \text{OD}_{\text{negative control}}}{\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}}}.$$

The distribution of ODR was slightly skewed (coefficient of skewness -0.249). Several transformations were investigated [$\log_{10}(x)$, square root(x), $1/(x)$, or $(x)^2$] but did not decrease the skewness; therefore, the data were not transformed.

A univariate mixed model was fitted to the ODR data using ASREML software (Gilmour et al., 2006). Various fixed, random, and nested effects (herd, area of

country, year, month and season of sample, time of sample, parity, and days postpartum) were systematically investigated in the model. The final model included the fixed effects of herd ($n = 229$) and season of sample ($n = 4$), and the random effect of sire of animal ($n = 461$; mean $\pm$ SD: 2.76 $\pm$ 2.99 daughters per sire, range of 1 to 27). Analysis of the data with a full animal model was not possible because of data limitations (lack of dam identification and some sire pedigree information). Thus, only the most simple sire model was used. The final model was as follows:

$$Y_{ijkm} = \alpha + H_i + M_j + S_k + \varepsilon_{ijkm},$$

where Y_{ijkm} = observation of ODR for animal m in herd i , season j , and with sire k ; α = intercept; the fixed effects were H_i = herd ($i = 1$ to 229) and M_j = season (j = December–February, March–May, June–August, and September–November); and the random effects were S_k = random effect of sire k [distributed $N(0, \sigma_s^2)$] and ε_{ijkm} = random error term [distributed $N(0, \sigma_e^2)$].

Heritability (h^2) was calculated as

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2},$$

where σ_s^2 = sire variance and σ_e^2 = residual variance.

The significance of the fixed effects was assessed using Wald F -statistics obtained from ASREML. The significance of the variance components was determined by the likelihood ratio test by removing the component from the full model and comparing $-2 \times$ change in likelihood to the chi-squared statistic with 1 degree of freedom. A P -value < 0.05 was considered statistically significant.

The heritability and variance component estimates are shown in Table 2. The ODR was heritable and the estimate was significantly different from zero ($h^2 \pm \text{SE}$: 0.13 $\pm$ 0.12; $P < 0.05$). Although previous genetic analyses of anti-*O. ostertagi* antibody levels in milk have not been carried out, the estimate in the present study is comparable to estimates of antibody response in serum (anti-*Escherichia coli* and ovalbumin $h^2 = 0.13$ to 0.88; Wagter et al., 2000; anti-*Mycobacterium avium* ssp. *paratuberculosis* $h^2 = 0.09$ to 0.15; Gonda et al., 2006; anti-*M. avium* ssp. *paratuberculosis* $h^2 = 0.09$ to 0.13; Hinger et al., 2008). Furthermore, the present study confirms that sire and herd have a significant effect on *O. ostertagi* antibody level, as found in a smaller study ($n = 9$ sires) using pooled milk samples per sire in New Zealand dairy cattle (Morris et al., 2002).

The effect of days postpartum, area of the country, parity, sample time (a.m. or p.m.), sample month, and year on ODR were not significant (following full investigation in the model) and therefore were not included in the final univariate model. Season had a significant ($P < 0.0001$) effect, with ODR being the largest during the summer months (June to August) and smallest in winter (December to February). Previous studies have reported a seasonal pattern in antibody levels (Agneessens et al., 2000; Charlier et al., 2007). This pattern is thought to reflect the ingestion of infective larvae, which are present on pasture and whose concentrations typically increase throughout the grazing season.

The effect of herd was significant ($P < 0.0001$) and accounts for many possible sources of variation including diet, herd size, management techniques including anthelmintic treatment, and level of parasite exposure. *Ostertagia ostertagi* is present in most, if not all, dairy herds in which the cows have access to pasture or outside exercise areas (Charlier et al., 2005; Forbes et al.,

Table 2. Variance components and heritability (h^2) estimate plus standard error and significance for optical density ratio (ODR)¹

Variance component ²	Variance	$h^2 \pm \text{SE}$
σ_a^2	0.0064	0.127* $\pm$ 0.118
σ_p^2	0.0505	
σ_e^2	0.0489	

¹ODR = (OD_{sample} - OD_{negative control}) / (OD_{positive control} - OD_{negative control}).

² σ_a^2 = additive genetic variance ($4 \times$ sire variance); σ_p^2 = phenotypic variance; σ_e^2 = residual variance.

* $P < 0.05$.

2008). However, there are several sources of variability due to, among other things, geographical location and related factors such as climate, grazing management, and stocking density (Charlier et al., 2005; Fox et al., 2007; Forbes et al., 2008). Figure 1 shows distribution of herds and ODR across geographic regions of the United Kingdom.

Area	N	Mean ODR $\pm$ sd
North West	340	0.685 $\pm$ 0.296
North East	15	0.592 $\pm$ 0.239
Yorkshire	91	0.577 $\pm$ 0.266
East Midlands	61	0.594 $\pm$ 0.277
East of England	60	0.629 $\pm$ 0.270
South East	96	0.666 $\pm$ 0.273
South West	265	0.713 $\pm$ 0.257
West Midlands	193	0.658 $\pm$ 0.294
Wales	182	0.699 $\pm$ 0.256

Figure 1. Map showing geographical regions in the United Kingdom and the associated mean optical density ratio (ODR; mean $\pm$ SD; before fitting the univariate model), where ODR = (OD_{sample} - OD_{negative control}) / (OD_{positive control} - OD_{negative control}).

The possible use of genetic variation in *O. ostertagi* total IgG antibody response in genetic selection depends on the relationship between antibody level and other important traits such as milk production, but also insofar as they may reflect a level of protective immunity against *O. ostertagi* (i.e., parasitic resistance or resilience). As discussed earlier, total IgG levels in serum have been related to acquired immunity to *O. ostertagi* in cattle (Kloosterman et al., 1984), and animals with a greater IgG titer have fewer and shorter worms with fewer ova per female, and more females with reduced vulval flaps (Kloosterman et al., 1984).

Work by Gasbarre et al. (1990) suggested that IgG₁ and IgG₂ antibodies are the most accurate indication of a calf's immune system response to parasite infection. As IgG₁, IgG₂, and eggs per gram (EPG) are under genetic control and acquired after exposure to parasites, the immune response, measured as IgG₁ and IgG₂, may also be associated with EPG levels. Furthermore, differing antibody levels have been found in young cattle according to different levels of infection; that is, high antibody levels in the high exposure group. However, within infection levels, the resistant animals have high antibody levels and low EPG, whereas susceptible animals have low antibody levels and high EPG (H. W. Ploeger; cited in Kloosterman et al., 1992). When exposed to the same level of parasite challenge, susceptible animals will have a high worm burden (i.e., high EPG) but will have only a weak immune response (low antibody level), whereas more resistant animals will have a lower worm burden as a result of a strong immune response (Morris et al., 2003).

The key to understanding some of the patterns of infection and immunity observed in parasitized ruminants may lie in a greater understanding of the nutritional costs of immunity (Lochmiller and Deerenberg, 2000). The paradoxical observations that the apparently more immune animals (lambs selected for low EPG or dairy cows with high antibody levels to *O. ostertagi*) exhibit inferior performance could be a manifestation of nutrients being partitioned away from growth or milk production and toward the immune response (Colditz, 2002). There is some support for this hypothesis in the general scheme proposed by Coop and Kyriazakis in young animals, but less so for adults (Coop and Kyriazakis, 1999). In support of this, recent work in lambs by Greer et al. (2005a,b) has shown that the adverse effects of gastrointestinal parasite infection (*Trichostrongylus colubriformis* and *Teladorsagia circumcincta*) on voluntary food intake, feed conversion efficiency, and liveweight gain can be completely abolished by immunosuppressive treatment despite the presence of large numbers of parasites in the gut, suggesting that the immune response itself could be heavily involved in

the inferior performance observed. It is clear that the complex relationships between parasitic infection, EPG, immune response, and productivity require a greater understanding before genetic selection is considered.

CONCLUSIONS

This study found that anti-*O. ostertagi* total IgG antibody response is under genetic control and also highlighted the significant effect of season of sample and herd or geographical location. These sources of environmental variation would need to be considered in future investigations into the potential use of antibody response in genetic selection and in parasite control programs. The possibility of anthelmintic resistance may mean that, in the future, producers may use a combination of management and genetic selection to control parasitic infection and susceptibility. Because of the relatively small amount of data available and the very simplified genetic model applied, further large-scale genetic studies need to be carried out to fully dissect the relationship between parasitic susceptibility and antibody response and the genetic and nongenetic factors affecting them.

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