OVINE PRION PROTEIN GENOTYPE PROFILES OF MERINO RAMS IN A ROMANIAN FARM

M. R. GURAU, S. BARAITAREANU & D. DANES

Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Agronomical Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania

Summary


The objective of this study has been the highlight of the rams’ scrapie resistant genotype profile in a Romanian sheep farm from the southeast area. Twenty-five breeding rams were genotyped. Most of the rams were in second class (56%), meaning sheep genetically resistant to scrapie, but asking a special attention to be used in selection programmes. In the first class (genotype ARR/ARR) we found only one ram (4%) that makes very difficult the genetic selection. The ARQ variant was the most frequent (56%). The results of this study prove the importance of sequence-based genotype investigations in the sheep-breeding management. Those data support the scrapie control and eradication programmes based on the gradual elimination of alleles associated with scrapie susceptibility and the use of the ARR/ARR genotype rams in breeding programmes.

Key words: genotyping, ovine genetic selection, scrapie, TSEs

Transmissible spongiform encephalopathies (TSEs), fatal diseases affecting the central nervous system, have been described in both humans and animals (Prusiner, 1999).

The codons 136, 154 and 171 have been proved to have a major importance in genetic determinism of the classical scrapie (Anonymous, 2001; 2003; Benedstad et al., 2008). The genotypes that have implication in susceptibility to this disease are assigned in five classes, according to the UK National Scrapie Plan (DEFRA, 2001). These five classes are gradually expressed from the greatest resistance to the highest susceptibility to scrapie (Agrimi et al., 2003). Class 1, very resistant, comprises only the ARR/ARR genotype. Class 2 animals are resistant to scrapie but they need attention in selection programmes. They depict the ARR/AHQ, ARR/ARH, ARR/ARQ genotypes. The sheep with lower genetic resistance to scrapie are ARQ/ARH, ARQ/AHQ, AHQ/ARH, AHQ/ARQ, ARQ/ARQ (Class 3). The sheep sensitive to scrapie are ARR/VRQ (Class 4), and the highest sensitivity is found in AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ (Class 5).

The aim of the study was to analyse genotype frequencies in rams from a Ro-
Ovine prion protein genotype profiles of merino rams in a Romanian farm

Romanian sheep farm by the PrP gene polymorphisms at codons 136, 154, 171 and to estimate the genotype-specific relative risks to become a detected scrapie case.

Blood samples, collected from 25 rams, in 3-mL tubes containing K$_2$-EDTA, originated from a southeast Romanian farm.

For the DNA extraction, the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) was used in accordance with the manufacturer’s instructions. Briefly, 200 µL whole blood has been mixed with 200 µL Binding Buffer and 40 µL Protease K, incubated for 10 min at 70 °C; 100 µL isopropanol was added, mixed well and applied to a High Pure filter tube. The mixture was centrifuged (1 min; 8,000× g), treated with 500 µL Inhibitor Removal Buffer and washed two times with 500 µL Wash Buffer; template DNA has been recovered from High Pure filter with 50 µL Elution Buffer (70 °C). Purified Template DNA was stored at –80 °C.

RT-PCR genotyping of ovine prion protein gene has been done by using the protocol from LightCycler® Scrapie Susceptibility Mutation Detection Kit (TIB MOLBIOL, Berlin, Germany) with Roche FastStart reagents LightCycler® FastStart DNA MasterPLUS HybProbe (Roche, Mannheim, Germany). LightCycler® 2.0 Real-Time PCR System (Roche, Germany) performed the melting curve analysis.

The studied farm was in the southeast Romanian area and housed 625 animals, from which 600 sheep and 25 rams. The Palas Merinos breed rams, 1–7 years of age, were from own breeding. The PrP genotyping of rams revealed all classes of susceptibility.

Most of the rams were in class 2 (56%), meaning sheep genetically resistant to scrapie, but asking a special attention to be used in selection programmes. In class 1 (genotype ARR/ARR) we found only one ram (4%), making very difficult the genetic selection. We advise to use guided mating, i.e. breeding ewes from the heterozygous class 2 (ARR/*) only with rams from class 1 (ARR/ARR) and so, there are 75% odds for the “new generation” to be in class 1. However, precautions should be taken to prevent transmission of scrapie by secondary sources, as suggested by the experimentally transmission study results (Simmons et al., 2007).

In the same herd we identified 8 rams (32%) in class 3, 1 ram (4%) in class 4 and 1 ram (4%) in class 5.

The previous Romanian genotype studies, focused on specific sheep breed (Turcana breed, Sibian ecotype), showed similar results in the herd structure of males: 19.25% genotype ARR/ARR (class 1), but different results in other classes: 27.68% from class 2; 40.69% from class 3; 5.53% from class 4 and 6.84% from class 5 (Constantinescu et al., 2009). Studies performed in herds from foreign countries showed heterogeneous data for all classes: the frequency of genotype ARR/ARR (class 1) was 21.3% in Great Britain, 15.9% in Norway, 11% in Canada and 9.3% in Japan (Ikeda et al., 1995; Baylis et al., 2004; Moum et al., 2005; Harrington et al., 2010).

All haplotypes were found in the studied rams. The ARQ variant was the most frequent (56%). The ARR variant was present only in one ram. The VRQ haplotype frequency in rams was 8%, this being highly similar with data previously published, where this haplotype missed or was registered at a low rate (Agrimi et al., 2003; Acín et al., 2004; Acutis et al., 2004).
From all fifteen genotypes, the present study identified only nine genotypes of PrP gene. The genotypes AHQ/AHQ, AHQ/ARH, ARH/ARH, ARR/VRQ, AHQ/VRQ and VRQ/VRQ have not been identified. The frequency of PrP risk genotypes – susceptible and highly susceptible to scrapie (classified in risk groups 4 and 5, respectively), was 4% for both ARH/VRQ and ARQ/VRQ. PrP genotypes associated also with scrapie susceptibility (risk group 3) showed a prevalence of 16% AHQ/ARQ, 4% ARH/ARQ and 16% ARQ/ARQ. The sheep PrP gene genotyping by PCR with melting curve analysis revealed the presence of all five classes of scrapie susceptibility, proving the high variation of susceptibility in the non-genetically selected herd. Most of the rams were in class 2 (56%), meaning sheep genetically resistant to scrapie, but asking a special attention to be used in selection programmes. In class 1 (genotype ARR/ARR) we found one ram (4%), making the genetic selection very difficult.

These results revealed the importance of sequence-based genotype investigations in sheep-breeding programmes. Those data support the scrapie control and eradication programmes based on the gradual elimination of haplotypes associated with scrapie susceptibility and the use of ARR/ARR genotype rams for breeding.

In our study, the presence of only one ram in class 1 is a challenge for the genetic selection, so we warn upon guided mating with ewes from the heterozygous class 2 (ARR/*) only with rams from class 1 (ARR/ARR) in order to obtain 75% odds for the “new generation” to be in class 1.

REFERENCES


Ovine prion protein genotype profiles of merino rams in a Romanian farm


Paper received 26.06.2013; accepted for publication 11.09.2013

Correspondence:
Mrs. Maria Rodica Gurau,
Faculty of Veterinary Medicine,
University of Agronomical Sciences and Veterinary Medicine Bucharest,
105 Splaiul Independentei, 5th district
Bucharest, Romania,
e-mail: guraumarodica@gmail.com