COMMUNICATION

Sequence polymorphism of PrP exon 3 gene in Istrian and crossbred sheep

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ABSTRACT - Polymorphisms in sheep PrP (prion protein) gene are known for scrapie susceptibility. We sequenced part of PrP exon 3 gene in 92 autochthonous Istrian (IS) and 38 crossbred sheep (CBS). ARQ, ARR and AHQ alleles were predominant with frequency of 0.674 (0.526), 0.228 (0.132) and 0.082 (0.263) in IS (CBS), respectively, while VRQ (0.011 in IS) and ARH (0.005 in IS and 0.079 in CBS) alleles were rare. We also found non-synonymous mutations at codons 112 (M→T), 127 (G→S) and 143 (H→R), and synonymous mutations at codons 231 (R) and 237 (L). Additional mutations were associated only with AHQ, ARH and ARQ alleles. The polymorphism of PrP gene in IS was not critical with respect to scrapie susceptibility and with some efforts number of "favourable" genotypes can be increased.

Key words: PrP gene, Sheep, Sequencing.

Introduction – Scrapie in sheep belongs to the group of transmissible spongiform encephalopathies or prion diseases characterized by the accumulation of abnormal protease-resistant isoform (PrP^{Sc}) of the cellular prion protein (PrP^{C}). PrP^{C} is encoded by the prion protein gene (PrP). Sheep PrP gene consists of three exons and two introns and is located on chromosome 13. The open reading frame is located in exon 3 and codes for protein product of 256 amino acids. While, high nucleotide diversity of PrP gene was identified in sheep (Goldmann *et al.*, 2005), susceptibility to scrapie is only related to variations at codons 136 (A/V), 154 (R/H) and 171 (Q/R/H), here SSHL ("scrapie susceptible haplotype locus"). Five alleles (ARR, ARQ, AHQ, ARH and VRQ), resulting in 15 genotypes, are known at SSHL (Baylis and Goldmann, 2004). While ARR allele is highly resistant; VRQ allele is the most susceptible to scrapie (Tongue *et al.,* 2004). No single case of scrapie disease has been reported in Croatia. Here, we present PrP gene polymorphism in autochthonous Istrian sheep (IS) and crossbreed system between East Frisien, Awassi and Istrian sheep (CBS).

Material and methods – The DNA was extracted from 92 IS and 38 CBS blood samples using QIAamp DNA Blood Mini Kit according to manufacturers' recommendations (Qiagen GmbH, Germany). A 628-bp fragment of exon 3 of PrP gene (EMBL U67922) comprising codons 112-211 was amplified by PCR using primers: upper (5'-CCGCTATCCACCTCAG-GGA-3') and lower (5'-TTGCCCCTATCCTACTATGAGA-3') according to Gmür *et al.* (2004). PCRs were performed in a 20μL volume containing 0.2μM of each primer and using Qiagen Multiplex PCR Kit (Qiagen GmbH, Germany) according to manufacturer's protocol. The amplification reactions were performed on a iCycler (Biorad, Germany) comprised of an initial denaturation at 95ºC for 15min, 44 cycles of denaturation at 94ºC for 1min, annealing at 64ºC for 1 min and extension at 72ºC for 1min and final extension at 72ºC for 7min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen GmbH, Germany) and sequenced directly by using an ABI PRISM® 3100-Avant Genetic Analyzer and the BigDye-terminator method with upper primer. Results were analysed by BioEdit 7.0.0. First, we estimated alleles (parental haplotypes for 136, 154, and 171 codons) for the SSHL using the Bayesian approach. Furthermore, we estimated genotype and allele frequencies for SSHL and other loci (codons) showing polymorphism. All calculations were done by PROC Haplotype and PROC Allele implemented in SAS/Genetics 9.1.3. (SAS Institute, Cary, NC).

Table 1. Allele frequencies (standard errors) of the PrP sequence (exon 3) polymorphism in 92 Istrian and 38 Crossbreed (Istrian, East Frisian, Awassi) sheep.

Locus[1]	Allele[2]	Istrian sheep (92)	Crossbreds (38)
136-154-171	AHQ	0.082 (0.019)	0.263 (0.052)
	ARH	0.005 (0.005)	0.079 (0.040)
	ARQ	0.674 (0.030)	0.526 (0.052)
	ARR	0.228 (0.030	0.132 (0.036)
	VRQ	0.011 (0.008)	-
112	$T_{[ACG]}$	0.038 (0.014)	0.040 (0.022)
	$M_{[ATG]}$	0.962(0.014)	0.961 (0.022)
127	$S_{[AGC]}$	0.016 (0.009)	0.066 (0.027)
	$G_{[GGC]}$	0.984 (0.009)	0.934 (0.027)
143	$R_{[CGT]}$	0.005 (0.005)	0.013 (0.013)
	$H_{[CAT]}$	0.995 (0.005)	0.987 (0.013)
231	$R_{[AGG]}$	0.913 (0.021)	0.829 (0.043)
	$R_{[CGG]}$	0.087 (0.021)	0.171 (0.043)
237	$L_{[CTC]}$	0.913 (0.021)	0.829 (0.043)
	$L_{[CTG]}$	0.087 (0.021)	0.171 (0.043)

[1]*Loci are defined according to the codon polymorphism.* [2]*Alleles are named according to the coded amino acids or underlined when mutations are synonymous.*

Results and conclusions – At SSHL we estimated five and four alleles in IS and CBS populations, respectively (Table 1). The most frequent allele was ARQ (0.674 in IS and 0.526 in CBS), followed by ARR (0.228) in IS and AHQ (0.263) in CBS. The frequency of allele VRQ was extremely low (two heterozygotes in IS) resulting in an extremely low frequency of highly scrapie susceptible genotypes (Table 2). In addition to the well explored polymorphism at SSHL, in both IS and CBS populations, we observed polymorphism: a) non-synonymous mutation at codons 112 (M→T), 127 (G→S) and 143 (H→R), and b) synonymous mutation at codons 231 (R→R) and 237 (L→L), for exact point mutations see Tables 1 and 2.

Table 2. Genotype frequencies of the PrP sequence (exon 3) polymorphism in 92 Istrian and 38 Crossbred (Istrian, East Frisian, Awassi) sheep.

Locus1	Genotype2,3	Istrian sheep	Crossbreds
136-154-171	ARR/ARR (1)	0.044	-
	ARR/AHQ (2)	0.022	-
	ARR/ARH (2)	-	0.053
	ARR/ARQ (2)	0.348	0.211
	AHQ/AHQ (3)	-	0.079
	AHQ/ARQ (3)	0.141	0.368
	ARH/ARH (3)	-	0.053
	ARH/ARQ (3)	0.011	-
	ARQ/ARQ (3)	0.413	0.237
	ARQ/VRQ (4)	0.022	-
112	$T_{[ACG]}/M_{[ATG]}$	0.076	0.079
	$M_{[ATG]}/M_{[ATG]}$	0.923	0.921
127	$S_{[AGC]}/G_{[GGC]}$	0.033	0.132
	$G_{[GGC]}/G_{[GGC]}$	0.967	0.868
143	$R_{[CGT]}/H_{[CAT]}$	0.011	0.026
	$H_{[CAT]}/H_{[CAT]}$	0.989	0.974
231	$R_{[AGG]}/R_{[AGG]}$	0.837	0.684
	$R_{[AGG]}/R_{[CGG]}$	0.152	0.290
	$R_{[CGG]}/R_{[CGG]}$	0.011	0.026
237	$L_{[CTC]}/L_{[CTC]}$	0.837	0.684
	$L_{[CTC]}/L_{[CTG]}$	0.152	0.290
	$L_{[CTG]}/L_{[CTG]}$	0.011	0.026

[1]*Loci are defined according to the codon polymorphism.* [2]*Alleles are named according to the coded amino acids or underlined when mutations are synonymous.* [3]*Scrapie risk category (see Tongue et al., 2004).*

Frequency of scrapie susceptible genotypes at SSHL was very rare in IS and CBS populations and a small increase of ARR alleles will improve the frequency of resistant genotypes. Non-synonymous polymorphism observed at codons 112, 127 and 143 requires further investigation with respect to scrapie susceptibility.

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