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Genetic Parameters of Immune Traits in Dairy Cattle

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ABSTRACT Previous work has identified a number of immune traits which are associated with dairy cow health. The purpose of the present study was to determine the genetic parameters of these immune traits. Blood samples were collected from 465 Holstein-Friesian dairy cattle and analysed for 13 different serological and cellular immune traits. Data were modelled using a repeatability mixed linear model. Heritability estimates and the variance components were obtained and ranged from 0.147 (NAb KLH) to 0.497 ($CD4^+$). Heritability of the T cell subsets was highest with 0.497, 0.250 and 0.314 for $CD4^+$, $CD8^+$ and $CD4^+$:$CD8^+$ ratio respectively. Furthermore, the ratio of permanent environmental variance to total phenotypic variance (c^2) was estimated and ranged from 0.028 (%Eosinophils) to 0.623 (TNF-α). Finally, the repeatability of each trait was estimated and ranged from 0.207 (NAb KLH) to 0.701 ($CD8^+$).
Keywords: dairy cattle; immune traits; heritability; genetics

Introduction

Health and welfare of dairy cattle are areas of increasing importance and identifying and predicting the occurrence of such events is crucial in maintaining a high level of production within a healthy herd. The use of immune traits for the purpose of predicting changes in health, fertility and production has been highlighted (Banos et al. 2013). In their study, Banos et al. (2013) analysed 16 immune traits with the aim of identifying associations between immune traits in blood and dairy cow health, productivity and reproduction. These immune traits included levels of serum haptoglobin, natural antibodies (NAb KLH) and Tumour Necrosis Factor Alpha (TNF-α). %Peripheral blood mononuclear cell (PBMC), %Eosinophils, %Lymphocytes, %Monocytes and %Neutrophils within the circulating leukocyte population, the % PBMC expressing CD14, CD21, CD335, CD3, CD4, CD8 or γδ-TCR (gamma delta T-cell receptor) and the $CD4^+$:$CD8^+$ ratio. Of the 16 immune traits originally analysed by Banos et al., (2013), 12 will be the main focus of the present study. In addition to the natural antibody type studied previously (NAb KLH), animals in the present study are generating data on a second NAb type, NAb LPS, giving a total of 13 immune traits of interest. This research aims to verify and estimate the heritability of these immune traits as well as the ratio of permanent environmental variance to total phenotypic variance and repeatability.

Materials and Methods

Animals. All animals involved in this study were Holstein-Friesians from the Langhill lines of dairy cattle housed at the SRUC Dairy Cattle Research Centre at Crichton Royal Farm, Dumfries in Scotland. These animals are currently involved in an ongoing selection experiment (genetic line × feeding systems) that has been running for over 30 years (Bell et al. 2011). Cows are divided between two genetic groups, a control I and a select (S). Those in the C group are selected for producing milk in line with the UK average genetic merit for kg fat and kg protein. In contrast, those in the S group are selected for production of milk with the highest levels of kg fat and kg protein (Pryce et al. 1999; Bell, et al., 2011, Banos, et al., 2013). Within each genetic group cows are also divided among two distinct feed groups, a high concentrate low forage diet, simulating high-input commercial systems, or a low concentrate high forage diet, simulating low-input grazing systems (Pryce et al. 1999; Bell et al. 2011; Banos et al. 2013).

Data. Animals in the Langhill herd are routinely and extensively monitored in areas such as productivity, health, welfare and reproduction, thus, generating a wealth of phenotypic data for use in statistical analyses. For the present study, a sample population was extracted from the Langhill database that contained all cows recorded as having being in first or subsequent lactation from January 2009 onwards. At the time of the study this totalled 713 individual animals that were present in the herd between July 2003 and January 2014. Thus, this study population contained cohorts and contemporaries of the cows studied in the previous immune trait study (Banos et al. 2013) as well as those in the present. A phenotype dataset was created for subset of this study population and contained all animals with immune data, i.e., 465 individuals from both past and present studies. Full pedigree spanning seven generations was available.

Immune traits. Immune trait data for the 13 immune traits of interest were matched to corresponding weekly milk production records. Data for immune traits generated from blood samples collected between July 2010 and March 2011 (samples collected at two-monthly intervals on five separate occasions) from the previous study (Banos et al. 2013) are readily available in the SRUC Langhill database for use and comparison with that currently being generated for the present study. Both sets of data are included in the phenotype dataset. Cellular immune

trait data from the former study are available for animals within both genetic groups but only those on the high concentrate diet (Banos et al. 2013). Data currently being generated is derived from both serum and milk samples, whereas previously data was generated from serum samples only (Banos et al. 2013). Hence, only data from serum is considered in this work. Additionally, the Haptoglobin and TNF-α assays used in the present study differ in that they are now analysed using in house ELISAs instead of the commercial kits used previously. Firstly, in the case of TNF-α, the use of the new assay appears to have a better dynamic range with less samples being below the detection limit. Secondly, in the case of Haptoglobin, a colorimetric assay was used to generate data for the previous study instead of an ELISA. The colorimetric assay had a small dynamic range and there was also the possibility of other substances interfering with the sample. The ELISA assay used to generate data in the present study has a greater dynamic range.

Statistical analyses. Following the methods used by Banos et al. (2013) statistical analysis of the 13 immune traits was carried out using a repeatability mixed linear animal model (1).

$$y_{ijklmnop} = \mu + C_i + F_j + G_k + L_l + W_m + T_n + a_o + p_o + e_{ijklmnop} \quad (1)$$

Where $y_{ijklmnop}$ is the immune trait observation; μ is the overall mean; C_i is the fixed effect the i^{th} year by month of calving interaction; F_j is the fixed effect of the j^{th} food group; G_k is the fixed effect of the k^{th} genetic group; L_l is the fixed effect of the l^{th} lactation number by age at calving; W_m is the fixed effect of the m^{th} lactation week; T_n is the fixed effect of the n^{th} assay technique; a_o is the random additive genetic effect of the o^{th} individual cow; p_o is the random permanent environmental effect of the o^{th} individual cow; and $e_{ijklmnop}$ is the random residual effect. Assay technique was fitted as a fixed effect to account for the variation between the methods used to generate the immune data in the previous and current studies. Total phenotypic variances (V_P), as well as corresponding additive genetic (V_A), permanent environmental (V_E) and residual (V_R) variance components, for the individual immune traits (Table 3) were obtained by carrying out a univariate analysis of the immune data via (1) using the statistical package ASReml (Gilmour, et al., 2009). Heritability, ratio of the phenotypic variance due to permanent environment (c^2) and repeatability were estimated from these variance components.

Table 1. Summary of the phenotype dataset used in all model analyses.

Description	Total
Records in dataset	1,559
Animals in dataset	465
Lactations	3†
Animals in pedigree	2632
Sires in pedigree	529
Dams in pedigree	2103
Pedigree levels	7

† Lactations ≥ 3 are grouped into the lactation 3 class.

Table 2. Descriptive statistics of the immune traits. Number of records, mean, standard deviation and coefficient of variation (%).

Trait	No. Records	Mean	SD	CV, %
HAPT[1]	1356	92.731	182.300	196.590
NAb KLH	1363	1.110	0.569	51.234
NAb LPS	507	1.170	0.387	33.085
TNF-α	1356	1351.100	5695.200	421.523
$CD4^+$	644	21.461	7.430	34.622
$CD8^+$	662	9.941	3.228	32.472
$CD4^+$:$CD8^+$	644	2.257	0.778	34.482
$CD335^+$	663	2.084	2.043	98.033
PBMC[2]	668	55.584	9.990	17.973
EOSIN[3]	668	2.353	2.923	124.224
LYMP[4]	667	37.669	14.276	37.899
MONO[5]	667	17.263	12.743	73.817
NEUT[6]	668	41.353	9.683	23.416

1 Haptoglobin
2 % peripheral blood mononuclear cell
3 % Eosinophils
4 % Lymphocytes
5 % Monocytes
6 % Neutrophils

Table 3. Results from the univariate analysis. Additive genetic (V_A), permanent environmental (V_E), residual (V_R), and phenotypic variances (V_P), with standard errors, for the 13 individual immune traits.

Trait	V_A (±s.e)	V_E (±s.e)	V_R (±s.e)	V_P (±s.e)
HAPT[1]	N.E¥	0.14 (0.08)	2.66 (0.12)	2.80 (0.11)
NAb KLH	0.04 (0.02)	0.02 (0.02)	0.22 (0.01)	0.27 (0.01)
NAb LPS	N.E¥	0.05 (0.01)	0.09 (0.01)	0.14 (0.01)
TNF-α	N.E¥	0.21 (0.02)	0.13 (0.01)	0.34 (0.02)
$CD4^+$	7.57 (3.41)	1.11 (2.87)	6.55 (0.51)	15.22 (1.23)
$CD8^+$	2.29 (1.49)	4.14 (1.44)	2.74 (0.21)	9.16 (0.73)
$CD4^+$:$CD8^+$	0.16 (0.08)	0.17 (0.08)	0.17 (0.01)	0.50 (0.04)
$CD335^+$	0.35 (0.99)	N.E¥	1.68 (0.11)	2.04 (0.12)
PBMC[2]	19.20 (4.77)	N.E¥	59.74 (4.29)	78.94 (4.96)
EOSIN[3]	1.05 (0.75)	0.13 (0.69)	3.68 (0.26)	4.86 (0.31)
LYMP[4]	N.E¥	N.E¥	0.18 (0.01)	0.18 (0.01)

MONO[5]	N.E[¥]	N.E[¥]	140.82 (8.06)	140.82 (8.06)
NEUT[6]	19.51 (4.51)	N.E[¥]	54.51 (3.90)	74.02 (4.69)

¥ Not estimable
1 Haptoglobin
2 % peripheral blood mononuclear cell
3 % Eosinophils
4 % Lymphocytes
5 % Monocytes
6 % Neutrophils

Results and Discussion

Descriptive statistics. The immune trait data are summarised in Table 2. The coefficient of variation of the immune traits was substantial and ranged from 18% (PBMC) to 422% (TNF-α).

Genetic parameters. Variance components for the 13 immune traits are given in Table 3. Estimates of heritability, c^2 and repeatability are shown in Table 4.

Table 4. Heritability estimates (h^2), ratio of permanent environmental variance (c^2) and repeatability (Rep), with standard errors, for the 13 immune traits.

Trait	h^2 (±s.e)	c^2 (±s.e)	Rep (±s.e)
HAPT[1]	N.E[¥]	0.050 (0.028)	N.E[¥]
NAb KLH	0.147 (0.068)	0.060 (0.065)	0.207 (0.031)
NAb LPS	N.E[¥]	0.328 (0.055)	N.E[¥]
TNF-α	N.E[¥]	0.623 (0.025)	N.E[¥]
$CD4^+$	0.497 (0.203)	0.073 (0.191)	0.570 (0.044)
$CD8^+$	0.250 (0.158)	0.452 (0.156)	0.701 (0.031)
$CD4^+$:$CD8^+$	0.314 (0.162)	0.340 (0.158)	0.655 (0.037)
$CD335^+$	0.172 (0.045)	N.E[¥]	N.E[¥]
PBMC[2]	0.243 (0.052)	N.E[¥]	N.E[¥]
EOSIN[3]	0.216 (0.150)	0.028 (0.142)	0.244 (0.050)
LYMP[4]	N.E[¥]	N.E[¥]	N.E[¥]
MONO[5]	N.E[¥]	N.E[¥]	N.E[¥]
NEUT[6]	0.264 (0.052)	N.E[¥]	

¥ Not estimable
1 Haptoglobin
2 % peripheral blood mononuclear cell
3 % Eosinophils
4 % Lymphocytes
5 % Monocytes
6 % Neutrophils

From the results obtained via model outputs, h^2 refers to the heritable proportion of variance and can be used to predict future progeny performance. Furthermore, repeatability refers to the overall proportion of variance due to the individual animal and can be used to predict future performance of the same animal. Heritability estimates ranged from 0.147 (NAb KLH) to 0.497 ($CD4^+$) and c^2 estimates ranged from 0.028 (%Eosinophils) to 0.623 (TNF-α). Finally, repeatability estimates ranged from 0.207 (NAb KLH) to 0.701 ($CD8^+$). The most significant estimates of heritability obtained were 0.497, 0.250 and 0.314 for the T cell subsets $CD4^+$, $CD8^+$ and $CD4^+$:$CD8^+$ ratio respectively. These traits also had the most significant estimates of repeatability (0.570, 0.701 and 0.655 respectively). Importantly, from the literature $CD4^+$:$CD8^+$ ratio has been found to exhibit a high level of repeatability and is significantly negatively correlated with somatic cell count (Banos et al. 2013). Somatic cell count is often considered as an indicator of mastitis and other intra-mammary infections (Mrode and Swanson 1996; Mrode and Swanson 2003). $CD4^+$:$CD8^+$ has also been associated with mastitis (Park et al. 2004) supporting the opinion that genetic selection of immune traits may be useful in improving resistance to disease (Thompson-Crispi et al. 2012).

Conclusion

The work in the present study has highlighted the potential heritability and repeatability of immune traits associated with health in dairy cattle. The highest and most significant heritabilities were seen in the T cell subsets $CD4^+$, $CD8^+$, $CD4^+$:$CD8^+$. This agrees in part with the literature concerning other species such as pigs (Clapperton et al. 2009; Flori et al. 2011) and humans (Hall et al. 2000). Unfortunately, genetic parameters were not estimable for all immune traits; however, initial model outputs suggest that the majority of immune traits of interest in the present study are indeed heritable.

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Literature Cited

Banos, G., Wall, E., Coffey, M. P., et al. (2013). *Plos One*, 8(6), e65766

Bell, M. J., Wall, E., Russell, G. C., et al. (2011). *J. Dairy Sci*. 94:3662–3678

Clapperton, M., Diack, A. B., Matika, O., et al. (2009). *Genet Sel Evol*. 41:54.

Flori, L., Gao, Y., Laloë, D., et al. (2011). *Plos One*, 6(7): e22717

Gilmour, A. R., Gogel, B. J., Cullis, B. R., et al. (2009). ASReml, Release 3.0 VSN International Ltd. www.vsni.co.uk

Hall, M. A., Ahmadi, K. R., Norman, P., et al. (2000). *Genes Immun.* 1:423-427
Mrode, R. A., Swanson, G. J. T. (1996). *Anim Breed Abstr.* 64:847-857
Mrode, R. A., Swanson, G. J. T. (2003). *Livest Prod Sci.* 79:239-247
Park, Y. H., Joo, Y. S., Park, J. Y., et al. (2004). *J Vet Sci.* 5:29-39
Pryce, J. E., Nielsen, B. L., Veerkamp, R. F., et al. (1999). *Livest Prod Sci*. 57:193-201
Thompson-Crispi, K. A., Sewalem, A., Miglior, F., et al. (2012). *J Dairy Sci.* 95:401-409.