QTL affecting innate immunity in Dutch dairy cattle

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Introduction

Innate immunity plays an important role in preventing (barrier function) or combating infection (effector function). Knowledge of innate immunity in cattle is scarce. A potentially important humoral component of innate immunity is formed by Natural Antibodies (**NAb**) (Elluru *et al.* 2008). In mammals, NAb are preferentially derived from CD5+ B (B1) cells (Casali and Notkins, 1989) and are mainly present in the Immunoglobulin (Ig) M isotype class (Boes 2000). NAb are defined as antibodies present in non-immunized individuals and characterized by a broad specificity repertoire with usually low binding affinity. A high proportion of NAb binds so-called pathogen associated molecular patterns (**PAMP**); these represent antigens that are shared by 'classes' of microbes. Important PAMP are: lipopolysaccharides (**LPS**) present on Gram-negative bacteria, such as *E. coli* or *Salmonella spp*; lipoteichoic acid (**LTA**) present on Gram-positive bacteria, e.g. *S. aureus*; and peptidoglycan (**PGN**) present on Gram-negative and Gram-positive bacteria. A relation has been suggested between titers of NAb binding keyhole limpet hemocyanin (**KLH,** the model antigen) and LPS in the peri-partum period on the one hand, and body condition, energy balance, milk yield, and plasma cholesterol concentration on the other hand (Van Knegsel *et al.* 2007). Increased incidences of (infectious) diseases, such as mastitis, endometritis, and laminitis, have been related to suboptimal immune function in the periparturient period (Mallard *et al.* 1998) and reflected in diminished mitogen-induced lymphocyte proliferation (Kehrli *et al.* 1989), decreased serum IgG concentrations (Detilleux *et al.* 1995), and lowered antibody responses (Mallard *et al.* 1997). A study of Ploegaert *et al.* (2010a) showed that NAb titers to LTA, LPS, and PGN as components of bacteria cell-walls, can be reliably measured in bovine milk. Furthermore, such parameters may represent a relevant image of the current animal and the mammary gland condition. Ploegaert et al (2010b) found heritability estimates ranging from 0.13 to 0.53 for NAb in milk of Dutch Holstein-Friesian (HF) heifers. The highest heritabilities were found for total NAb binding KLH and LTA, and IgM and IgA binding LTA in milk. Increased level of NAb binding antigen reflects the cow's ability to mobilize an immune response against infection. This could enable the identification of cows which are at risk of developing mastitis during their productive life. Detecting genes responsible for the biological relation between NAb and mastitis occurrence can be used in cow selection (Detilleux *et al.* 2006). Gene(s) associated with improved response of innate immune system to pathogens might not only help in breeding healthier animals, but would also reduce costs of the mastitis treatment.

Therefore, NAb measured in milk might be a good parameter of humoral innate immune system, and they might shed light on the involvement of humoral innate immunity in dairy cattle disease resistance. Here we report the QTL analysis for NAb in the scope of the Dutch Milk Genomics Initiative.

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Materials and methods

Animals and data collection. In total 1,912 Holstein-Friesian cows in their first parity were used. All animals were between 66 and 282 days of lactation. Morning milk samples were collected for analyses between February and March 2005.

Genotypes. DNA was available for cows from five large families (animal numbers 199, 188, 180, 179, and 100) and two smaller families (with 29 and 24 cows). The set of 3,073 SNP was available for four sires from large half-sib families. Further the linkage maps for all 29 bovine autosomes (BTA) were built in CriMap. More details in Schopen *et al.* (2009).

Traits/phenotypes. Total NAb titers (for PAMP: KLH, LPS, LTA, PGN) and Immunoglobulin (Ig) isotypes of NAb binding LTA (IgG1, IgM, IgA) were determined by ELISA in defrosted milk samples of all 1,912 cows. In total seven traits were used in QTL analysis. More details in Ploegaert *et al.* (2010a).

QTL analysis was performed on data of 849 genotyped cows from five large half-sib families and two small half-sib families with a set of 1,341 SNP. The NAb titers were adjusted for several systematic environmental effects: day of lactation, age at calving, season of calving, and herd. QTL analysis was based on multi-marker regression approach for half-sib families (Knott *et al.* 1996) and performed for each of 7 traits on each BTA in Fortran 95. Test statistic used across families calculated every 0.1 cM of the chromosome to test for the presence of a QTL. 10,000 permutations of phenotypic data within all 7 half-sib families were used to determine significance ($P_{chromosome}$) of chromosome-wise QTL. Genome-wise significance was estimated with Bonferroni correction (de Koning *et al.* 1999). Moreover, 1,000 bootstraps were performed to estimate 95% confidence interval for all the locations of QTL. The power of the design applied in this study is larger than 0.8.

Results

Detected QTL. In total nine significant QTL ($P_{genome}<0.05$) were detected on 8 different chromosomes (Table 1). In addition, 17 suggestive QTL were detected ($P_{chromosome}<0.05$) (no details given).

Table 1: Genome-wise (P_{genome}) significant locations with confidence interval (CI) of the most suitable QTL for Natural Antibodies binding PAMP: KLH, LPS, LTA, PGN and isotypes of antibodies binding LTA: IgG1, IgM, IgA.

BTA	Trait	QTL location (cM)	CI for QTL location	F-value	P_{genome}
3	KLH	52.8	4.8-123.0	4.81	0.009
3	LTA	125.9	46.5-125.9	4.93	0.002
6	LTA-IgM	30.0	28.3-122.3	3.50	0.034
7	LTA-IgM	0.0	0.0-125.5	3.38	0.045
8	PGN	81.5	32.7-91.0	5.18	0.001
9	LTA-IgM	59.8	0.0-76.5	3.68	0.021
12	LPS	31.6	21.5-75.4	4.91	0.002
18	LTA-IgM	71.1	5.4-82.8	3.61	0.026
23	LTA-IgA	20.9	0.0-51.8	3.70	0.030

QTL effects. The QTL explaining the largest proportion (4.1%) of phenotypic variance was found for NAb binding PGN on BTA8 (Table 2). For IgM isotype of NAb binding LTA, four significant QTL were found which in total explained 11.0% of the phenotypic variance which corresponds to about half of the genetic variance in that trait. Other significant QTL were present only once for each trait on non-overlapping chromosomal locations (Table 2). The heritabilities for NAb, estimated in the same material by Ploegaert *et al.* (2010b), ranged from 0.13 to 0.53 (Table 2).

Table 2: Overall heritabilities (h^2) and proportion (V%) of phenotypic variance explained by significant QTL (V%) (h^2 from Ploegaert *et al.* 2010b)

BTA	Trait	QTL location (cM)	V%	h^2
3	KLH	52.8	3.6	0.42
3	LTA	125.9	4.0	0.32
6	LTA-IgM	30.0	2.7	0.47
7	LTA-IgM	0.0	2.6	
8	PGN	81.5	4.1	0.13
9	LTA-IgM	59.8	2.9	
12	LPS	31.6	4.0	0.15
18	LTA-IgM	71.1	2.8	
23	LTA-IgA	20.9	2.9	0.53

Discussion

No other study was yet conducted to investigate QTL affecting NAb titers binding KLH, LPS, LTA, and PGN in dairy cattle. Genetics underlying NAb titers binding KLH, LPS, and LTA was studied in two different populations of laying hens (Siwek *et al.* 2003, 2006: Biscarini *et al.* 2009). These studies demonstrated QTL affecting LPS, LTA, and KLH. Recently a QTL study on calves was conducted by Maltecca *et al.* (2009) revealed suggestive QTL for serum IgG levels on BTA 20.

Large confidence intervals (CI) were observed for all traits (Table 1), often comprising the length of the whole chromosome. It does not allow a direct identification of specific genes underlying the detected QTL. However, on chromosomes on which significant QTL were found, in other studies genes related to mastitis resistance and immune traits were detected. A search for genes was done using www.ncbi.nlm.nih.gov database with the 1 cM = 1 Mbp (10^6 bp). Regions on two chromosomes BTA12 and BTA23 seem to link with B cells. These associations are interesting, because most probably NAbs are derived from CD5+ B cells (Casali and Notkins 1989). Moreover, on BTA23 several genes involved in immune response are located. First, alleles of BoLA *DRB3* locus associated with mastitis have been reported at position 26,329 – 26,410 kbp (Dietz *et al.* 1997). Second, the gene for interleukin 17 (*IL17*) at position 25,119 – 25,121 kbp. *IL17* comes from cytokines family involved in several immune functions, for example, stimulation and production of other cytokines: chemokines and prostaglandins (Iwakura *et al.* 2008). *IL17* was recently found to be associated with NAb in laying hens (Biscarini *et al.* 2009). Additionally, in the study of Sahana *et al.* (2008), QTL affecting clinical mastitis (mapped between the markers *BM4208* and *INRA084*) on BTA9

was found to be pleiotropic and affecting also milk yield in one of the studied Nordic red cattle breeds.

Conclusion

A number of QTL were found for NAb titers in cattle binding KLH, LPS, LTA (plus its isotypes: IgM and IgA), and PGN. These QTL can help to increase our understanding of the biology of innate immunity and to improve the efficiency of selection with improved innate immunity. The results agree with finding in laying hens. Further research is needed to find the genes underlying the QTL and the impact on disease resistance.

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