

Genetic Structure at PRN-P Locus and Framing Risk Classes for Scrapie Resistance in Merinos of Cluj Breed

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Abstract: The present paper shares the results of genetic structure analysis of the PRN-P gene, carried out in an ovine nucleus of Merinos of Cluj breed. Biological samples (blood) were collected from 20 rams (Merinos of Cluj breed) from a herd in genetics conservation belonging to SDE Cluj-Napoca and were genotyped at the PRN-P locus by means of primer extension analysis, and the final products were analyzed on a Genetic Analyzer ABI Prism 3130 xl. The frequency of genes by haplotypes were identified, and the individuals were categorized in risk groups based on the frequency of the identified genotype, with a view to applying assisted selection at the molecular level.

Keywords: scrapie, genetic structure, PrP locus, Merinos of Cluj

Introduction. Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of fatal neurodegenerative diseases including sheep and goat scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeld-Jakobdisease (CJD) in humans. Prions are the transmissible pathogens that cause these neurodegenerative diseases in mammals. During the course of prion disease, a largely protease resistant aggregated form of PrP, designated PrP^{Sc}, accumulates mainly in brain, and may be the main or only constituent of the prion (Prusiner, 1999). With over 30 SNPs already reported, the ovine prion gene (PrP) shows an unusually high level of genetic variation. Increased resistance to classical scrapie is associated with a prion protein gene (*PRN-P*) haplotype allele encoding one alanine (A) and two arginine (R) at codon positions, respectively 136, 154 and 171 (i.e.ARR). Generally the possible combination at the 5 amino acids encoded by the 3 different codons will determine the existence of the 15 possible genotypes at the PrP locus [5,6]:

- ARR/ARR-sheeps very resistant at scrapie;
- ARR/AHQ, ARR/ARH, ARR/ARQ-sheeps genetic resistant to scrapie but they need especial attention for using in selection programs,
- ARQ/ARH, ARQ/AHQ, AHQ/AHQ, ARH/ARH, AHQ/ARH, ARQ/ARQ-sheeps with lower genetic to scrapie,
- ARR/VRQ-sheeps sensitive to scrapie,
- AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ-sheeps very sensitive to scrapie.

Aims and Objectives. They focused on determination of genetic structure of PRN-P gene in Merinos of Cluj breed, previously unknown situation and framing risk classes regarding resistance to scrapie of genotype rams.

Materials and Methods. Blood samples were collected from 20 rams (Merinos of Cluj breed) belonging to a herd in genetics conservation belonging to SDE Cluj-Napoca. In order to accomplish this goal several methods are used, the most accuracy methods are based on sequencing, and in this study we use the primer extension technique. In order to perform

the primer extension technique for genotypization at PrnP locus is necessary to follow some steps (Cosier *et al.*, 2008):

- DNA extraction from the blood samples;
- determination of the DNA quality and quantity;
- template amplification;
- purification of the PCR products;
- primer extension technique- performing the capillarity electrophoresis to determine the polymorphism (Genetic AnalyzerABI Prism 3130 xl);
- the analysis of results for electrophoretic profiles with GeneMapperR software.

Results and Discussions. From the data obtained in Table 1, the frequency of the allele ARR in Merinos of Cluj breed is 40%, allele AHQ is 0%, ARH 5%, ARQ 50%, VRQ 5%, VHQ 0% and VRH 0%.

Tab. 1

Allele frequency at the PrP locus in Merinos of Cluj breed

Allele	ARR	AHQ	ARH	ARQ	VRQ	VHQ	VRH
Frequency	40%	0%	5%	50%	5%	0%	0%

After genotyping at Prn-P locus the 20 male sheeps Merinos of Cluj breed, we reach at the follow results (Tab. 2):

- 3 rams (15%) had the ARR/ARR genotype, which is the genotype related to the highest resistance to scrapie (Class I)
- 10 rams (50%) had the genotypes ARR/AHQ, ARR/ARH, ARR/ARQ, which are the genotype correlated with resistance to scrapie, but those require a particular attention to be use in selection programs (Class II)
- 5 rams (25%) had the genotypes ARQ/ARH, ARQ/AHQ, AHQ/AHQ, ARH/ARH, AHQ/ARH, ARQ/ARQ, which are the genotype correlated with lower resistance to scrapie (Class III)
- 0 rams with genotype ARR/VRQ (Class IV)
- 2 rams (10%) have been found with genotypes related with lower resistance to scrapie: AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ, and is recommended to be sacrifice (Class V).

Tab. 2

Framing risk classes in Merinos of Cluj breed

RISK INTENSITY															
Risk class	I	II			III						IV	V			
Genotypes	ARR/ARR	ARR/AHQ	ARR/ARH	ARR/ARQ	ARQ/ARH	ARQ/AHQ	AHQ/ARH	AHQ/ARH	ARQ/ARH	ARQ/ARQ	ARR/VRQ	AHQ/VRQ	ARH/VRQ	ARQ/VRQ	VRQ/VRQ
Proportions	15%	0%	10%	40%	0%	0%	0%	0%	0%	25%	0%	0%	0%	10%	0%
Risk group scale proportions	15%	50%			25%						0%	10%			

I-very resistant rams genetically to scrapie; **II**- resistant rams genetically to scrapie, but that requires a particular attention for use in selection programs; **III**-rams with low genetic resistance to scrapie-their use must be avoided in selection programs; **IV**-rams with low genetic resistance to scrapie; **V**-very susceptible to scrapie rams, which must be castrated or slaughtered

Conclusion. The results obtained in Table1 indicate a relatively low frequency of the allele, which confers increased resistance –ARR (40%) as compared to other breeds (Suffolk 52.4%; Texel 48.3%, Coburg Fox 50.2%, Rhoen 65.9%). Concerning local breeds the

frequency of allele ARR is slightly higher (Tzurcana breed, Sibian ecotype 35.4%, Tzurcana breed, Hateg ecotype 34.15%, Tzurcana breed, Bala of Bistrita ecotype 38.03%) (Constantinescu *et al.*, 2009; Cosier *et al.*, 2008).

Also the obtained results indicate a medium proportion (5%) of the allele, which confers minimum resistance to scrapie (VRQ) compare to Tzurcana breed, Sibian ecotype-6.06%, Tzurcana breed, Hateg ecotype 8.94%, Tzurcana breed, Bala of Bistrita ecotype 2.82%).

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