Sheep prion protein gene (*PRNP*) genotyping in the Czech Republic

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Introduction

Scrapie genetics in sheep

Susceptibility of sheep to scrapie, the naturally occuring transmissible spongiform encephalopathy (TSE) of small ruminants, is known to be strongly associated with polymorphisms in codons 136, 154 and 171 of the prion protein gene (*PRNP*) [1]. Polymorphisms in these codons form five basic haplotypes of sheep *PRNP* designated by aminoacids encoded by the three codons, showing diverse associations with scrapie (Tab.1). An atypical form of scrapie was described in Norway in 1998, named Nor98. For this type of the disease, polymorphism in codon 141 of *PRNP* ($AF_{141}RQ$ haplotype) is associated with susceptibility, while the ARR haplotype seems to provide no resistance [2].

About a hundred of other *PRNP* polymorphisms have been described in various sheep populations world-wide, such as AHR, ARK, TRQ or VRR, mostly in very low frequencies and unknown associations with scrapie (reviewed in [3]).

Tab.1: Basic sheep *PRNP* haplotypes

haplotype	association with scrapie
ARR	resistant
ARQ ARH AHQ	depends on genotype and environment
VRQ	susceptible

Scrapie resistance breeding programmes

Promoted by EU legislation in 2004 [4], breeding programmes for resistence of sheep to scrapie have been under way in nearly 20 European countries in the last 15 years. In Czech Republic, the programme started in 2002. As a guideline for selection of animals, the 15 genotypes of *PRNP* formed by the five basic haplotypes are classified into 5 scrapie risk categories: R1-R5 (Tab.2). The goal of the programmes is to increase the frequency of the ARR haplotype while lowering the frequency of the VRQ haplotype in sheep populations. So far, codon 141 is not taken into consideration in the breeding programmes.

Tab.2: Classification of sheep *PRNP* genotypes into scrapie risk groups

risk group	*PRNP* genotypes	implications for selection
R1	ARR/ARR	most suitable
R2	ARR heterozygotes (except R4)	suitable
R3	other	to be considered
R4	ARR/VRQ	to be considered
R5	VRQ carriers (except R4)	not allowed

Sheep *PRNP* genotyping

Correct *PRNP* genotyping is essential for proper animal selection in the frame of the breeding programmes. Several methods for this purpose have been described in the early stages of the breeding programmes (Tab.3) (reviewed in [5]). The methods vary in therms of laboratory equipment demands, laboriousness and time effectiveness. In the Czech Republic, State Veterinary Institute Jihlava (SVIJ) is the only laboratory providing *PRNP* genotyping service. Several techniques have been used for *PRNP* genotyping in SVIJ in the course of the breeding programme to ensure punctual delivery of precise genotyping information to sheep breeders.

Tab.3: Some techniques proposed for sheep *PRNP* genotyping

genotyping technique / shortcut
PCR - restriction fragment length polymorphism / PCR-RFLP
denaturing gradient gel electrophoresis / DGGE
amplification refractory mutation detection system / ARMS
HyBeacon and HybProbe assays
direct Sanger sequencing

Materials and methods

Routine sheep *PRNP* genotyping in the course of the breeding programme in the Czech Republic

period	sheep genotyped	DNA extraction system	genotyping technique / note	codons genotyped
~2003 - ~2005	5 150	High Pure PCR Template Preparation Kit (Roche)	PCR - single stranded conformational polymorphism (PCR-SSCP) (Hořín et al. 2002, unpublished) / a 221 bp DNA amplicon comprising codons 136, 154 and 171	136, 154, 171
~2006 - ~2011	32 839	MagNA Pure LC instrument (Roche)	LightTyper Sheep PrP Gene Mutation Detection Kit (Roche) / a HybProbe assay using the LC480 instrument (Roche); PCR-SSCP protocol used for haplotype determination, if needed	136, 154, 171
~2012 - ~2015	21 388	MagNA Pure 96 instrument (Roche)	LightMix(R)480HT Scrapie Susceptibility Mutation Detection (TIB Molbiol) / a HybProbe assay using the LC480 instrument (Roche); PCR-SSCP protocol used for haplotype determination, if needed	136, 141, 154, 171

PRNP sequencing of unclear samples

sheep *PRNP* target region / amplicon length
the whole coding sequence (CDS) - 771 bp / 896 bp
primer sequences 5'- 3'
For - CCA ACC TGG CAA AGA TTA AG Rev - CAT TAT GCT GCA GAC TTT AAG TG
sequencing chemistry / instrument
BigDye® Terminator v3.1 Cycle Sequencing Kit / 3500 Genetic Analyser (Applied Biosystems)

Results

Fig.1: Sheep sample flowthrough in SVIJ in 2015

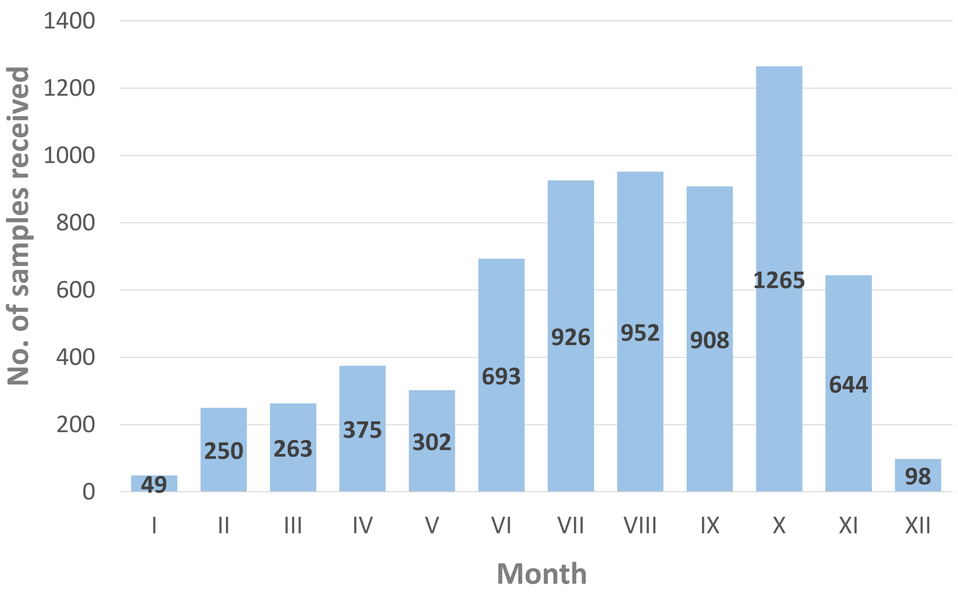


Fig.3: Unclear genotyping results observed using the HybProbe assay (TIB Molbiol)

in green: heterozygote controls; in red: samples with unclear melting profiles

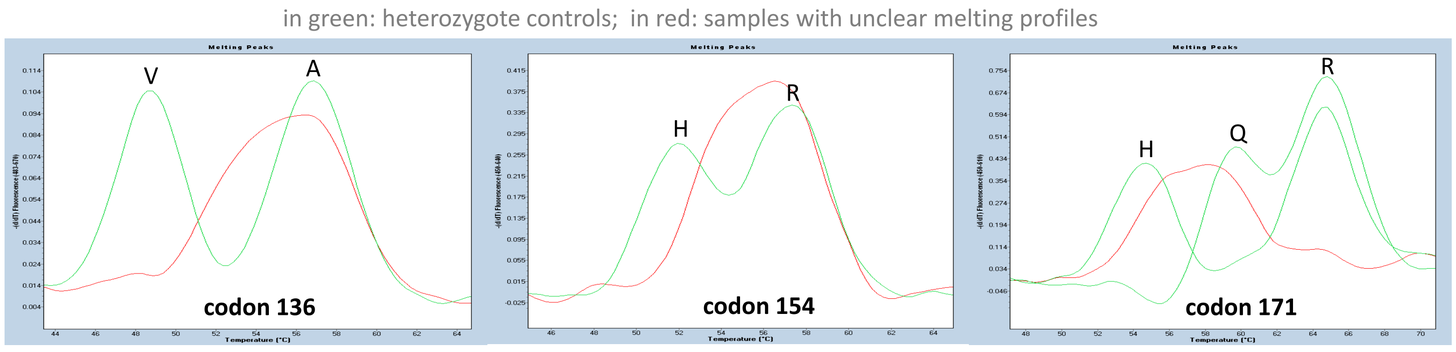


Fig.4: Unclear genotyping results resolved by Sanger sequencing

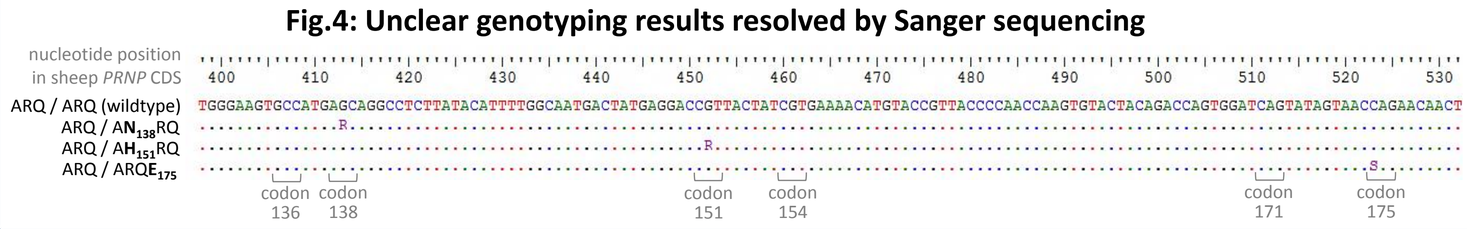


Fig.2: *PRNP* haplotype determination using the PCR-SSCP protocol

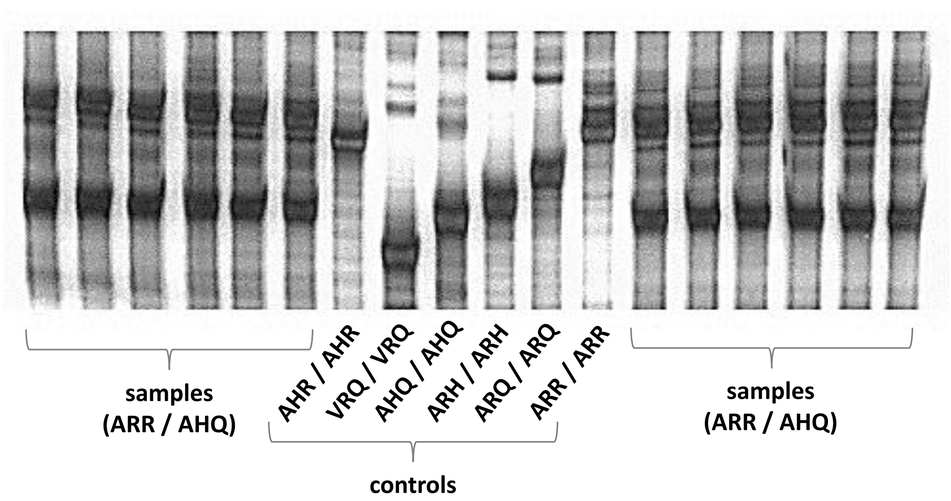
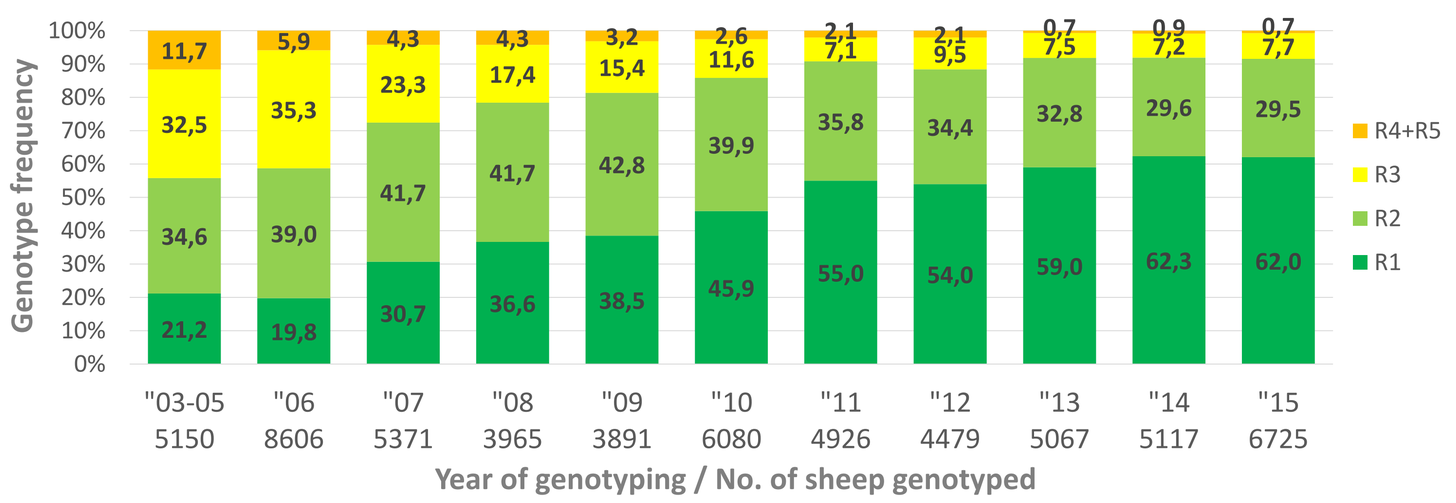


Fig.5: *PRNP* genotype frequencies in sheep analysed in the course of the breeding programme



Discussion

PRNP genotyping

As seen from the pattern of sheep sample numbers received by SVIJ throughout the year 2015 (Fig.1), summer and autumn are the most demanding seasons for *PRNP* genotyping in the Czech Republic. In some instances more than 500 samples have been received by SVIJ in one week during these seasons (not shown). Under such demands, the HybProbe genotyping assays and automated DNA extraction systems utilised in SVIJ proved to be efficient and indispensable in both therms of time and labour costs.

Although the more time costly PCR-SSCP protocol is still essential for haplotype determination in case of 136A/A-154R/H-171R/Q results (Fig.2), number of these cases is relatively small and is shrinking further with ongoing selection for the ARR haplotype.

Rare *PRNP* polymorphisms

PRNP polymorphisms in close proximity to codons 136, 154 and 171 may hinder the genotyping process by deforming the melting curves in the HybProbe assays (Fig.3). In such samples, sequencing protocol developed in SVIJ for this purpose was successfully used for accurate genotype determination (Fig.4).

All of the rare polymorphisms detected this way in Czech sheep have been reported previously in other countries [1]. Because of their low frequencies, these polymorphisms have no significance for *PRNP* genotyping, nor for the breeding programme in the Czech Republic. Moreover, since the rare polymorphisms are linked to the ARQ haplotype, their elimination in Czech sheep populations is likely due to genetic drift and selection towards the ARR haplotype.

The breeding programme in the Czech Republic

The pattern of *PRNP* genotype frequencies observed during the last 12 years of genotyping (Fig.5) supports the idea that the scrapie resistance breeding programme has had the desired effect on sheep populations in the Czech Republic, similarly to other European countries, where such breeding programmes have been implemented [6]. As seen in Fig.5, the frequency of the susceptible VRQ haplotype carriers (risk group R4+R5) diminished to less than 1% in animals genotyped in 2015, while the total frequency of ARR carriers (risk group R1+R2) in Czech sheep exceeded 90%. However, the target frequency of the resistant ARR haplotype in sheep populations both in the Czech Republic and other European countries is still a matter of debate [6]. At the moment, the breeding programme and sheep *PRNP* genotyping in the Czech Repubic is ongoing.

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References

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4th EAVLD Congress Prague, Czech Republic 2016