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Mapping QTL Controlling Milk Somatic Cell Counts in Sheep and Goat Support the Polygenic Architecture of Mastitis Resistance

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ABSTRACT: QTLs for milk SCC were detected in two dairy sheep and goat designs including 1009 AI rams and 2254 Alpine and Sanen goats sired by 20 AI sires, respectively. Animals were genotyped with the 50K ovine and caprine SNP chips. Linkage and Linkage disequilibrium analyses were performed using the QTLmap software. The high number of chromosomal regions detected tended to confirm the polygenic architecture of the mastitis resistance trait. Many of these QTLs were population specific. Hence, it is difficult to tell extent to which the genetic architecture of resistance truly differs between species and breed. High significance combined to narrow confidence intervals of a few QTLs (OAR3 in sheep) and commonalties between breeds and species (OAR11 and CHI19) might help prioritizing chromosomal regions for further fine mapping and functional characterization
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Introduction

Amongst infectious diseases in dairy ruminants, mastitis is of major importance because of its high frequency and related costs. In dairy small ruminants mastitis is the primary health reason for involuntary culling, and prevalence of subclinical mastitis is 20-30% per lactation. Accumulating research results over the last decades give strong evidence that the host's response to control udder health is under genetic control in dairy ruminants (Rupp and Foucras (2010)). Existence of QTL for mastitis resistance in cattle has been reported on almost all chromosomes (Rupp and Foucras (2010); www.animalgenome.org/QTLdb/). Among studies, the common mastitis resistance phenotype was milk somatic cell count (**SCC**), measured periodically over lactation, and clinical mastitis occurrence.

In contrast to cattle, very little literature exists in sheep (Gutiérrez-Gil et al., (2007); Raadsma et al., (2009)) and no information is available in goat. Because of the multifactorial nature of the resistance trait and specificity of the disease according to the species, it is unlikely that QTL for mastitis resistance found in one dairy species can be directly transposed to the other species. Indeed, dairy sheep and goat mastitis is mainly characterized by subclinical cases caused by Negative Coagulase Staphyloccoci with few clinical mastitis cases or streptococci– caused infections when compared to dairy cattle. In addition, whereas the SCC means in both sheep and cattle species are similar (300 to 500 *103 cells/mL), SCC in goat is higher (around 1 million cells/mL). In goat, several specific features in the biological interpretation of SCC do exist, such as apocrine milk secretion, cellular sub-populations, and importance of non-bacterial variation factors. For goat, there was no commercial SNP tool available before 2011. In 2010, however, the release of the goat sequence by the Beijing Genomics Institute (Shenzen, China) paved the way to an international effort toward constructing project and tools for goat genomics. The International Goat Genome Consortium (**IGGC**) was created. In the frame of the IGGC, and with the support of 3SR and the French dairy goat industry, a 50K SNP goat chip was released by Illumina end of 2011 (Tosser Klopp et al. (2014)).

The objective of this paper was to report QTL detection for SCS in sheep and goat, based on two French programs using high density (≈50K) SNP chips in ovine Lacaune and caprine Alpine and Saanen commercial populations.

Materials and Methods

Sheep and goat QTL populations. The QTL population in sheep consisted in a total of 1,009 rams of the Lacaune sheep breed distributed in 33 half-sib families in a so called grand-daughter design. Family size averaged 30.2 (±9.7) sons and ranged from to 18 to 54. For further confirmation of QTL, an additional set of rams (n=117) among those present in the two Lacaune breeding center were genotyped.
QTL detection in goat was performed in a daughter-design population of French dairy goat from Alpine and Saanen breeds. Genotyped animals consisted in a total of 2254 goats from 209 herds and sired by 20 Alpine and Saanen artificial insemination bucks (11 Alpine and 9 Saanen families).

Mastitis phenotype. The phenotypic measurement was the milk somatic cell count (SCC) measured for lactating females and recorded as part of the milk recording system. A lactation average was then defined for sheep and goat. In sheep, the SCC was collected on average three times per lactation in first and second parity. SCC was then log-transformed to somatic cell score, SCS [log2 (SCC/100) +3] for normalizing the data distribution and averaged per lactation to LSCS as described by (Rupp et al. (2003)). In goat milk SCC were measured monthly over a 250-day lactation period. The lactation average was defined

as the weighted mean of SCS adjusted for lactation stage as described in (Rupp et al. (2011)). For mapping in the sheep granddaughter design, we used twice the daughter yield deviation (DYD) calculated for SCS from the national genetic evaluation procedure. In the goat daughter design, the studied trait was the yield deviation for the LSCS. Both were derived from national genetic evaluation programs.

Genome-wide genotyping. All 1009 rams were genotyped using the Illumina Ovine SNP50 BeadChip assay. The SNP quality control included check for SNP call rate (>0.97%), minor allele frequency (>1%) and Hardy-Weinberg equilibrium ($p<10^{-6}$) and pedigree consistency. After edits, a total of 41,501 autosomal SNPs, distributed on ovine chromosomes OAR1 to OAR26 were genotyped. The marker order and positions were based on the Ovine Assembly v2.0 (http://www.livestockgenomics.csiro.au/cgi-bin/gbrowse/oarv2.0/).
The 2274 goats were genotyped with the 50K SNP Illumina goat chip (Tosser-Klopp et al. (2014)). SNP were mapped on the 29 caprine autosomes using the assembly information provided by the Chinese group of Dong et al. (2013). Quality control of genotype included SNP call rate (>99%), minor allele frequency (>1%), Hardy Weinberg equilibrium ($p<10^{-6}$) and pedigree consistency. After editing, a total of 49,647 SNP were validated for further analyses out of the 53,347 synthetized SNPs.

QTL detection. QTL detection was based on linkage analyses (**LA**) or linkage disequilibrium analyses (**LD)** using the QTLmap software (Elsen et al., 1999). Both LA and LD methods involved a first step for the estimation of the most likely phase for sires (Elsen et al. (1999)). For LA, interval mapping was performed by a likelihood ratio test (LRT) using within-sire linear regression (Elsen et al. (1999)). For LD, a regression analysis of the phenotypes on founders' haplotypes is computed for every haplotypes of 4 consecutive SNP along the chromosome (Legarra et Fernando (2009)). Chromosome- and genome-wise significance thresholds were estimated by permutation test (LA) and simulation (LD). In goat, detection were implemented for the mixed population of independently for each of the two Alpine and Saanen breeds.

Results and Discussion

QTL detection for LSCS in sheep. The phenotypic measurement was the milk somatic cell count (SCC) measured for Using LA, seven QTL for LSCS, on OAR3, 8 10, 11, 14, 16 and 20, were significant at the chromosome wide threshold of 5% (Figure 1). Many other QTL regions were detected by LD: eight reaching genome wise significance of 0.1% on OAR 3, 4, 8, 9, 11, 16, 19 and 26. (Figure 1 ; Figure 2). For the above mentioned regions, however, there was no correspondence with previously published QTLs in AwassiXMerinos backcross (Raadsma et al. (2009)) or Churra sheep (Gutiérrez-Gil et al. (2007)).

The highly significant QTL on OAR3 (Figure 2), whose localization was identical with both LA and LD and with narrow confidence interval (LD 95% CI = 200 kb) was selected for further confirmation. Accordingly, a new population of 117 genotyped Lacaune rams, that could be phase on the QTL position with certainty, was analysed. Phases (four successive SNPs) carried by the animals were categorized into four groups: very unfavorable (1 phase) ; intermediate; favorable (3 phases) and very favorable (2 phases). An ANOVA was performed to test the effect of the phase on the EBV for SCS. The model also included an effect of the sire of rams (n=12). EBV for SCS are expressed in genetic standard deviation with the sign inverted, thus ranging from -3 to +3 in the global population (means=0.30±0.59 in the selected sample of young rams). SCS EBVs of rams carrying the most favorable phase were significantly higher (+0.82) than for rams carrying the most unfavorable phase, thus confirming the QTL effect in a different population.

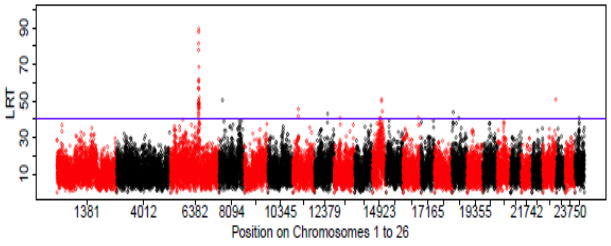

Figure 1: Manhattan plot for the discovery genome-wide association (likelihood ratio test, LRT) of lactation average SCS in sheep (Lacaune breed). The blue horizontal line refers to the 1% genome-wise significant threshold (average over 26 chromosomes).

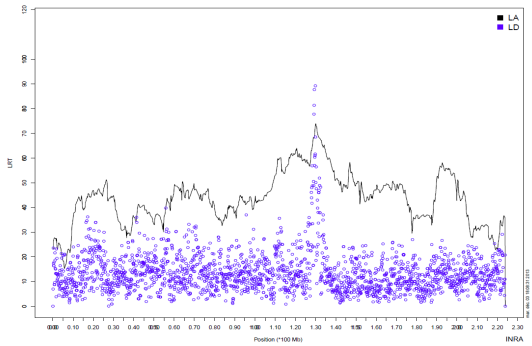

Figure 2: Profile of the likelihood ratio test ((likelihood ratio test, LRT) values for lactation average SCS on ovine chromosome OAR3 for both linkage (LA) and association (LD) analyses.

QTL detection for LSCS in goat. The number of significant chromosomal regions detected using LA or LD is shown in Table 1. The number of QTL for LSCS found varied according to the method (LD vs LA) and to the population analyzed (full population vs Alpine and Saanen breeds separately). For LA, 5%

chromosome-wise significant QTL were detected on CHI1 (Alpine), CHI17 (mixed breed) CHI19 (Saanen and mixed breed) and CHI22 (Saanen). Many more significant QTLs were found with LD method when compared to LA method (Table 1 and Figure 3). A higher number of QTLs was found in the Saanen breed than in the Alpine breed. This results was in agreement with the higher heritability of LSCS in the Saanen breed (h^2=0.24) when compared to the Alpine breed (h^2=0.20), as reported earlier (Rupp et al. (2011)).

Table 1 : Number of significant chromosomal regions for lactation average SCS according to the population studied, for both linkage (LA) and association (LD) analyses.

	Sheep	Goat		
Method	Lacaune	All	Alpine	Saanen
LD[1]	7	16	27	17
LA[2]	8	2	1	2

[1] 1% genome-wise significant QTL [2] 5% chromosome-wise significant QTL.

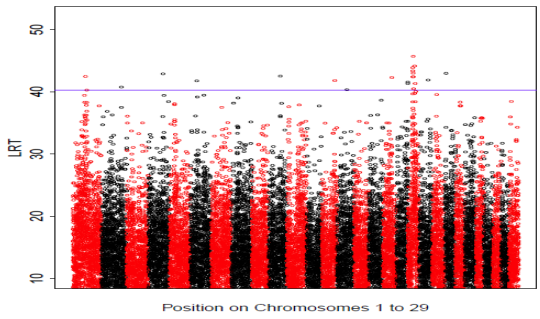

Figure 3: Manhattan plot for the discovery genome-wide association (likelihood ratio test, LRT) of the lactation average SCS in goat (Mixed population of the Saanen and Alpine breeds). The blue horizontal line refers to the 1% genome-wise significant threshold (average over 29 chromosomes).

Among the numerous regions detected in goat, three regions on chromosome 16, 19 and 21 were of high interest because they were detected in the two breeds and because the significance level was especially high (CHI 19). Those regions are of primary interest for further fine mapping and validation in independent populations. In these three cases, however, the position from the LD analyses were approx. 10 Mb distant between breeds (7, 7.10 and 9.40 Mb apart for chromosome 16, 19 and 21, respectively) making it necessary to conduct a fine mapping step with additional SNPs before any candidate gene and causal mutation can be hypothesized.

Comparison across sheep and goat. Comparative mapping between the sheep and goat mastitis QTL regions showed that the QTL on goat chromosome 19 was in the homologous sheep region carrying a QTL for LSCS in the Lacaune dairy sheep population OAR11. For both goat and sheep, however, the latter QTL showed a large confidence interval so that comparative mapping might not help narrowing down the causative genes. Also the region is particular rich in gene, making the nomination of candidate genes difficult.

Conclusion

The methods and designs used in this study have been successful in identifying QTL for mastitis resistance (using LSCS phetnotype) in sheep and goat using the 50K SNP chips. The high number of chromosomal regions detected tends to confirm the polygenic architecture of the resistance trait. Hence, from the population-specific results, it is difficult to tell extent to which the genetic architecture of resistance truly differs between species and breed. Narrow confidence intervals (OAR3 in sheep) and a commonalties between breeds and species (OAR11 and CHI19) might help prioritizing chromosomal regions for further fine mapping and functional characterization.

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