PRNP GENOTYPE PREVALENCE IN LATVIAN DARKHEADED SHEEP BREED

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ABSTRACT
Scrapie is fatal neurodegenerative sheep and goat disease, belonging to the group of transmissible spongiform encephalophaties (TSE). Cause of scrapie is infectious cellular protein isoform, originally encoded by *PRNP* gene. When connection between *PRNP* gene and susceptibility to disease was discovered, European Commission established special breeding programs aiming to eliminate scrapie-susceptible genotypes from sheep herds. Main sheep breed in Latvia is Latvian darkheaded (LD); and currently there is a lack of information about level of susceptibility of this sheep breed both to classical and recently discovered atypical scrapie strains. In order to estimate level of susceptibility of LD, 645 pure-breed sheep were genotyped for the polymorphisms in codons 136, 141, 154 and 171 of *PRNP* gene. Based on obtained genotype and allele frequencies, estimated level of susceptibility both to classical and atypical scrapie strains was found low.

KEY WORDS: scrapie, *PRNP*, Latvian darkheaded, genotyping.

INTRODUCTION
Scrapie is fatal neurodegenerative sheep and goat disease belonging to the group of TSEs. Disease is caused by accumulation of infectious proteins called prions. Prions lack nucleic acid and propagate by converting cellular prion protein (PrP^{C}) into pathogenic form (PrP^{Sc}) (Prusiner et al. 1998).

Scrapie occurs in many European countries and historically it is considered as endemic in Great Britain (Dawson et al. 1998, Fediaevsky et al. 2008). Complete epidemiology of scrapie is still unknown; however many studies showed that there is a connection between disease susceptibility in different sheep populations and *PRNP* genotype, as cellular prion protein is encoded by *PRNP* gene (accession No. U67922) (Benestad et al., 2008; Prusiner et al., 1998). Single nucleotide polymorphisms (SNPs) in three codons of *PRNP* (136, 154 and 171) are shown to be of particular importance in susceptibility to classical scrapie. Based on SNPs in these positions five main haplogroups have been determined – ARR, ARQ, AHQ, VRQ and ARH (Goldmann, 2008; Vaccari et al. 2009). ARR homozygous animals are shown to be resistant to classical scrapie and up to date there are only few reports of ARR/ARR scrapie positive cases (Baylis et al. 2002; Tranulis, 2002). Also some rare combinations with no clear evidence of showing any influence on disease susceptibility or resistance have been reported – ARK, VRR and AHR (Vaccari et al. 2009). According to the National Scrapie Plan (NSP) for Great Britain all genotypes, regarding classical PrP^{Sc} strain, are distributed in five groups from R1 to R5, where R1 is resistant (ARR/ARR), and R5 is the most susceptible (VRQ/VRQ) and therefore should not be used for breeding in the context of controlled breeding programme. In a similar way, there was an attempt to group sheep genotypes by levels of genetic risk for atypical scrapie (Nor98) (Fediaevsky et al. 2009). For instance, genotypes, including V136, considered to be in R4 and R5 groups of NSP for classical scrapie, are highly resistant to atypical scrapie strain. Also amino acid changes in codons 141 and 154 are crucial. In reported cases of atypical scrapie sheep with

F141 and H154 are observed in a high frequency (Benestad et al. 2008; Simmons et al. 2009; Vaccari et al. 2009).

European Commission established special breeding programs in order to eliminate susceptible genotypes and produce scrapie resistant sheep population (1003/202/EC). In Latvia such program was introduced in 2004 with accession to the European Union.

Main sheep breed in Latvia is Latvian darkheaded (LD), but currently there is a lack of publications showing main genotypes of *PRNP* in this important sheep breed (Sild et al. 2006). This study was carried out in order to describe *PRNP* variations in LD sheep breed and to estimate theoretical resistance level against both classical and atypical scrapie strains.

MATERIAL AND METHODS

Blood samples (n = 645) collected from randomly selected healthy LD sheep during annual state genotyping program from 2004 to 2011 were used in this study. Genomic DNA was isolated using FlexiGene DNA Kit (Qiagen) following instructions of manufacturer. Samples obtained in 2004 to 2007 were analysed for polymorphisms in *PRNP* codons 136, 154 and 171 using ASO probe assay (Van Poucke et al. 2005). Starting from 2008, as suggested by European Reference Laboratory for TSEs (AHVLA, Weybridge, UK), standard method was changed to direct sequencing of part of *PRNP* gene including codons 136, 141, 154 and 171 (Acutis et al. 2010 with minor modifications). In order to determine SNPs in desired codons, obtained sequences were compared with ovine *PRNP* gene wild type genotype available in GeneBank (accession No. AJ000739.1) (Goldmann et al. 2005).

RESULTS AND DISCUSSION

The aim of this work was to observe and investigate diversity of *PRNP* genotypes in LD sheep breed and to evaluate theoretical resistance levels both to classical and atypical scrapie strains. Alleles and genotypes were coded using standard amino acid nomenclature: codon 136 (A/V/T), codon 154 (R/H), codon 171 (Q/R/H/K). Additionally codon 141 (L/F) was analyzed (Simmons et al. 2009, Vaccari et al. 2009).

In total, regarding codons 136, 154 and 171, eight genotype variations representing all five risk groups for classical scrapie were determined (Table 1). Three genotypes were prevailing: ARR/ARQ (49.2%), representing high resistance level to classical scrapie disease due to presence of ARR allele (Goldmann, 2008), wild-type ARQ/ARQ (23.9%), belonging to the medium resistance level (R3 group) and finally ARR/ARR (14.7%) – resistant genotype. Among genotypes unwanted for selection (R4 and R5), only two were observed: ARR/VRQ and ARQ/VRQ, however both of them were in low frequencies.

Polymorphisms in codon 141 (L/F) as well as codon 154 (R/H) are considered to be connected with susceptibility to atypical scrapie (Vaccari et al. 2009). In total 318 samples were tested, including codon 141 into the analysis. The results showed that only nine samples (2.83%) belonged to R4 and R5 groups, which are considered to be most susceptible to atypical scrapie, and only three sample genotypes (0.94%) contained phenylalanine (F) instead of leucine (L) in position 141. Regarding susceptibility to atypical scrapie associated with codon 154 of *PRNP* gene, in 4.95% of all samples tested R/H polymorphism was observed (Table 1 and 2). Regarding all four codons of ovine *PRNP* (Table 2) 13 different genotypes were found and three dominant genotypes were observed: ALRR/ALRQ (41.82%), ALRQ/ALRQ (23.9%) and ALRR/ALRR (21.7%).

Table 1

***PRNP* genotypes (codons 136, 154 and 171) in LD breed sheep**

Genotype	n	Frequency (%)	Risk group Classical scrapie
ARR/ARR	48	14.7	R1
ARR/ARQ	161	49.2	R2
ARR/AHQ	19	5.8	R2
ARQ/AHQ	6	1.8	R3
ARQ/ARQ	78	23.9	
AHQ/AHQ	1	0.3	
ARR/VRQ	3	0.9	R4
ARQ/VRQ	11	3.4	R5
Total	**327**	**100**	

In order to gain deeper insight in LD sheep population resistance/susceptibility to both classical and atypical scrapie strains, frequencies of *PRNP* haplotypes including three and four codons were determined (Table 3). Alleles associated with high level of resistance (ARR and ALRR) to classical scrapie are observed in very high frequencies, in turn, alleles associated with high levels of susceptibility to classical or atypical PrP^{Sc} strains are found to be in very low frequencies. Taken together these findings indicate high level of resistance of Latvian Darkheaded sheep breed to classical and Nor98 scrapie strains. However, LD breeding program executors should take into account that genotypes found to be resistant to classical scrapie strain are more susceptible to atypical scrapie (Green et al. 2007).

Table 2

***PRNP* genotypes (codons 136, 141, 154 and 171) in LD breed sheep**

Genotype	n	Frequency (%)	Risk group Classical scrapie	Risk group Atypical scrapie
ALRR/ALRR	69	21.7	R1	R2
ALRR/ALRQ	133	41.82	R2	R1
ALRR/ALHQ	2	0.63		R4
ALRR/AFRQ	1	0.31		
ALRR/ALRH	7	2.2		R2
ALRQ/ALRQ	76	23.9	R3	R1
ALRQ/ALRH	11	3.46		
ALRH/ALRH	1	0.31		R3
ALHQ/ALRQ	4	1.26		R4
AFRQ/AFRQ	2	0.63		R5
ALRQ/VLRQ	8	2.52	R4	R1
ALRR/VLRQ	3	0.94		
ALHQ/VLRQ	1	0.31		R3
Total	**318**	**100**		

Table 3

***PRNP* allele frequencies in LD breed sheep**

Allele	n	Frequency (%)	Allele	n	Frequency (%)
ARR	279	42.66	ALRR	284	44.51
ARQ	334	51.07	ALRQ	309	48.43
AHQ	27	4.13	ALHQ	7	1.10
VRQ	14	2.14	ALRH	20	3.13
			VLRQ	13	2.04
			AFRQ	5	0.78
Total	**654**	**100**	**Total**	**638**	**100**

CONCLUSIONS

1. Predominant genotypes in LD breed regarding codons 136, 154 and 171 of *PRNP* gene were ARR/ARQ (49.2%), ARQ/ARQ (23.9%) and ARR/ARR (14.7%);
2. Predominant genotypes regarding codons 136, 141, 154 and 171 of *PRNP* gene were ALRR/ALRQ (41.82%), ALRQ/ALRQ (23.9%) and ALRR/ALRR (21.7%);
3. Predominant haplotypes regarding codons 136, 154 and 171 were ARQ (51.07%) and ARR (42.66%);
4. Predominant haplotypes regarding codons 136, 141, 154 and 171 were ALRQ (48.43%) and ALRR (44.51%);
5. Estimated level of susceptibility of Latvian Darkheaded sheep breed to classical and atypical (Nor98) scrapie strains are low;
6. Taking into account total level of resistance of predominant Latvian sheep breed, Latvia would benefit economically by helping to establish a fully scrapie resistant breed.

LITERATURE

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