QTL Detection For Resistance To *Haemonchus contortus* In Sheep: A Preliminary Study Using The OvineSNP50 Beadchip

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Introduction

Emergence of anthelmintics resistances and arising consumers concern about drug residues in food tend to make parasitic infection a major veterinary issue into a curse for the sheep meat industry. Over the past few years, studies using microsatellites markers have reported several QTL for resistance to nematodes in sheep (Dominik 2005; Bishop & Morris 2007). One of these studies mapped 14 QTL for resistance against *Haemonchus contortus* (*H.contortus*) in a backcross design between a resistant and a susceptible breed (Moreno *et al.* 2006). Using this latter design, this paper reports the first results of a whole-genome QTL detection study using the OvineSNP50 Beadchip.

Material and methods

Animals and experimental infection. The experimental population was the same as already described in Moreno et *al.* (2006). Four out of the 5 F1 Martinik Black Belly*Romane rams that had been backcrossed with purebred Romane ewes at the La Sapinière experimental farm (Osmoy, France) were considered. The 1,002 considered lambs were experimentally infested with 10,000 *H.contortus* larvae in 2 successive infections separated by a drenching and a 15 days recovery period. At days 25 and 35 Fecal Egg Count (FEC) were measured in both infections while Packed Cell Volume (PCV) were determined both at day 0 and day 41 during the 1st infection (PCV1) and at day 41 during the 2nd infection (PCV2). Differences between PCV while being infested and PCV at day 0 were also considered (Var-PCV1 and Var-PCV2 for the 1st and 2nd infection respectively). FEC values were applied a fourth square root transformation to fit normality, and the mean values during infection, *i.e.* FEC1 and FEC2 for 1st and 2nd infection respectively, were considered for subsequent analyses.

Molecular data. All lambs were genotyped using the Illumina, Inc. OvineSNP50 Beadchip (http://www.illumina.com/products/ovine_snp50_whole_genome_genotyping_kits.ilmn).

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Genotyping of the 54,977 SNPs was performed by LABOGENA (Jouy-en-Josas, France). To ensure the quality of the genotypes, control parameters were chosen in accordance with recent literature (Miyagawa *et al.* 2008; Neale & Purcell 2008), *i.e.* minor allele frequency (MAF) and SNP call rate. In addition parents/offspring inheritance discordances were checked based on inheritance Mendelian rules. The SNP50_Breedv1.map, provided by the International Sheep Genomics Consortium (ISGC, http://www.sheephapmap.org/), was used as reference (49,034 SNPs).

Statistical analyses. For every lamb/SNP couple, we inferred which allele was inherited by lambs from their sire. This was thus feasible if the sire was heterozygous and its lamb homozygous (dams were not genotyped). The null hypothesis, *i.e.* no difference of effect on resistance traits between the 2 paternal inherited SNP alleles, was then tested against the alternative hypothesis, *i.e.* difference of effect. For both hypotheses, sire was fitted as a fixed effect, as well as the environmental effects: sex, management group, born litter size and reared litter size. Two restrictive significance thresholds were considered using a Bonferroni correction (Lander & Kruglyak 1995). For a targeted genome-wide significant threshold (at the 5% level), a stringent p-value cutoff was fitted as p< (0.05/No. informative SNPs), *i.e.* $p<1{,}5.10^{-6}$. A second suggestive threshold was chosen (cutoff p-value of $3{,}0.10^{-5}$) that corresponds to the probability that one SNP is declared associated by chance, *i.e.* p< (1/No. informative SNPs).

Results and discussion

Marker information. A 99.96% reproducibility was observed thanks to 50 duplicates. Every genotyped lamb had a call rate beyond 98%. Only one sire whose call rate was too weak (92.8%) had his genotype inferred from its progenies. Monomorphic or ungenotyped SNPs (n=4532) were removed. An additional subset of 1220 SNPs that were not found in the SNP50_Breedv1.map was also discarded. In addition, 27 SNPs were eliminated since more than 5% of the genotyped individuals exhibited Mendelian inheritance discordance for these markers.

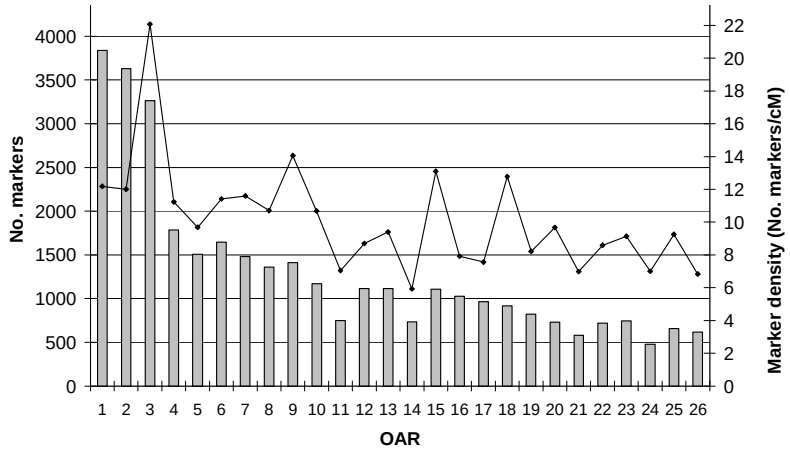

Figure 1. Informative SNP distribution along the genome after correction

Edits further included 82 SNPs with a call rate >90% as well as 11 SNPs with a MAF<5%. SNPs with unknown location (n=304) and SNPs located on gonosomes (n=1503) were not considered for analysis. Finally 34,177 informative SNPs out of the 54,977 originally genotyped SNPs remained for analysis (Figure 1). Every chromosome carried around 1000 SNPs except the first 3 that carried more than 3000 SNPs. SNP density was in a 6 to 13 SNPs/cM range, except for OAR3 (22 SNPs/cM). In addition, at least 2 sires were informative for 60% of the SNPs.

QTL for resistance to *H. contortus*. Sixteen SNPs were associated with one of the resistance traits, mostly with FEC measures (Table 1). In comparison with the previous study based on microsatellites (Moreno et *al.*, 2006), 4 out of 6 regions were confirmed using SNPs. The previously mapped QTL on OAR5 for PCV1 was also detected but for another trait, *i.e.* Var-PCV2 (considering that 1Mbp=1cM). In addition, OAR7 had an overwhelming weight in this study, with 11 SNPs being associated with FEC1. Eight of these SNPs were located in the 35-60 Mbp range thus corresponding to the QTL found by Moreno et *al.* (2006). Only two microsatellites had been genotyped on OAR7 in this previous study, thus certainly explaining that this QTL had only been detected at a suggestive linkage.

Table 1. QTL detected in the microsatellite study (Moreno & *al.* 2006) and using SNPs

OAR	Microsatellite study	SNP study	
	QTL : trait (location at LRT_{max} in cM)	**QTL: trait (No. suggestive SNPs, location in Mbp)**	**P-value[a]**
3	FEC2 (168)	Var-PCV2 (1 SNP,146)	$4{,}1.10^{-6}$
5	FEC2 (53), PCV1 (90)	Var-PCV2 (1 SNP, 89)	$2{,}5.10^{-5}$
7	FEC1 (34)	FEC1 (11 SNPs, 20-82)	$3{,}0.10^{-6}$ -$3{,}0.10^{-5}$
12	FEC1 (61)	FEC2 (2 SNP, 35-44)	$2{,}5.10^{-5}$-3.10^{-5}
14	-	FEC2 (1 SNP, 7)	$5{,}6.10^{-6}$

a: *: SNP genome-wide suggestive p-value

Moreover, 2 SNPs located on OAR12 around 40 Mbp were associated with FEC2 trait (Table 1), and an additional SNP association with FEC1 at 60 Mbp almost reached suggestive threshold ($p=5{,}8.10^{-5}$). These findings, in agreement with the QTL found by Moreno et *al.* (2006) for FEC1, make this area a strong candidate involved in resistance in both 1st and 2nd infection.

Surprisingly only 1 SNP was detected on OAR3 for Var-PCV2 around 150 Mbp, *i.e.* 10 Mbp upstream from the *IFNG* locus, while this gene was in the confidence interval of the QTL mapped by Moreno et *al.* for FEC2 (Moreno, personal communication). We did not find any suggestive association with SNPs in the *IFNG* locus, as usually reported in other QTL studies for resistance to *H.contortus* (Bishop & Morris 2007). In the same way, the Major Histocompatibility Complex (*MHC* locus), often proposed as a functional candidate for resistance to *H.contortus* (Dominik 2005), could not be significantly associated with any of the traits. This lack of SNP association on OAR20 is in agreement with findings of the

previous study (Moreno et *al.* 2006). An additional suggestive association on OAR14 was found for FEC2.

It is noteworthy that no SNP association could reach the significant threshold, and that no QTL could be found on OAR13 and OAR23 as did Moreno et *al*. (2006). However, given the marker density, every SNP are not independent between each other as assumed while considering a Bonferroni correction. As a result the significant threshold must be lower than the considered one. Very interestingly, considering a p-value cutoff of 10^{-4} resulted in detecting some putative QTL on OAR13 for FEC1 and FEC2, on OAR2 for Var-PCV1 and FEC1, and on OAR3 and OAR9 for FEC2. Hopefully these regions will be confirmed using more elaborated analyses like LD-LA approach.

Conclusion

This study is one of the first examples of QTL detection based on the ovineSNP50 Beadchip. Sixteen SNPs were found in association with resistance traits to *H.contortus* infection. The QTL previously mapped on OAR3, 5, 7 and 12 using microsatellites (Moreno *et al.* 2006) were confirmed. So far we only performed a single marker linkage analysis and applied very restrictive thresholds. It is therefore very likely that additional QTL will be found in further more elaborated analyses using multi-marker and LD-LA approaches.

Acknowledgements

The authors are grateful to the staff of the INRA experimental farm of La Sapinière (Osmoy, France) for managing the experimental flock, and to F. Guillaume for his advice while handling SNP data. LABOGENA is acknowledged for genotyping the animals. This project was supported by the french Region Centre, the Animal Genetics department of INRA, ANR and APISGENE ('SheepSNPQTL' project) and EU grants ('FEOGA' 024/m/1873).

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