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Immune associated traits measured in milk of Holstein-Friesian cows as proxies for blood serum measurements

By Denholm et al. The opportunity to use routinely collected milk samples as a substitute for blood presents a low-cost, and more importantly, less-invasive approach to obtain valuable and informative data from dairy cows. Notably, no additional labor costs or changes in management need occur to collect such data. Using this method to collect immunological data will not only improve animal welfare but also provide an indication of the animal’s health status. Moreover, such immunological data have the potential act as biomarkers of disease allowing early intervention and as such have the potential to guide breeding programs for improved health, fertility and disease resistance.

USING MILK IMMUNOLOGICAL DATA AS A PROXY FOR BLOOD

Immune associated traits measured in milk of Holstein-Friesian cows as proxies for blood serum measurements

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ABSTRACT
Previous work has highlighted that immune-associated (IA) traits measurable in blood are associated with health, productivity and reproduction in dairy cows. The aim of the present study was to determine relationships between immune associated traits measured in blood serum and those simultaneously measured in milk as well as their association with disease phenotypes. All animals were Holstein-Friesian cows from the Langhill research herd (n=546) housed at the SRUC Dairy Research Centre in Scotland. Milk and serum samples were collected on 20 separate occasions between July 2010 and March 2015 and analyzed by ELISA for haptoglobin (Hp), tumor necrosis factor alpha (TNF-α) and for natural antibodies binding keyhole limpet hemocyanin (NAb_{KLH}) and lipopolysaccharide (NAb_{LPS}). Data were analyzed using mixed linear models that included pedigree information. Analyses revealed positive phenotypic correlations between milk and serum NAb (0.59 ≤ r ≤ 0.77), Hp (r=0.37) and TNF-α (r=0.12). Milk and serum NAb were also found to have a strong genetic correlation (0.81 ≤ r ≤ 0.94) and were both genetically correlated with cow lameness (0.66 and 0.79 for milk NAb_{KLH} and serum NAb_{LPS} respectively). Clinical mastitis was found to be phenotypically correlated with both milk and serum Hp (0.09 ≤ r ≤ 0.23). Serum Hp was also strongly genetically correlated with other cellular immune-associated traits such as %NKp46⁺ (0.35) and %PBMC (-0.90). Similarly, genetic correlations were found to exist between serum TNF-α and %NKp46⁺ (0.22), %PBMC (0.41) and %lymphocytes (0.47). Excluding serum Hp, all milk and serum IA traits were repeatable ranging from 0.11 (milk Hp) to 0.43 (serum NAb_{LPS}). Between-animal variation was highest in milk and serum NAb (0.34 – 0.43) and significant estimates of heritability were also observed in milk and serum NAb (0.17 – 0.37). Our findings show that certain immune associated traits, such as NAb_{KLH} and NAb_{LPS}, found in milk and serum are strongly correlated and highlight the potential of using routinely collected milk samples as a less invasive and cost-effective source of informative data for predictive modeling of animal immune associated traits.

**Keywords:** natural antibody, immune-associated trait, correlation, dairy cow
INTRODUCTION

Animal health and welfare is an important issue, especially in the case of farmed animals involved in intensive production systems such as the modern high-yielding dairy cow. Maintaining a healthy herd is vital, both ethically and financially. The UK dairy industry is under increasing financial pressure due to the cost of production against the lower level of returns generated via milk sales (Colman and Harvey, 2004; Bate, 2016; ADHB, 2017). Further financial losses and pressures also emerge in the form of animal disease, culling and infertility within the herd. With greater cow to staff ratios and an increased use of automation it is crucial for dairy farmers to record, monitor and manage losses due to disease and resultant culling and hence maximize profitability (Macrae and Esslemont, 2015).

The prospect of using immune associated (IA) traits as markers of health and fitness in agricultural production systems has attracted an increase in interest due the fact that such traits are readily measurable and can be used in the prediction of susceptibility and general immunity of individuals (Clapperton et al., 2008, 2009; Flori et al., 2011; Thompson-Crispi et al., 2012b; a; van Knegsel et al., 2012; Banos et al., 2013; Denholm et al., 2017). Moreover, previous work has shown that serological and cellular IA traits found in serum are associated with dairy cow health, fertility and production (Eckersall et al., 2006; Banos et al., 2013; Denholm et al., 2017).

Serological IA traits such as the acute phase protein (APP) haptoglobin (Hp), natural antibodies (NAb) binding keyhole limpet hemocyanin (NAbKLH) and lipopolysaccharide (NAbLPS) and the pro-inflammatory cytokine Tumor Necrosis Factor Alpha (TNF-α) are not only present in the blood of dairy cows but can also be measured in the milk (Bannerman et al., 2004; Eckersall et al., 2006; van Knegsel et al., 2007; Tassi et al., 2013). These serum IA traits have been shown to be associated with important health and production traits in dairy cows: for example serum Hp was positively correlated with clinical mastitis (Eckersall et al., 2006), whereas circulating levels of NAbKLH were negatively correlated with liveweight as well as both feed and dry matter intake (Banos et al., 2013). Additional studies have also
linked NAb with dairy cow health, e.g., energy balance, somatic cell count and mastitis (van Knegsel et al., 2007, 2012; Ploegaert et al., 2010), as well as production, e.g., milk yield and diet (van Knegsel et al., 2007). Relationships between NAb and age have also been highlighted (Srinivasan et al., 1999; van Knegsel et al., 2007; de Klerk et al., 2015).

Furthermore, serum TNF-α, Hp and NAb have all been shown to be associated with metabolic health (Ohtsuka et al., 2001; Ametaj et al., 2005; van Knegsel et al., 2012)

Focusing on NAb, Hp and TNF-α, the present study aimed to analyze longitudinal immunological data collected from repeated blood and milk sampling in order to determine if any relationships exist between IA traits measurable in blood (serum) and concurrent IA traits measurable in milk. The overall objective was to determine whether routinely collected milk samples can provide a less invasive method of obtaining useful IA trait data relevant to dairy cow health and productivity, and hence, reduce the requirement for blood sampling.

**MATERIALS AND METHODS**

**Animals**

Data were collected from 546 Holstein-Friesian dairy cows from the experimental Langhill herd between July 2010 and March 2015. All cows were raised at the SRUC Dairy Research Centre at Crichton Royal Farm, Dumfries in Scotland and were involved in a long running, and on-going, selection experiment, genetic line x feeding system, following a 2 by 2 factorial design (Veerkamp et al., 1994).

Within the Langhill herd, cows are evenly distributed between two distinct genetic lines, termed Control and Select. Cows in the Control line are progeny of sires with UK average genetic merit for production of milk for kilograms of fat plus protein whereas Select cows are progeny of sires with the highest genetic merit for kilograms of fat plus protein. Within each genetic group, cows are randomly assigned one of two diets: a high concentrate low forage diet simulating high-input commercial systems; and a low concentrate high forage diet simulating low-input grazing systems. Put simply, the two diets can be thought of as a
Concerning the animals involved in the present study, 46% were cows sampled as part of a previous pilot study by our group. These pilot study cows \((n=253)\) were born between January 2003 and March 2009 and were between their 1\textsuperscript{st} and 6\textsuperscript{th} lactation inclusive (Banos \textit{et al.}, 2013). All remaining cows \((n=358)\) were born between May 2007 and September 2012 and ranged from 1\textsuperscript{st} and 5\textsuperscript{th} lactation (inclusive). A small number of cows \((n=65)\) were contemporaneous with both studies.

\textit{Ethics Statement}

Blood sample collection was conducted in accordance with UK Home Office regulations (PPL No: 60/4278 Dairy Systems, Environment and Nutrition) and procedures were approved by the SRUC Animal Experimentation Committee. Otherwise, the study was restricted to routine on-farm observations and measurements that did not inconvenience or stress the animals.

\textit{Sampling Protocol}

Blood and milk samples were collected on 15 separate occasions between April 2013 and March 2015 and included summer and winter samplings. Samples were collected at four bi-monthly intervals between April 2013 and October 2013, seven monthly intervals between January 2014 and July 2014 and a further four bi-monthly intervals between September 2014 and March 2015. This resulted in 2,687 blood samples and 2,667 milk samples. Additional blood samples were collected previously as part of a pilot study, between July 2010 and March 2011, the methods of which are described by Banos \textit{et al.} (2013). These additional samples accounted for approximately 25\% (886 samples) of the total number of samples collected (6,240 samples, which included 3,581 blood samples and 2,667 milk samples). For serological analyses, whole blood was collected into plain Vacutainers (BD) and blood allowed to coagulate before centrifugation at \(2,000 \times g\) for 10 min. Milk was centrifuged at
3,000 × g for 30 minutes and the skimmed milk fraction was retained. Samples were stored at 
-20°C prior to analysis.

Blood and milk samples from the current study were analyzed by ELISA for natural 
antibodies (NAb_{KLH} and NAb_{LPS}), Hp and TNF-α as described below. Samples from our 
previous study (Banos et al., 2013) had previously been analyzed for NAb_{KLH}, Hp and TNF-
α. Cellular IA trait data were derived according to the methods in Denholm et al. (2017).

Measurement of Natural Antibodies, Haptoglobin and TNF-α

NAb binding either KLH or LPS were quantified by indirect ELISA in serum and 
skimmed milk as follows: microtitre plates (Immulon 2HB, Thermo Electron Corporation, 
Milford, MA) were coated with 100µl/well of either 1µg/ml Keyhole Limpet Hemocyanin 
(Calbiochem®, Nottingham, UK) or 4µg/ml LPS O55:B5 (Sigma-Aldrich) in 0.1M carbonate 
coating buffer at pH 9.6 overnight at 4°C. After washing in PBS, pH 7.4, containing 0.05%
Tween 20® (PBS/T), non-specific binding sites were blocked by incubating wells with PBS 
containing 3% fish gelatin (Sigma-Aldrich, St. Louis, MO) for 1 hr. at 37°C. Plates were 
subsequently incubated with serum diluted 1:40 or skim milk diluted 1:2.5 in PBS/NaCl/T 
(PBS containing 0.5 M NaCl and 0.5% Tween 80®) for 1 hr. at 37°C. For each plate, 8 wells 
were incubated with PBS/NaCl/T alone (blank), a known positive control sample was 
analyzed, and each test sample was analyzed in duplicate. After washing in PBS/T, plates 
were incubated for 1 hr. at 37°C with sheep anti-bovine IgG (H+L chain) conjugated to 
horseradish peroxidase (AbD Serotec, Kidlington, UK) diluted in PBS/NaCl/T. After a final 
wash in PBS/T, color reactions were developed by addition of Sigma-Fast OPD substrate 
(Sigma-Aldrich). Reactions were terminated by addition of 2.5 M H₂SO₄, and the optical 
density (OD) at 492 nm (OD@492) measured using a Sunrise™ microplate reader (Tecan, 
Mannedorf, CH). The dilutions of serum and skim milk were chosen following serial dilution 
of representative samples to ensure that the OD@492 for all samples was on the linear part of 
the curve. For each plate, the mean OD@492 of the blank wells was subtracted from the 
sample readings, and inter-plate variation was normalized to the positive control sample.
Hp levels in serum and milk were quantified by sandwich ELISA as follows: 96-well microtitre plates were coated with 0.125μg/ml rabbit anti bovine Hp (Life Diagnostics Inc., West Chester, USA) in carbonate coating buffer overnight at 4°C. Following washing in 0.02M Tris-HCl, 0.05% Tween 20® (TBS/T), wells were incubated with 10% non-fat milk powder in wash buffer for 1 hour at 37°C. Following washing in TBS/T, wells were incubated with serum or skim milk samples, diluted 1:40 or 1:10 for serum and skim milk, respectively, for 1 hr. at 37°C with shaking (500rpm). Eight serial dilutions of recombinant bovine haptoglobin standard (Life Diagnostics Inc., West Chester, PA, USA) ranging from 8 to 1025 ng/ml were included on each plate. After a further wash in TBS/T, plates were incubated with a 1:7500 dilution of rabbit polyclonal anti-bovine haptoglobin (Life Diagnostics Inc.) which had been conjugated to alkaline phosphatase using a Lightning-Link® Alkaline Phosphatase Labeling Kit (Innova Biosciences Ltd., Cambridge, UK). Plates were washed then developed using BluePhos® Microwell phosphatase substrate system (KPL, Gaithersburg, USA) for 15 minutes before stopping with APstopTM solution (KPL) and measuring OD@620nm using a Sunrise™ microplate reader (Tecan).

TNF-α was quantified in serum and skimmed milk as previously described (Tassi et al., 2013). Both serum and milk samples were analyzed neat and 8 serial dilutions of recombinant bovine TNF-α standard (R&D Systems, Abingdon, UK) ranging from 125 to 8000pg/ml were included on each plate.

To quantify levels of Hp and TNF-α, a four point Marquardt analysis of the standard curve was used to calculate concentrations within the samples. For Hp and TNF-α, ELISAs were repeated at lower or higher sample dilution if the sample OD did not fall within the range of ODs of the standard curve. Samples which still fell below the standard curve were assigned a concentration half of the lowest standard (i.e. 4ng/ml for Hp and 62.5pg/ml for TNF-α).

There were some differences between the techniques used previously (Banos et al., 2013) and those used in present study to take the blood and milk measures of the immune associated traits. For Hp and TNF-α, the assays used in the present study differed from
Firstly, in the case of Hp, a colorimetric assay (Tridelta PHASE™ Haptoglobin Assay kit, Tridelta Development Ltd, Maynooth, IR) was used to generate data in (Banos et al., 2013) instead of the in-house ELISA used in this study. The main difference between the two assays was the smaller dynamic range of the colorimetric assay. Secondly, an in-house ELISA was used to quantify TNF-α in this study whereas in (Banos et al., 2013) a commercial ELISA kit was used (Bovine TNF-α DuoSet ELISA kit, R&D Systems, Inc., Minneapolis, MN), however, the same TNF standards were used in both assays and as such the values are comparable. There were no differences in the methods used to generate NAb$_{KLH}$ data between the two studies although NAb$_{LPS}$ was not measured in (Banos et al., 2013).

Derivation of Health Traits

Each cow in the study population had detailed longitudinal health records spanning birth to the time of the present study. These data were used to create a phenotypic dataset matching the immune profile of each individual cow to relevant health event information. Health events were grouped into 3 groups: clinical mastitis, reproductive problems and cow lameness. Due to the low incidence of some disorders/diseases within the Crichton herd (e.g., metabolic) these conditions (including ketosis, displaced abomasum, hypocalcaemia, hypomagnesaemia, pyelonephritis etc.) were grouped into a fourth group simply termed “other”. Health and IA traits were matched such that animals were scored as 0 or 1 for absence or presence of a condition or treatment within one week (pre and post) of the immune traits sample date. Moreover, for each cow in the study, the number of distinct mastitis, reproductive and lameness episodes per lactation was calculated and added to the phenotypic dataset. Distinct episodes were calculated according to consecutive treatments more than 7, 21 and 28 days apart for mastitis, reproductive problems and lameness respectively (Banos et al., 2013; Denholm et al., 2017).

Data Preparation
Where possible all data were analyzed in their original raw format except in cases where data were not normally distributed, in which case transformations were applied. Milk and serum data for both Hp and TNF-α were highly positively skewed, and hence, Box-Cox transformation analyses were carried out in order to determine the optimum transformation required to achieve normality. The Box-Cox analyses yielded results close to zero; therefore, data relating to these traits underwent a log-transformation. In the case of milk and serum TNF-α, values that were below the detection limit of the assay (62.5*0.5 = 31.25pg/ml) were set close to zero (1x10^-11) before being transformed according to a log-transformation. No transformations were applied to milk or serum NAb before formal analyses.

**Statistical Analysis**

All statistical analyses of IA traits were carried out using ASReml version 3 (Gilmour et al., 2009) using repeated measures mixed linear animal models. In general models were of the form in (1):

\[
y = Xa + Z_1 b_{-a} + Z_2 b_{-w} + Z_3 c + e
\]

(1)

Where \( y \) is a vector of trait observations; \( a \) is a vector of fixed effects; \( b_{-a} \) is a vector of random permanent environmental effects across lactations; \( b_{-w} \) is a vector of random permanent environmental effects within lactation; \( c \) is a vector of random additive genetic effects; \( e \) is a vector of random residual effects; and, \( X, Z_1, Z_2, Z_3 \) are incidence matrices linking phenotypic records to fixed, permanent environmental across lactations, permanent environmental within lactation and additive genetic effects, respectively.

The general model included the following as fixed effects: genetic line, diet group, lactation week (WIM), year-by-month of record interaction, year-by-month of calving interaction and lactation number-by-age at calving interaction. Assay technique was also fitted as a fixed effect (where applicable) to account for the variation between the methods used to generate IA trait data in (Banos et al., 2013) and in the present study. Treatment status
was fitted as a binary fixed effect to account for whether or not the individual cow was receiving medical treatments at time of IA trait sampling (where 0 represents no treatment and 1 otherwise). Cow was fitted as a random effect to account for the random additive genetic effect of the \( n \)th individual cow (including pedigree data for 2,793 animals). The permanent environmental effect of the \( n \)th individual cow was fitted as a random effect to account for repeated sampling of the same animal across and within lactation. A further random effect of lactation by permanent environmental effect (of the \( n \)th individual cow) interaction was also fitted to account for repeated sampling of the same animal within a lactation (Bormann et al., 2002; Carthy et al., 2015).

**Variance Components and Ratios**

Univariate models were used to estimate variance parameters and ratios. The model in (1) partitioned the variance into its additive genetic (\( \sigma_a^2 \)), permanent environmental (\( \sigma_{pe}^2 \)) and residual error (\( \sigma_e^2 \)) components. Variance components and corresponding standard errors were estimated via the Restricted Maximum Likelihood (REML) approach using the statistical software ASReml version 3 (Gilmour et al., 2009). ASReml outputs were used to calculate heritability \( (h^2) \); repeatability \( (rep) \), i.e., the between-individual variance; and, the ratio of permanent environmental variance to total phenotypic variance \( (c^2) \) of milk and blood serological IA traits.

**Associations Between Defined Traits**

In order to investigate and determine associations between traits, bivariate, trivariate and multivariate models were used. Univariate analysis of the general model (1) was used to determine which fixed effects to include in the \( n \)-trait models \( (n=2, 3, 4, \text{Table S1}) \). Additive genetic, phenotypic, permanent environmental and residual correlations were calculated along with their corresponding standard errors via \( n \)-trait analyses of the general model in (1) without the random permanent environment within lactation effect fitted. This was due to
problems with convergence (with sensible variance or covariance estimates). As the variance, and subsequent parameters, from the univariate analyses (with both p. e. term fitted) only had a marginal impact on the variances we used the single p. e. (across all lactations) term in the multivariate analyses.

Correlations between immune and health traits, including clinical mastitis, lameness and reproductive disorders, were also investigated.

**RESULTS**

Summaries of the milk and serum serological IA traits are given in Table 1. On average, lower NAb levels were observed in milk compared to those observed in serum. NAb levels in milk were also seen to increase with lactation number. In serum, however, no significant effect of lactation was observed in NAbKLH and only a slight decrease in NAbLPS was observed. Variability of IA traits (determined by the coefficient of variation) ranged from 32 to 44% for NAbKLH; 45 to 72% for NAbLPS; 441 to 659% for Hp; and 349 to 363% for TNF-α. Trait variability was observed to be higher in milk than in serum for all IA traits in general (Table 1).

**Variance Components and Ratios**

Estimates of variance components of the defined milk and serum serological IA traits as well as corresponding variance ratios are presented in Table 2 and summarized below.

**Natural Antibodies**

In both milk and serum, genetic variation and permanent environmental variation were relatively small and close to zero (Table 2). Between-animal variation accounted for less than 50% of total phenotypic variance and repeatability of NAb in milk (0.34 and 0.38 for NAbKLH and NAbLPS respectively) was less than in serum (0.34 and 0.43 for NAbKLH and NAbLPS respectively). Moreover, repeatability of NAb in both milk and serum was highly significant ($P<0.001$, Table 2). Variance parameter calculations also yielded significant
heritabilities for both milk and serum NAb (Table 2). In serum, \( NAb_{\text{KLH}} \) and \( NAb_{\text{LPS}} \) had heritability estimates of 0.21 and 0.24 respectively (\( P<0.01 \)). Similarly, in milk \( NAb_{\text{KLH}} \) and \( NAb_{\text{LPS}} \) had heritability estimates of 0.17 and 0.37 respectively (\( P<0.05 \)). Heritability and repeatability of \( NAb_{\text{LPS}} \) was observed to be greater than that of \( NAb_{\text{KLH}} \) regardless of whether measured in milk or serum.

**TNF-\( \alpha \)**

Genetic variation did not contribute much to the total phenotypic variation); however, a significant contribution was observed in the permanent environmental variance within lactation (Table 2).

Between-animal variance accounted for up to 21\% of total phenotypic variation. Repeatability of both milk and serum TNF-\( \alpha \) was highly significant (0.21 and 0.11 for milk and serum respectively, \( P<0.01 \)). Due to the small contribution of additive genetic variation, estimates of heritability were low and not significant in both milk and serum (0.02, 0.04, respectively).

**Haptoglobin**

Similar to NAb and TNF-\( \alpha \) above, the additive genetic variance component was small and did not contribute very much to the total phenotypic variance in serum (0.03) and was only slightly higher in milk (0.14). Between-animal variance was low and not significant in serum with a repeatability of 4\%, whereas in milk between-animal variance accounted for a much larger proportion of total variance with an estimated repeatability of 11\%. Heritability of Hp in both serum and milk was low and non-significant (0.02 and 0.07 respectively).

**Associations Between Milk and Serum IA Traits**

Strong positive additive genetic correlations (\( P<0.01 \), Table 3) were found between \( NAb_{\text{KLH}} \) and \( NAb_{\text{LPS}} \) in both milk (0.88) and in serum (0.99). Similarly high correlations were also observed between milk and serum \( NAb_{\text{KLH}} \) (0.84) as well as between milk and serum.
Moreover, serum NAb_{KLH} was also positively correlated with milk NAb_{LPS} (0.81), as was serum NAb_{LPS} and milk NAb_{KLH} (0.91). Similar strong positive phenotypic correlations ($P<0.01$) were observed (0.59 – 0.77).

Model outputs (Table 4) also highlighted a highly significant ($P<0.01$), positive phenotypic correlation between TNF-$\alpha$ concentrations in milk and serum (0.12) and Hp concentrations in milk and serum (0.37). Phenotypic associations ($P<0.05$) between milk and serum Hp and milk NAb were also observed (0.15 for both NAb_{KLH} and NAb_{LPS}).

**Associations Between Serological and Cellular IA Traits**

Multivariate analyses (5-trait) were carried out to determine if any relationships exist between serological (milk and serum) and cellular IA traits. In the serum (Table 4), significant negative genetic associations were found between NAb_{KLH} and % peripheral blood mononuclear cells (PBMC, -0.39) and NAb_{LPS} and %eosinophils (-0.64). Positive genetic correlations were observed between NAb_{KLH} and %neutrophils (0.50). Interestingly, NAb_{KLH} was seen to have a negative association with %lymphocytes (-0.36), whereas NAb_{LPS} had a positive one (0.15). Regarding TNF-$\alpha$, significant genetic correlations were observed with NKp46$^+$ (0.22) a natural killer (NK) cell marker; %PBMC (0.41); and, %lymphocytes (0.47). A significant phenotypic association was also observed between TNF-$\alpha$ and the CD4$^+$:CD8$^+$ ratio (0.36). Hp was found to have a strong, negative, additive genetic correlation with %PBMC (-0.90) as well as moderate positive, additive genetic correlations with NKp46$^+$ (0.35). Remaining significant correlations between serum serological and cellular immune associated traits were low and are presented in Table 4.

In milk (Table 4), statistically significant correlations at the phenotypic level were found to exist between NAb_{LPS} and %eosinophils (-0.07), the CD4$^+$:CD8$^+$ ratio (-0.03), and NKp46$^+$ (0.10). Furthermore, NAb_{KLH} was found to be significantly correlated at the phenotypic level with %eosinophils and the CD4$^+$:CD8$^+$ ratio (-0.08, -0.05 respectively). A strong additive genetic association was also observed between milk Hp and %eosinophils (0.64).
Associations Between Serological IA Traits and Health Events

Bivariate analyses (Table 5) yielded significant positive genetic correlations between NAb and lameness on the week of immune analysis (0.66, P<0.05; and 0.79, P<0.01 for milk NAb\textsubscript{KLH} and serum NAb\textsubscript{LPS} respectively). Significant positive phenotypic correlations (P<0.001) were also observed between clinical mastitis and Hp (0.09 and 0.15 for serum and milk respectively); and, mastitis episodes and Hp (0.10 and 0.23 for serum and milk respectively).

DISCUSSION

The present study sought to determine the feasibility of using milk samples as a proxy for blood samples in order to more easily collect valuable trait information from dairy cows. The possibility of using routine milk samples to collect important and informative data is both less invasive to the cow and relatively inexpensive in comparison to collecting blood samples on a regular basis. In this study, four IA traits were investigated, NAb\textsubscript{KLH}, NAb\textsubscript{LPS}, Hp, TNF-α, and a genetic analysis performed.

Natural Antibodies

Significant additive genetic and phenotypic parameters, as well as correlations were estimated between NAb levels measured in milk and serum. Natural antibodies are derived from CD5+ B-1 cells (Casali and Notkins, 1989) and are an important part of the innate immune system. NAb are found in all healthy animals in the absence of antigenic stimulation and are defined as antigen-binding antibodies present in non-immunized individuals (Panda and Ding, 2015). They are characterized by broad antigen specificity, usually binding antigen with low affinity (Baumgarth et al., 2005). The premise for quantifying NAb binding KLH and LPS was that cows are highly unlikely to encounter KLH, which is derived from the hemolymph of the giant keyhole limpet, *Megathura crenulata*, and therefore NAb binding
this antigen reflects the capacity of the innate antibody response to respond to pathogen challenge. In contrast, exposure to LPS is highly likely and thus NAb binding LPS may better reflect the active status of the innate humoral immune system (van Knegsel et al., 2007).

Levels of NAb in milk have been shown to increase with parity (van Knegsel et al., 2007; de Klerk et al., 2015) whereas no significant change in levels in blood have been witnessed (de Klerk et al., 2015). Furthermore, NAb levels in milk are generally lower than in blood (van Knegsel et al., 2007; Ploegaert and Tijhaar, 2011; de Klerk et al., 2015); results from the present study confirm these observations for both NAb\textsubscript{KLH} and NAb\textsubscript{LPS}. From the genetic analysis, all milk and serum NAb were found to be repeatable \((P<0.01)\), agreeing with the results of Ploegaert and Tijhaar (2011), although estimates in the present study appear slightly lower than in Ploegaert and Tijhaar (2011). In both milk and serum, NAb\textsubscript{KLH} repeatability was lower than NAb\textsubscript{LPS}; Ploegaert and Tijhaar (2011) observed a similar trend in blood but not in milk. Moreover, heritability of NAb\textsubscript{KLH} in both milk and serum was similar in magnitude to those estimated by de Klerk et al. (2015). Furthermore, we found that heritability estimates of NAb\textsubscript{KLH} in serum \((0.21)\) were higher than in milk \((0.17)\), as was the case in de Klerk et al. (2015). In the case of NAb\textsubscript{LPS}, however, we found the opposite was true with higher heritability estimates seen in milk compared to serum, \(i.e., 0.37 \,(h^2\text{milk})\) and \(0.24 \,(h^2\text{serum})\).

Our analysis also highlighted, at the genetic level, NAb\textsubscript{KLH} and NAb\textsubscript{LPS} within serum have a correlation of approximately 1, and whilst phenotypically these traits differed \((0.61, P<0.01)\), they were always positively correlated (Table 3). The results of the analysis suggest NAb in milk and serum are strongly and positively correlated, confirming the findings of other studies (Ploegaert and Tijhaar, 2011; de Klerk et al., 2015) and suggest that recording of milk NAb will provide accurate information on corresponding levels of NAb in serum. All phenotypic correlations were highly significant \((p<0.01)\) and in agreement with the literature (de Klerk et al., 2015). Genetic correlations ranged from 0.81 – 0.99, similar to the results of de Klerk (de Klerk et al., 2015), indicating that selective breeding for one NAb type will have a strong influence on the other. Within milk, both NAb\textsubscript{KLH} and NAb\textsubscript{LPS} were also found to be
strongly correlated with phenotypic and genetic correlations of 0.54 – 0.58 and 0.85 – 0.93, respectively. Genetic correlations were greater than phenotypic correlations as in de Klerk et al. (2015).

Furthermore, results of the present study found genetic correlations between NAb\textsubscript{KLH} and \%PBMC (-0.39) and \%lymphocytes (-0.36), which was similar to our previous smaller pilot study (Banos et al., 2013) which reported correlations of -0.29 both for \%PBMC and \%lymphocytes with NAb\textsubscript{KLH} but did not include pedigree information. We also identified significant positive genetic correlations between lameness episodes and both milk NAb\textsubscript{KLH} and serum NAb\textsubscript{LPS}. Together with our observations reported in Banos et al., (2013), in which serum NAb\textsubscript{KLH} was found to be negatively correlated with dry matter intake, feed intake and live weight at the phenotypic level, these data suggest that selection for low NAb levels, either using milk or serum measurements, may influence both nutritional traits and cow lameness.

**Haptoglobin**

Haptoglobin is an acute phase protein (APP) and part of the non-specific innate immune response (Murata et al., 2004). It is a positive APP meaning concentrations of Hp become elevated as a result of external and internal challenges such as tissue damage, stress and infectious and inflammatory disease (Murata et al., 2004; Lomborg et al., 2008). In healthy cattle, blood Hp is either non-detectable or measurable only in very low concentrations, i.e., <100 µg/ml (Panndorf et al., 1976). Moreover, Smith at al. (2010) found cows that tested positive for Hp had concentrations ranging between 370 and >1000 µg/ml.

The present study estimated a highly significant ($P<0.01$) phenotypic correlation between milk and serum levels (0.37), whereas the genetic correlation was found to be negative and not significant (-0.50). From the literature the phenotypic correlation between Hp measured in milk and blood has been estimated as 0.6 (Hiss et al., 2009).

The present study also highlighted positive significant phenotypic correlations ($P<0.001$) between Hp and clinical mastitis (0.09 and 0.15 for serum and milk respectively);
as well as, between Hp and mastitis episodes (0.10 and 0.23 for serum and milk respectively), suggesting either milk or serum Hp could be used as a potential biomarker of bovine mastitis. These correlations, however, were much weaker than those found in a previous study by our group, which observed a phenotypic correlation between serum Hp and clinical mastitis of 0.32 (Banos et al., 2013). The difference in magnitude of the correlation in (Banos et al., 2013) is most likely due to the inclusion of pedigree information in the present study as well as fitting a permanent environmental random effect. Moreover, whereas the previous results were obtained from data collected on 5 occasions, the present study made use of a much larger pool of data collected on 20 occasions which included 15 distinct sample periods, corresponding to a four-fold increase in records.

Serum Hp was found to have a positive genetic correlation with NKp46+ (0.35), a natural killer (NK) cell marker (Sivori et al., 1997; Storset et al., 2004) that has been linked to reproductive outcome in both humans (Michou et al., 2003; Kwak-Kim and Gilman-Sachs, 2008; Kwak-Kim et al., 2010; Seshadri and Sunkara, 2014) and cattle (Denholm et al., 2017).

**TNF-α**

TNF-α is a pro-inflammatory cytokine and is connected to many immune system cells including B and T lymphocytes and natural killer (NK) cells (Benedict et al., 2003) and plays a key role in the induction of the bovine acute phase response (Kushibiki *et al.* 2003).

Model analyses yielded a highly significant positive phenotypic correlation (0.12, *P*<0.01) between milk and serum TNF-α and, similar to serum Hp, serum TNF-α was also found to be genetically predisposed to increase when circulating levels of NKp46+ were elevated. Significant positive genetic correlations were also found to exist with %PBMC (0.41) and %lymphocytes (0.47), both %PBMC and %lymphocytes have been previously associated with milk fat % at the genetic level, such that levels are elevated with increasing milk fat (Denholm et al., 2017).

**CONCLUSIONS**
Outcomes from the genetic analysis in the present study suggest that immune-associated traits present in the milk of dairy cows are heritable, repeatable and have the potential to describe profiles in the blood, especially in the case of NAb. The strong genetic correlations found between the milk and serum NAb suggests there is potential for using NAb in the milk as a marker for NAb in the blood. Furthermore, the relationships found between NAb, Hp and TNF-α with both health and cellular IA traits are promising and warrant further study. Presently, milk samples are routinely collected for milk recording purposes, and hence, offer a less invasive and cost-effective way to sample and collect valuable and informative IA trait data for use in a variety of areas of predictive modeling.

ACKNOWLEDGMENTS

This research was funded by the Biotechnology and Biological Sciences Research Council (BBSRC, grant no., BB/K002260/1). The Langhill experiment at Crichton Dairy Research Centre, SJD, EW, TMcN, MPC and GR are supported by the Scottish Government Rural Affairs, Food and the Environment (RAFE) Strategic Research Portfolio 2016-2021. The authors gratefully acknowledge all staff at Crichton farm (SRUC, Dumfries, Scotland) for collecting samples and managing animals. Ian Archibald (SRUC, Edinburgh, Scotland) is also gratefully acknowledged for managing the Langhill database and assisting with data extraction.

REFERENCES


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http://dx.doi.org/10.3168/jds.2008-1632.


March, M.D., L. Toma, a. W. Stott, and D.J. Roberts. 2016. Modelling phosphorus...


http://dx.doi.org/10.1016/0301-6226(94)90202-X.
Table 1. Descriptive statistics for the milk and serum immune associated traits

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trait</th>
<th>No. Records</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>CV(^1) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Hp (μg /ml)</td>
<td>3,561</td>
<td>83.87</td>
<td>369.79</td>
<td>440.89</td>
</tr>
<tr>
<td>Serum</td>
<td>NAb(_{KLH})</td>
<td>2,687</td>
<td>0.94</td>
<td>0.30</td>
<td>31.79</td>
</tr>
<tr>
<td>Serum</td>
<td>NAb(_{LPS})</td>
<td>3,570</td>
<td>1.15</td>
<td>0.52</td>
<td>44.90</td>
</tr>
<tr>
<td>Serum</td>
<td>TNF-α (pg/ml)</td>
<td>3,568</td>
<td>1841.74</td>
<td>6435.26</td>
<td>349.41</td>
</tr>
<tr>
<td>Milk</td>
<td>Hp (μg /ml)</td>
<td>2,667</td>
<td>0.97</td>
<td>6.39</td>
<td>659.36</td>
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<tr>
<td>Milk</td>
<td>NAb(_{KLH})</td>
<td>2,667</td>
<td>0.81</td>
<td>0.35</td>
<td>43.84</td>
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<tr>
<td>Milk</td>
<td>NAb(_{LPS})</td>
<td>2,667</td>
<td>0.34</td>
<td>0.24</td>
<td>71.61</td>
</tr>
<tr>
<td>Milk</td>
<td>TNF-α (pg/ml)</td>
<td>2,667</td>
<td>103.29</td>
<td>375.23</td>
<td>363.29</td>
</tr>
</tbody>
</table>

\(^1\) Coefficient of variation
Table 2. Variance components and ratios of milk and serum serological immune associated traits. Estimated variance components (additive genetic, $\sigma_a^2$; permanent environmental across lactations, $\sigma_{pe-a}^2$; permanent environmental within lactation, $\sigma_{pe-w}^2$; residual, $\sigma_e^2$; and total phenotypic, $\sigma_p^2$) of milk and serum immune associated traits obtained from univariate analyses. Ratios of additive genetic variance to total phenotypic variance (heritability, $h^2$) and individual animal variance to total phenotypic variance (repeatability across lactations, rep) calculated from variance components obtained from univariate analyses. Statistically significant values ($P<0.05$) are given in bold, standard errors are presented in parenthesis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trait</th>
<th>$\sigma_a^2$</th>
<th>$\sigma_{pe-a}^2$</th>
<th>$\sigma_{pe-w}^2$</th>
<th>$\sigma_e^2$</th>
<th>$\sigma_p^2$</th>
<th>$h^2$</th>
<th>rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Hp (μg /ml)</td>
<td>0.03</td>
<td>0.04</td>
<td><strong>0.12</strong></td>
<td>1.56</td>
<td>1.75</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.03)</td>
<td>(0.05)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.02)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>Serum</td>
<td>NAb\text{KLH}</td>
<td><strong>0.05</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.12</strong></td>
<td><strong>0.22</strong></td>
<td><strong>0.21</strong></td>
<td><strong>0.34</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.00)</td>
<td>(0.01)</td>
<td>(0.05)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Serum</td>
<td>NAb\text{LPS}</td>
<td><strong>0.02</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.04</strong></td>
<td><strong>0.08</strong></td>
<td><strong>0.24</strong></td>
<td><strong>0.43</strong></td>
</tr>
<tr>
<td></td>
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<td>(0.01)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.09)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Serum</td>
<td>TNF-α (pg/ml)</td>
<td>5.89</td>
<td>8.76</td>
<td><strong>16.18</strong></td>
<td><strong>103.47</strong></td>
<td><strong>134.30</strong></td>
<td>0.04</td>
<td><strong>0.11</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.85)</td>
<td>(5.03)</td>
<td>(4.16)</td>
<td>(2.81)</td>
<td>(3.79)</td>
<td>(0.03)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Milk</td>
<td>Hp (μg /ml)</td>
<td>0.14</td>
<td>0.08</td>
<td><strong>0.53</strong></td>
<td>1.22</td>
<td>1.98</td>
<td>0.07</td>
<td><strong>0.11</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.11)</td>
<td>(0.12)</td>
<td>(0.10)</td>
<td>(0.04)</td>
<td>(0.08)</td>
<td>(0.06)</td>
<td>(0.05)</td>
</tr>
<tr>
<td>Milk</td>
<td>NAb\text{KLH}</td>
<td><strong>0.01</strong></td>
<td><strong>0.01</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.17</strong></td>
<td><strong>0.34</strong></td>
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<tr>
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<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.07)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Milk</td>
<td>NAb\text{LPS}</td>
<td><strong>0.04</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.06</strong></td>
<td><strong>0.11</strong></td>
<td><strong>0.37</strong></td>
<td><strong>0.38</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.01)</td>
<td>(0.08)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>Milk</td>
<td>TNF-α (pg/ml)</td>
<td>1.85</td>
<td><strong>23.11</strong></td>
<td><strong>17.24</strong></td>
<td><strong>76.81</strong></td>
<td><strong>119.02</strong></td>
<td>0.02</td>
<td><strong>0.21</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.26)</td>
<td>(6.73)</td>
<td>(4.40)</td>
<td>(2.41)</td>
<td>(4.66)</td>
<td>(0.04)</td>
<td>(0.04)</td>
</tr>
</tbody>
</table>
Table 3. Results from a multivariate analysis of milk and serum NAb. Additive genetic and phenotypic correlations between milk and serum natural antibodies. Heritabilities are presented on the diagonal and are highlighted in bold with genetic and phenotypic correlations presented above and below the diagonal respectively. All values were statistically significant and standard errors are presented in parenthesis.

<table>
<thead>
<tr>
<th>Immune associated trait</th>
<th>NAb&lt;sub&gt;KLH&lt;/sub&gt; (serum)</th>
<th>NAb&lt;sub&gt;LPS&lt;/sub&gt; (serum)</th>
<th>NAb&lt;sub&gt;KLH&lt;/sub&gt; (milk)</th>
<th>NAb&lt;sub&gt;LPS&lt;/sub&gt; (milk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAb&lt;sub&gt;KLH&lt;/sub&gt; (serum)</td>
<td><strong>0.21</strong> (0.05)</td>
<td>0.99 (0.09)</td>
<td>0.84 (0.09)</td>
<td>0.81 (0.12)</td>
</tr>
<tr>
<td>NAb&lt;sub&gt;LPS&lt;/sub&gt; (serum)</td>
<td>0.69 (0.01)</td>
<td><strong>0.17</strong> (0.06)</td>
<td>0.91 (0.13)</td>
<td>0.94 (0.05)</td>
</tr>
<tr>
<td>NAb&lt;sub&gt;KLH&lt;/sub&gt; (milk)</td>
<td>0.77 (0.01)</td>
<td>0.59 (0.02)</td>
<td><strong>0.16</strong> (0.05)</td>
<td>0.88 (0.10)</td>
</tr>
<tr>
<td>NAb&lt;sub&gt;LPS&lt;/sub&gt; (milk)</td>
<td>0.61 (0.02)</td>
<td>0.77 (0.01)</td>
<td>0.75 (0.02)</td>
<td><strong>0.23</strong> (0.07)</td>
</tr>
</tbody>
</table>
Table 4. Results from multivariate analyses of milk and serum serological immune associated traits and serum cellular immune associated traits. Individual multivariate analyses (5-variate) were carried out for each individual cellular immune-associated trait with 4 serological immune-associated traits. Additional bivariate analyses were carried out between milk and serum serological immune-associated traits but not within. Statistically significant values ($P<0.05$) are given in bold, standard errors are presented in parenthesis

<table>
<thead>
<tr>
<th>Immune associated trait</th>
<th>Phenotypic correlations</th>
<th>Additive genetic correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hp (serum)</td>
<td>NAb$_{LPS}$ (serum)</td>
</tr>
<tr>
<td>Hp (milk)</td>
<td>0.37 (0.02)</td>
<td>0.01 (0.03)</td>
</tr>
<tr>
<td></td>
<td>NAb\textsubscript{LPS} (milk)</td>
<td>NAb\textsubscript{KLH} (milk)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>(0.02)</td>
<td>(0.01)</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
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<td>(0.02)</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.01</td>
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<tr>
<td></td>
<td>(0.02)</td>
<td>(0.03)</td>
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</tbody>
</table>

\textsuperscript{1}KLH, LPS, α (milk); \textsuperscript{2}α (milk)
<table>
<thead>
<tr>
<th>%monocytes</th>
<th>0.02</th>
<th>0.03</th>
<th>0.04</th>
<th>-0.02</th>
<th>0.22</th>
<th>-0.13</th>
<th>-0.17</th>
<th>-0.31</th>
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<tbody>
<tr>
<td></td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.39)</td>
<td>(0.21)</td>
<td>(0.25)</td>
<td>(0.45)</td>
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<tr>
<td>%neutrophils</td>
<td>0.05</td>
<td>0.03</td>
<td>0.00</td>
<td>-0.03</td>
<td>0.06</td>
<td>0.03</td>
<td>0.10</td>
<td>-0.28</td>
</tr>
<tr>
<td></td>
<td>(0.03)</td>
<td>(0.04)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.30)</td>
<td>(0.19)</td>
<td>(0.22)</td>
<td>(0.55)</td>
</tr>
<tr>
<td>%eosinophils</td>
<td>0.02</td>
<td>-0.07</td>
<td>-0.08</td>
<td>0.06</td>
<td>0.64</td>
<td>-0.16</td>
<td>-0.29</td>
<td>0.46</td>
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<tr>
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<td>(0.04)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.31)</td>
<td>(0.26)</td>
<td>(0.26)</td>
<td>(0.56)</td>
</tr>
<tr>
<td>CD4⁺</td>
<td>-0.03</td>
<td>-0.04</td>
<td>-0.03</td>
<td>0.03</td>
<td>0.05</td>
<td>0.00</td>
<td>0.01</td>
<td>0.38</td>
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<tr>
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<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.30)</td>
<td>(0.18)</td>
<td>(0.22)</td>
<td>(0.43)</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>-0.01</td>
<td>-0.01</td>
<td>0.04</td>
<td>0.02</td>
<td>0.23</td>
<td>0.05</td>
<td>0.18</td>
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</tr>
<tr>
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<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.31)</td>
<td>(0.19)</td>
<td>(0.22)</td>
<td>(0.56)</td>
</tr>
<tr>
<td>CD4⁺:CD8⁺</td>
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<td>-0.03</td>
<td>-0.05</td>
<td>-0.01</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
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<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>NKp46⁺</td>
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<td>0.00</td>
<td>-0.02</td>
<td>0.17</td>
<td>-0.18</td>
<td>-0.66</td>
</tr>
<tr>
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<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.30)</td>
<td>(0.17)</td>
<td>(0.21)</td>
<td>(0.81)</td>
</tr>
</tbody>
</table>

1 % Peripheral Blood Mononuclear Cells
2 % of total leukocytes that were PBMC, eosinophils, lymphocytes, monocytes or neutrophils
3 % of PBMC that were CD4, CD8 and NKp46 positive
n.e. Not estimable
Table 5. Results from bivariate analyses of milk and serum serological immune associated traits and health traits. Phenotypic and additive genetic correlations observed between serum and milk serological immune associated traits and health traits. Only phenotypic and additive genetic correlations that were statistically significant (P<0.05) are presented.

<table>
<thead>
<tr>
<th>Health trait</th>
<th>Serological trait</th>
<th>Correlation</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastitis</td>
<td>TNF-α (milk)</td>
<td>0.04 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Mastitis</td>
<td>Hp (milk)</td>
<td>0.15 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Mastitis</td>
<td>Hp (serum)</td>
<td>0.09 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Reproductive problems</td>
<td>NAbLPS (serum)</td>
<td>-0.03 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Lameness</td>
<td>NAbKLH (milk)</td>
<td>0.66 2</td>
<td>0.28</td>
</tr>
<tr>
<td>Lameness</td>
<td>NAbLPS (serum)</td>
<td>0.79 2</td>
<td>0.28</td>
</tr>
<tr>
<td>Lameness</td>
<td>Hp (serum)</td>
<td>0.05 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Other health problems</td>
<td>Hp (serum)</td>
<td>0.06 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Mastitis episodes</td>
<td>Hp (milk)</td>
<td>0.23 1</td>
<td>0.03</td>
</tr>
<tr>
<td>Mastitis episodes</td>
<td>Hp (serum)</td>
<td>0.10 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Reproductive problems episodes</td>
<td>TNF-α (serum)</td>
<td>-0.08 1</td>
<td>0.03</td>
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</tbody>
</table>

1 Phenotypic correlation
2 Additive genetic correlation