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Validation of QTL Affecting Resistance to Nematodes in Sheep Identified in a Back-Cross Design in a Pure Breed Population

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ABSTRACT: Gastro-intestinal nematodes are of major concern to sheep health worldwide. Identifying the causative genes responsible for resistance would augment the efficiency of selection. Previously, back-cross (BC) sheep (Black Belly * Romane breeds) were genotyped with a 50K SNP chip and were measured for faecal egg count after two successive experimental challenges by *H. contortus*. The most significant QTL were identified on chromosomes 5, 12, 13, 21. A customized assay including 1000 SNP was created to increase the density of marker coverage in these regions and others major QTL regions affecting parasitism resistance in natural infection conditions. In the present study, 277 Romane lambs were experimentally challenged with H contortus and genotyped with the dedicated 1000 SNP. Six out of the eight tested QTL regions have an effect in the Romane pure Breed population.
Keywords: Nematode; Resistance ; Sheep ; SNP

Introduction

Gastrointestinal nematodes constitute a major cause of production losses and mortality in grazing ruminants (Mandonnet et al. (2005); Davies et al. (2006)). Anthelminthic drenching has been the main control strategy for the last 50 years. However, the ecotoxicology of some drugs has been clearly demonstrated and several parasite populations throughout the world have developed resistance to one or several classes of anthelmintic. Thus, selection of animals naturally resistant to intestinal nematodes appears as a complementary control strategy for nematode fight.

Resistance is classically measured by faecal egg count (FEC), an indirect measure of worm population and in relation to the ability of the host to regulate establishment, development, fecundity and survival of worms. However, FEC-based genetic selection requires selection candidates to be challenged. Moreover, FEC is time consuming and expensive because of the large number of coprological examinations required. Classical or genomic selection based on FEC is therefore difficult to implement on a large scale. Selecting for resistance would be substantially simplified if genes determining resistance and susceptibility were known, making it possible to select animals on their genotype.

QTL having an effect on resistance to internal parasites in sheep have been accumulated since 10 years. In the 3SR EU project, the main QTL regions were detected on chromosomes 4, 5, 7, 12, 13, 14 and 21 (Sallé et al. (2012); Riggio et al. (2013)). These regions were used to design a dedicated 1000-SNP assay.

Since 4 of these 8 QTL were detected by Sallé et al. (2012) in a back-cross Black Belly*Romane protocol, the idea of the present paper is to check the existence of these QTL in the commercial French Romane breed.

Materials and Methods

Population. The Romane (ex-INRA 401 line, cross Romanov x Berrichon) lambs were produced at the INRA experimental farm "La Sapinière" (Osmoy, Cher, France). 26 males and 179 females were used to procreate 148 male and 129 female Romane lambs. Lambs were kept inside to overcome possible nematode infections.

Genotyping. A 1000-SNP assay was designed to validate the QTL detected on chromosomes 3, 4, 5, 7, 12, 13, 14 and 21 in previous studies performed in frame of the 3SR EU project. Approximately 110 SNP within 5 centimorgans (cM) were chosen by QTL region, using SNP existing in 50K and 800 K SNP chips by mapping them to Oar-v3.1 by BLAST using flanking sequences provided by Illumina and ISGC. Two exceptions were made: on chromosome 12, 262 markers were chosen on 10 cM because the confidential interval was large and on chromosome 21 where 10 SNP were chosen in a region of an obvious candidate gene: pepsinogen. Genotyping was performed using the KASP™ (Kompetitive Alelle Specific PCR) technology ((LGC, Teddington, UK).

Data. The six-month-old sheep were orally infected twice with 10,000 *H. contortus* infective larvae, the two experimental challenges lasting four weeks and being terminated by an oral drench of ivermectin (Oramec®, Merial, 0.2 mg/kg BW). The second infection took place after a

two-month washout period. Faecal samples were taken directly from the rectum on day 24 and day 35 post each infection (FEC1, FEC2, FEC3 and FEC4). FEC were determined using the modified MacMaster technique (Raynaud (1970)) in saturated salt solution with a lower limit of detection of about 30 eggs per gram of faeces. The mean of day 24 and day 35 FEC in first or second challenge were also considered (denoted FEC12 and FEC34 respectively) for further analyses.

Statistical analyses. Individual FECs were fourth-root transformed and averaged within each challenge to create variables FEC1t, FEC2t, FEC3t, FEC4t, FEC12t and FEC34t. A GLM analysis was performed to identify significant environmental effects: sex, litter size, artificial versus natural suckling, management group and all interactions between these effects. Then, SNP effect was tested following the EMMAX association analysis method (Kang et al. (2010)). This model simultaneously estimates the SNP effect while correcting for the fixed environmental effects and fitting a genomic relationship matrix to account for existing kinship. The SNP-based relationship matrix was estimated following the method of VanRaden et al. (2008) using every other SNP not located on the chromosome of interest. Because of the close location of SNP in each tested QTL regions, we considered a complete LD between SNP within the tested QTL regions. Consequently, the genome-wide thresholds were obtained by applying the Bonferroni correction $P_{genome\text{-}wide}=1-(1-P_{SNP})^n$, where n is the number of chromosomes.

Results and Discussion

Phenotype analysis. Means of FEC measured in the second infection were significantly reduced compared to the first infection. It is classically observed in this type of experimental infection (Sallé et al. (2012)). The dynamics and the intensity of FEC were different between the two infections. The main significant fixed effect was the sex: males were more infected than females which is classical (Zuk. (2009)).

QTL validation. 6 out of the 8 tested QTL regions in the present study showed significant effect in the considered Romane population. Among the four main QTL detected by Sallé et al (2012), the QTL located on chromosomes 5, 12 and 13 were confirmed as affecting the first infection FEC (Table 1). However, the effects of the QTL located on OAR12 and 13 on FEC at second infection were not confirmed. The significant markers positions were located at 1 or 2 cM from the maximum likelihood signal found by Sallé et al. (2012). The other tested QTL regions on chromosomes 3, 4 and 7 were chosen because they were identified in other naturally infected populations of the 3SR project (a back cross between Lacaune and Sarda Breeds and a Scottish Blackface population) (Riggio et al. (2014)). Interestingly, significant markers of chromosomes 3, 4 and 7 affect mainly resistance during the second infection which mimics naturally repeated infections (Table 1). None of SNP closed to pepsinogen affects significantly FEC traits, but blood concentration of pepsinogen will be soon available.

Table 1. Genome-wide P-values of significant markers for the first and/or the second experimental infection by *H. contortus*.

ch	SNP location (Mb)	FEC1t	FEC2t	FEC12t	FEC3t	FEC4t	FEC34t
3	88742735	NS	NS	NS	NS	0,04	0,03
4	7386559	NS	NS	NS	NS	0,03	0,04
4	7933563	0,06	NS	0,04	NS	NS	NS
4	9377742	NS	NS	NS	NS	0,03	0,02
4	9534833	0,05	0,05	0,05	NS	NS	NS
4	9574764	0,0006	NS	0,02	NS	NS	NS
5	87935483	0,02	NS	0,04	NS	NS	NS
5	88746609	0,02	NS	0,02	NS	NS	NS
5	89299546	0,005	NS	0,03	NS	NS	NS
7	54552055	NS	NS	NS	NS	NS	0,04
7	54661843	0,05	NS	NS	NS	0,02	0,02
7	55180628	NS	0,05	0,02	0,02	NS	0,03
12	55710246	NS	0,01	0,02	NS	NS	NS
12	55789009	NS	0,0008	0,007	NS	NS	NS
13	69104639	0,0003	0,001	0,001	0,01	NS	NS
13	72659097	NS	0,03	0,02	NS	NS	NS

Conclusion

The effects of three out of the four main QTL detected in a previous back cross between Black Belly and Romane breeds (Sallé et al. (2012)) were confirmed in a pure Romane breed population. Some other regions detected previously in two additional naturally infected populations also seem to exert significant effect on *H. contortus* infection. Haplotype-based analyses will be performed to confirm the similarity with the regions previously identified in the BC population.

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