GENETIC VARIATION FOR TOTAL AND DIFFERENTIAL NUMBERS OF LEUKOCYTES EXISTS IN GROWING PIGS

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INTRODUCTION

Additive genetic variation for resistance to clinical and sub-clinical diseases (i.e., lameness, respiratory diseases, diarrhoea, and arthritis) exists in growing pigs (Smith *et al.,* 1962 ; Lundeheim, 1988 ; Lingaas and Rønningen, 1991 ; Henryon *et al,*. 2001). This finding suggests that selecting pigs for resistance to clinical and sub-clinical disease could be successful. However, the level of success is largely dependent upon the reliability with which breeding values of the pigs can be estimated for resistance. Using the incidence of clinical and sub-clinical disease prior to selection as the sole selection criterion often results in unreliable breeding value estimates. Consequently, selective breeding would be more successful if other traits, which reflect resistance of the pigs, could be identified and used as additional selection criteria. Such traits may include total and differential numbers of white blood cells (i.e., leukocytes). Total and differential numbers of leukocytes may reflect resistance, as the immune system, with its complex interactions of innate and adaptive response mechanisms, depends upon the activities of leukocytes. In its simplest form, innate response largely depends upon granulocytes (i.e., neutrophils, eosinophils, and basophils) and monocytes, while adaptive response involves lymphocytes.

There are two prerequisites required for total and differential numbers of leukocytes to be suitable selection criteria. First, these traits need to express additive genetic variation. Second, they need to be genetically correlated with resistance to clinical and sub-clinical disease. Total and differential numbers of leukocytes may well fulfil the first of these prerequisites in growing pigs, namely the expression of additive genetic variation, as Edfors-Lilja *et al.* (1994) reported additive genetic variation for total number of leukocytes (h^2±s.e.=0.44±0.29) and number of granulocytes (0.87±0.41), though not for number of lymphocytes (0.00±0.00). However, these estimates were obtained using a small data set (i.e., 124 growing pigs from 12 sires and 31 dams). Therefore, in this study, the premise that additive genetic variation for total and differential numbers of leukocytes exists in growing pigs was tested as a first step to assessing the suitability of these traits as additional selection criteria for resistance to clinical and sub-clinical disease.

MATERIALS AND METHODS

Pigs. A total of 4204 male growing pigs from the Duroc, Landrace, and Yorkshire breeds were assessed for total and differential numbers of leukocytes when they were 52±3 (mean±s.d.) days old. The pigs were part of the nucleus breeding population of the Danish pig breeding programme (DanAvl). They were from 583 sires, 2835 dams, and 3082 litters. The total

number of individuals in the pedigree structure after tracing animals up to 20 generations back from the sires and dams of the pigs was 20 847.

Rearing of pigs. The pigs were assessed at Bøgildgård, the central test station of DanAvl. Approximately 90 pigs arrived at Bøgildgård each week from their respective breeding farms as 30±3 day old piglets weighing 8.3±1.1 kg. Upon arrival, the pigs were allocated to two stalls (i.e., stall groups) within an acclimatisation facility, where they remained for 5 weeks. The acclimatisation facility consisted of 14 stalls. Each stall was divided into four pens, and each pen maintained between 14 and 16 pigs. Pigs from the Duroc, Landrace, and Yorkshire breeds were allocated to each stall. However, with few exceptions, pigs allocated to the same pen within each stall (i.e., pen group) were from the same breed.

Measurements. The pigs were blood-sampled on day 22 following arrival at Bøgildgård. Blood was sampled from the cranial *vena cava*, and collected as stabilised blood in K3E EDTA tubes. Total and differential numbers of leukocytes in the blood were assessed on the day of sampling using a CELL-DYN® 3500 hemacytometer configured for pig blood. The hemacytometer counted the total number of leukocytes/L blood, and measured the proportion of leukocytes that were neutrophiles, eosinophils, monocytes, and lymphocytes. The numbers of neutrophiles, eosinophils, monocytes, and lymphocytes/L blood were derived from the total number of leukocytes and the proportions of neutrophiles, eosinophils, monocytes, and lymphocytes.

Statistical analysis.
Data transformations. The total number of leukocytes, and the numbers of neutrophiles, eosinophils, monocytes, and lymphocytes, were $\log_e$-transformed prior to analysis. Both the observed and transformed means are presented for each trait.

Model. Additive genetic variation was estimated by fitting a univariate linear animal model to each of the traits on the $\log_e$-scale. The model was:

$$\mathbf{y} = \mathbf{X_b b} + \mathbf{X_c c} + \mathbf{Z_f f} + \mathbf{Z_d d} + \mathbf{Z_s s} + \mathbf{Z_p p} + \mathbf{Z_m m} + \mathbf{Z_a a} + \mathbf{e} \quad (1)$$

where **y** is a vector of obervations, **b** is a vector of fixed breed effects, **c** is a vector of covariate effects, **f** is a vector of random breeding farm effects ~N(0, $\mathbf{I_f}\sigma_f^2$), **d** is a vector of random sampling day effects ~N(0, $\mathbf{I_d}\sigma_d^2$), **s** is a vector of random stall group effects ~N(0, $\mathbf{I_s}\sigma_s^2$), **p** is a vector of random pen group effects ~N(0, $\mathbf{I_p}\sigma_p^2$), **m** is a vector of random litter effects ~N(0, $\mathbf{I_m}\sigma_m^2$), **a** is a vector of random additive breeding values ~N(0, $\mathbf{A}\sigma_a^2$), **e** is a vector of random errors ~N(0, $\mathbf{I_e}\sigma_e^2$), $\mathbf{X_b}$, $\mathbf{X_c}$, $\mathbf{Z_f}$, $\mathbf{Z_d}$, $\mathbf{Z_s}$, $\mathbf{Z_p}$, $\mathbf{Z_m}$, and $\mathbf{Z_a}$ are known design matrices, **A** is a known matrix of additive genetic relationships among the animals, and σ_f^2, σ_d^2, σ_s^2, σ_p^2, σ_m^2, σ_a^2, and σ_e^2 are the unknown variances associated with the random breeding farm, sampling day, stall group, pen group, litter, additive breeding value, and error effects. The covariate effects (i.e., elements of **c**) differed when the model was fitted to the numbers of leukocytes and lymphocytes (i.e., age, body weight), neutrophils (body weight), eosinophils (race x age and race x body weight interactions), and monocytes (race x age interaction). Variance components

were estimated using an AI-REML algorithm (Jensen *et al.* 1997) included in the DMU package (Madsen and Jensen 2000).

Additive genetic variation. Additive genetic variance (σ_a^2) and heritability are presented for each trait, where the heritability was calculated as $h^2=\sigma_a^2/(\sigma_m^2 + \sigma_a^2 + \sigma_e^2)$.

Genetic and phenotypic correlations. Genetic and phenotypic correlations were estimated by fitting bivariate linear animal models [model (1)]. Phenotypic correlations were calculated using the breeding farm, sampling day, stall group, pen group, litter, additive breeding value, and error components of (co)variation. The genetic correlations were tested for significance from zero using a -2log(restricted likelihood ratio) test.

RESULTS

Numbers of leukocytes. The pigs had a total of 19.82 x 10^9 leukocytes/L blood (Table). Approximately half of these cells were lymphocytes, 42.1% were neutrophils, 1.7% were eosinophils, and 6.9% were monocytes. The remaining cells were basophils and dead cells.

Table 1. Observed mean, $\log_e$-transformed mean (±s.d.), additive genetic variation (σ_a^2±s.e.), heritability (h^2±s.e.), and genetic (±s.e.) and phenotypic correlations for total number of leukocytes, and numbers of neutrophils, eosinophils, monocytes, and lymphocytes in growing pigs. The additive genetic variation, heritability, and genetic and phenotypic correlations are presented on the $\log_e$-scale. Genetic correlations are above, and phenotypic correlations below, the diagonal

	Leukocytes	Neutrophils	Eosinophils	Monocytes	Lymphocytes
Observed mean[A]	19.82	8.34	0.34	1.36	9.76
$\log_e$ mean[B]	2.95±0.25	2.02±0.45	-1.29±0.67	0.19±0.49	2.24±0.28
σ_a^2	0.013±0.003	0.033±0.007	0.107±0.018	0.048±0.009	0.017±0.004
h^2	0.25±0.05	0.22±0.04	0.29±0.05	0.22±0.04	0.24±0.05
Leukocytes		0.80±0.06	0.41±0.11	0.68±0.09	0.71±0.07
Neutrophils	0.76		0.44±0.12	0.57±0.11	0.17±0.14[ns]
Eosinophils	0.28	0.20		0.30±0.12	0.09±0.13[ns]
Monocytes	0.44	0.35	0.19		0.26±0.14[ns]
Lymphocytes	0.66	0.07	0.15	0.08	

[A]Number of cells (x 10^9)/L blood [B]Mean of $\log_e$[Number of cells (x 10^9)/L blood]
[ns]Not significantly different from zero (P>0.05)

Additive genetic variation. Moderate amounts of additive genetic variation were detected for total number of leukocytes, and numbers of neutrophils, eosinophils, monocytes, and lymphocytes (Table 1). The heritability was 0.25 for total number of leukocytes, and ranged between 0.22-0.29 for numbers of neutrophils, eosinophils, monocytes, and lymphocytes.

Genetic correlations. Genetic correlations between total number of leukocytes and each of the differential numbers of leukocytes were positive and moderate-to-very strong (0.41-0.80, Table 1). By contrast, correlations among the numbers of neutrophils, eosinophils, monocytes, and

lymphocytes were positive and low-to-moderately strong (0.09-0.57). However, those involving the number of lymphocytes were not significantly different from zero. The 5 x 5 additive genetic (co)variance matrix was singular (determinant=8.8×10^{-11}).

Phenotypic correlations. Phenotypic correlations between total number of leukocytes and each of the differential numbers of leukocytes were positive and moderate-to-very strong (0.28-0.76, Table). By contrast, correlations among the numbers of neutrophils, eosinophils, monocytes, and lymphocytes were positive and low-to-moderately strong (0.07-0.35).

DISCUSSION

This study established that additive genetic variation for total and differential numbers of leukocytes exists in growing pigs. Moderate amounts of additive genetic variation were detected for total number of leukocytes, and numbers of neutrophils, eosinophils, monocytes, and lymphocytes. These results indicate that total and differential numbers of leukocytes will respond to selection, and may provide a suitable criterion by which to select pigs for resistance to clinical and sub-clinical disease.

The positive and moderate-to-very strong genetic and phenotypic correlations between total number of leukocytes and each of the differential numbers of leukocytes were detected because the total number of leukocytes was a composite of the differential numbers, and because the genetic and phenotypic correlations among each of the differential numbers were positive. From a selective breeding perspective, this suggests that selection only need be placed on total number of leukocytes for there to be simultaneous response in the numbers of neutrophils, eosinophils, monocytes,andlymphocytes

The additive genetic variation detected in this study demonstrates that total and differential numbers of leukocytes in pigs fulfil the first prerequisite required for these traits to be suitable selection criteria for resistance to clinical and sub-clinical disease. However, before they can be considered suitable selection criteria, it needs to be shown that total and differential numbers of leukocytes fulfil the second prerequisite, namely that they are genetically correlated with resistance.

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