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Comparison of natural antibodies measured in milk and blood samples of Dutch Dairy Cattle

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ABSTRACT: To improve resilience of dairy cows, parameters like levels of natural antibodies (NAbs) can be used. The present study aimed to identify differences between levels of NAbs in milk- and blood from dairy cows, and subsequently estimate genetic parameters for NAb immunoglobulin isotypes IgM and IgG. Titres of NAb binding Keyhole Limpet Hemocyanin (KLH), simultaneously measured in blood plasma and milk, were obtained using ELISA. Results revealed that factors like herd, parity and lactation stage influence NAb levels. Moderate positive phenotypic correlations (0.34-0.38) between milk- and blood NAbs were observed, indicating that NAbs from plasma and milk might reflect different aspects of a cow's immune status. Genetic correlations ranged from 0.80 to 0.89 between NAbs in milk and plasma. NAbs in plasma showed slightly higher heritabilities (0.18-0.28) compared to NAbs in milk (0.09-0.24), suggesting both might have potential for genetic selection.
Keywords: Dairy cattle; Natural Antibodies; Genetic parameters

Introduction

In mammals natural antibodies (NAbs) represent an important component of innate immunity, forming a first line of defence and linking innate and specific immunity (Ochsenbein and Zinkernagel 2000). NAbs are defined as immunoglobulins derived from self-renewing CD5+ B-1 cells (Baumgarth et al. 2005). NAbs are found in animals without intentional antigenic stimulation (Tizard, 2009) and have been proposed to reflect the ability of an animal to stay healthy and prolong survival (Boes et al. 2000; Star et al. 2007).

Increasing herd size at dairy farms in the Netherlands and the requirements to reduce antibiotics usage puts pressure on resilience of dairy cows. Together with the rising awareness of animal welfare, increased longevity of dairy cows will be beneficial from an economical as well as an ethical viewpoint. Parameters, such as levels of natural antibodies, might be a novel tool to measure the (general) ability of dairy cows to resist diseases. In chicken NAbs binding Keyhole Limpet Hemocyanin (KLH) were indicative for a higher probability of chickens to live longer during the laying period (Star et al. 2007, Sun et al. 2011). In cattle, NAb levels increase with age (Van Knegsel et al. 2007), and a relationship was suggested between NAbs and body condition, energy balance and milk yield of dairy cattle (Van Knegsel et al. 2007). Other studies on dairy cows showed that NAbs are heritable, with heritabilities ranging from 0.18 to 0.45 (Ploegaert et al. 2010; Thompsom-Crispi et al. 2013; Wijga et al. 2013).

Measurement of NAb levels in milk provide a non-invasive way to obtain a parameter that might be predictive for disease resistance of dairy cows. To study whether NAbs should be measured in plasma or milk, correlations between NAbs in plasma and in milk were obtained. Specific knowledge on this subject is still lacking, therefore in this study the relationship between NAbs binding KLH in milk and in plasma was determined. Also genetic parameters for selection on the level of NAbs binding KLH in both plasma and milk of dairy cattle were estimated.

Material & Methods

Animals and samples. Data used for this study are from the Dutch project 'WeerbaarVee'. In February and March (2011) blood and milk were sampled on the same day from 2919 dairy cows from 29 Dutch dairy farms (number of productive cows > 60) spread over the country. Milk samples were collected from all lactating cows, while blood samples were taken from ca. 70 randomly selected cows per farm (lactating and non-lactating). The pedigree file including 30436 animals for estimating genetic parameters was provided by CRV.

Natural Antibodies. NAb isotypes IgM and IgG binding *Megathura crenulata*-derived Keyhole limpet hemocyanin (KLH) were determined in individual plasma and milk samples by an indirect ELISA procedure. Flat-bottomed 96-well medium binding plates were coated O/N at 4ºC with 1 µg/ml KLH (MP Biomedicals Inc., Aurora, Ohio). After subsequent washing, plates were incubated for 1.5 hrs at room temperature (RT) with the samples (diluted 1:30, 1:90, 1:270, and 1:810). Binding of the antibodies to KLH was visualized using a 1:20.000 diluted rabbit anti-bovine IgGFc labelled with peroxidase (Nordic, Tilburg, The Netherlands) (RABo/IgGFc/PO) and 1:20.000 diluted rabbit anti-bovine IgM labeled with peroxidase (Nordic, Tilburg, The Netherlands) (RABo/IgM/PO). After washing, substrate (tetramethylbenzidine and 0.05% H_2O_2) was added, and 10 min later, the reaction was stopped with 2.5 *N* H_2SO_4. Extinctions were measured with a Multiskan (Labsystems, Helsinki, Finland) at a wavelength of 450 nm. Antibody levels (titers) were expressed as log2 values of the highest dilutions giving an extinction closest to 50% of EMAX, where EMAX represents the highest mean extinction of a standard positive reaction present on each flat-bottomed ELISA-plate (Ploegaert et al. 2010). EMAX was

calculated based on a blood calibrated line for each Elisa plate, for both milk and blood samples. An amount of 3 was added to all obtained milk NAb titers to create positive values.

Statistical analyses. Statistical analyses were performed using SAS-sofware (version 9.2) and *ASREML*-software (version 3.0, Gilmour et al. 2009). Variance components for different NAb samples were estimated with a linear animal model:

$$Y_{ijkl} = \mu + PAR_i + LS_j + herd_k + animal_l + e_{ijkl} \quad (1)$$

Where NAbs were taken as dependent variables and were corrected for herd, parity and lactation stage; where Y is NAb (i.e. Migg= IgG in milk, Migm= IgM in milk, Pigg= IgG in blood plasma and Pigm= IgM in blood plasma), μ is the population mean, PAR is fixed effect of parity (i=class of parities from 0 to 4, where 0 = heifers before first calving, 1=1, 2=2, 3=3 and 4 ≥4), LS is fixed effect of lactation stage (j=class of lactation stages in days in milk (dim), where 1=<60 dim, 2=60-120 dim, 3=120-200 dim, 4=200-305 dim and 5>305 dim), herd is fixed farm effect, animal is random cow effect and e is the random residual. With the estimated variance components narrow sense heritabilities (2) and correlations (3) were calculated:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \quad (2)$$

where σ_a^2 was the additive genetic variance and σ_e^2 was the residual variance.

$$r_g = \frac{\sigma_{a1,a2}}{\sqrt{(\sigma_{a1}^2 \times \sigma_{a2}^2)}} \quad (3)$$

where σ_{a1}^2 was the additive genetic variance of trait 1 and σ_{a2}^2 was the additive genetic variation of trait 2 and $\sigma_{a1,a2}$ was the genetic covariance between trait 1 and 2.

Results and Discussion

Descriptive statistics. All four NAbs (2 isotypes in both plasma and milk) used in this study were normally distributed. Isotype IgG showed lower means compared to IgM in both milk and blood samples (table 1), this may be due do the pentameric structure of IgM, resulting a higher affinity compared to IgG. IgG showed highest variation in blood, which might be explained by the fact that IgG is a more reactive component and influenced by environmental factors like exposure to bacteria and viruses. The present results are in line with previous studies (Wijga et al. 2013, Thompson-Crispi et al. 2013). Ploegeart (2010) found that NAbs in milk are related to mastitis. If mastitis occurs: more NAbs may be transported to the udder, causing a sort of biological filter between blood and milk NAb levels. Therefore it is possible that the observed NAb levels within milk reflect the udder health status of a cow at a certain moment whereas NAb levels measured in plasma reflect also other 'attacks'. Like other studies (Ploegaert et al. 2011, Thompson-Crispi et al. 2013) the present study also showed lower NAb levels in milk compared to levels in blood. This might be because NAb levels in milk are more reflective of local tissue (udder) and can react indirectly after being transported from blood into milk. On the other hand, NAb levels in plasma may reflect more the cow's overall (health) status. Consequently phenotypic correlations are moderate but positive. This indicates that NAbs measured in milk and plasma reveals different information regarding the immune status of (parts) of the cow.

Table 1: Overview of uncorrected means, maximal/minimal values and standard deviations (SD) of NAb levels of 2 isotypes (IgM and IgG) measured in milk (M) and bloodplasma (P)

Antibody	N	Mean	Max	Min	SD
M-IgM	2600	5.59	11.5	2.3	1.07
M-IgG	2600	3.25	10.2	0.4	0.83
P-IgM	2017	8.56	11.7	5.1	0.88
P-IgG	2015	5.03	10.8	1.2	1.24

Heritability. Positive heritabilities (0.09-0.28) were found for all NAbs included in this study. Heritabilities for both milk- and plasma NAbs were comparable (table 2). Where heritability of IgM in blood was highest and of IgG in blood was lowest. This corresponds with the hypothesis that IgM is more naturally present instead of being produced as a reaction to the environment (Ehrenstein & Notley, 2010) like IgG, and therefore more heritable. Ploegaert et al. (2010) showed across herd heritability of 0.36 and intra herd heritability of 0.42, but they did not discriminate between isotypes. Thompson-Crispi et al. (2013) found a heritability of 0.32 for IgG and 0.18 for IgM both binding KLH, which is opposite to current findings, possibly because in that study cows were immunized before sampling. We conclude that NAbs are heritable. This genetic background of NAbs can in the future possibly be used to develop a useful tool for breeding programs that focus on increasing resilience of a dairy cow.

Table 2: Differences of heritabilities and correlations[&] between 2 different NAb-isotypes (IgM and IgG) measured in Milk (M) and Blood plasma (P))

	M-IgM	M-IgG	P-IgM	P-IgG
M-IgM	**0.24**	0.37	0.38	0.09
M-IgG	0.58	**0.09**	0.09	0.34
P-IgM	0.80	0.19	**0.28**	0.31
P-IgG	0.28	0.89	0.11	**0.18**

[&]Heritabilities on diagonal, phenotypic correlations above diagonal and genetic correlations below diagonal.

C**orrelations.** Phenotypic correlations between plasma and milk NAb levels were positive but moderate, this might indicate that NAbs in milk reflect a more local part (udder), whereas NAbs in blood reflects the total health status of a cow. Genotypic correlations of IgG and IgM between blood and milk are highly positive

(0.80/0.89 resp.), so IgM and IgG measured in both blood and milk are expected to originate from the same (group of) genes to a substantial level. This suggests a subsequent common heritable background of the isotypes measured in plasma and milk, but that the isotypes have different and various tasks in blood or milk.

Conclusion

Simultaneously measured NAbs in both blood and milk only showed moderate phenotypic correlations and high positive genetic correlations, indicating that they are produced by the same gene(s). NAb isotypes IgG and IgM binding KLH in both milk and blood are heritable, where IgM is more heritable than IgG. Further research is needed to acquire detailed information on reasons for variability in NAb levels influenced by factors like farm management, genetic background and functional differences between milk- and blood NAbs, and especially correlations between NAbs and diseases.

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