

Changes in allele and genotype frequencies of *PrP* gene in breeds of sheep in Slovakia between 2004 and 2015

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The objective of the study was to analyze the changes in allele and genotype frequencies of *PrP* gene in auction purebred rams of various breeds in Slovakia between 2004 and 2015. Male genotyping was done within the National Scrapie Eradication Programme by the certified laboratories. Positive changes in all breeds were found during the analyzed period. As a result of strict positive selection for ARR allele and strict negative selection for VRQ and ARQ alleles in most numerous Improved Valachian (IV) and Tsigai (TS) breeds, 94.7% (IV) and 95.7% (TS) of animals were included in the first and second risk (R1 and R2) groups in 2015. Frequencies of ARR allele were above 0.9 in specialized meat breeds. In Lacaune breed, frequencies of ARR, ARQ and VRQ alleles were 0.896, 0.117 and 0.003 in 2015. All purebred East Friesian rams were included in R1 and R2 groups in 2015.

Keywords: sheep, scrapie, PrR gene, allele frequencies, risk groups

1 Introduction

Scrapie is a fatal neurodegenerative disease of the group of transmissible spongiform encephalopathies (TSE) in sheep and goats. Because of exceptional severity of this disease, the State Veterinary and Food Administration launched the breeding programme aimed at forming the scrapie resistant sheep populations. The National Scrapie Eradication Programme is based on the Law No. 39 of 2007 about veterinary care and the Regulation (EC) No. 999 of 2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. The most effective way of eradication of classical scrapie is genotyping of *PrP* gene and incorporation of this knowledge in selection (Dawson et al., 1998, Goldman, 2008). When eradication programmes in member states of EU were launched, it was supposed that animals with desirable *PrP* gene would have been resistant against any type of scrapie and that resistant animals would have not been the transmitters. So called classical scrapie is modulated by a combination of five possible alleles of *PrP* gene (ARR, AHQ, ARH, ARQ, VRQ), combination of which arises fifteen genotypes which are grouped in five risk groups: R1 to R5 (Dawson et al., 1998). Results from beginning of scrapie eradication programmes indicated

that animals with homozygous ARR alleles are of almost no risk of occurrence of scrapie with clinical signs (R1 group). In contrast, animals with VRQ allele are most susceptible (R4 and R5 groups). Animals with ARQ allele are intermediately susceptible to scrapie (Hunter, 1997; Goldman, 2008).

Results from sheep selection for resistance to classical scrapie in EU countries where sheep industry is highly developed indicate success (Hurtado et al., 2002; Hagensars et al., 2010), despite the fact that in the beginning of implementation of the national scrapie eradication programmes there were high differences among breeds as far as the occurrence of risk genotypes is related. Unfortunately, the above mentioned classification system of five risk groups does not work when so called atypical scrapie occurs (Fast and Groschup, 2013). In comparison to classical scrapie, animals susceptible to atypical scrapie are mostly included in R1 to R3 groups (Benestad et al. 2008).

The objective of this study was to analyze the changes in allele and genotype frequencies of *PrP* gene in sheep breeds in Slovakia between 2004 and 2015. The analyses were based on genotype determinations of *PrP* gene done by the State Veterinary and Food Administration on the yearly basis. This is provided to Sheep and Goat

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Breeders' Association who is authorized to implement the breeding programmes aimed at forming of scrapie resistant sheep populations.

2 Material and methods

2.1 Analyzed biological material

During the period between 2004 and 2015, the State Veterinary and Food Administration implemented the mandatory programme for sheep resistance against transmissible spongiform encephalopathies (scrapie) on the yearly basis. This programme specified conditions and requirements for including the flocks in breeding programme, for classification of *PrP* genotypes in risk groups, defined specific needs for male and female populations (which genotypes of *PrP* gene are appropriate for breeding and selection) and further veterinary conditions for flocks to be declared with statute of level I (including only animals with ARR/ARR genotype) and of level II (offspring only of sires with ARR/ARR genotype). Following the approved scrapie eradication programmes and regulations of the State Veterinary and Food Administration, blood samples of all breeding and auction males (intended for mating) in nucleus and multiplier flocks were taken in 2004. In further years, blood samples were taken from all auction males in flocks with production of breeding animals. Although female animals were sampled in certain years (mainly in flocks where animals which showed clinical signs of scrapie occurred), this study is aimed at analysis based on male animals.

Blood samples were taken and *PrP* genotypes were determined in certified laboratories (National Reference Laboratory for TSE which works under the State Veterinary and Food Institute in Zvolen and the State Veterinary and Food Institute in Dolný Kubín). The samples were labeled in accordance with central register (unique identification of each animal). Also in 2004 and 2005, only males of R1, R2 and R3 groups were allowed for mating. Males with genotype ARR/ARR (R1 group) or males in R2 group were preferred. Involving the males of further risk groups (R4 and R5) was based on decision of the Slovak Expert Commission for Scrapie Eradication in the beginning of programme implementation. The exceptions allowed by scrapie eradication programme referred to so called risk breeds (frequency of ARR allele under 25% or even 10%). The principles of including animals in risk groups were slightly modified during the period between 2004 and 2015. Following published studies, all animals with ARQ/ARQ genotype were included in R4 group since 2008. In the beginning of implementation of scrapie eradication programme, males in R3 group were also allowed for mating. Later, only males in R2

group were allowed. At present, mostly males in R1 group are allowed.

The dynamics of changes in allele frequencies of *PrP* gene and of changes in proportion of risk groups in purebred rams of Improved Valachian, Tsigai, Ile de France, Berrichon du Cher, Suffolk, Charollais and East Friesian breeds during the period between 2004 and 2015 is evaluated.

2.2 Method for determination of *PrP* gene

The genotype of *PrP* gene is determined from DNA isolated from white blood cells. Blood of each animal is taken by a veterinary doctor. The principle of genotyping is the amplification of specific regions of DNA encoding the prion protein, and the subsequent detection of the polymorphism of amino acids at position 136, 154, 171. The SSCP method and sequence analysis (or both) are employed.

3 Results and discussion

In the beginning of the scrapie eradication programme, both lower and higher frequencies of all five alleles of *PrP* gene for classical scrapie (ARR, AHQ, ARQ, ARH, VRQ) in purebred populations of Improved Valachian, Tsigai, Merino, Lacaune, Romanov breeds occurred. With meat sheep (Ile de France, Berrichon du Cher, Oxford Down, Suffolk, Charollais), all five alleles occurred only in Suffolk breed. Scrapie eradication was most successful in meat breeds during 12-year period. At present, two to three alleles occur in genotyped males. Almost no occurrences of AHQ and ARH alleles are observed, the occurrence of VRQ allele is very low. The frequency of ARR allele (minimal risk of classical scrapie) in all meat breeds was above 0.9 in 2015. The exception was Charollais breed with frequency of ARR allele equal to 0.773. The most desirable occurrence of alleles of *PrP* gene was observed in Berrichon du Cher breed. The frequency of ARR allele was equal to 0.946 in this breed. The frequency of (risk) ARQ allele was equal to 0.054. In accordance with expectation and specified requirements of scrapie eradication programme, the proportion of males included in R1 group increased to a greater extent. With all meat breeds in Slovakia, the proportion of males included in R1 group was above 80% in 2015. The exception is Chrollais breed with 57.6% proportion of males in R1 group, with 39.4% proportion of males in R2 group, with 3.0% proportion of males in R5 group. Males of Ile de France and Berrichon du Cher breeds occurred only in R1 and R2 groups in 2015.

Positive changes in allele frequencies of *PrP* gene and proportions of risk groups were also observed in most numerous breeds in Slovakia (Improved Valachian

Table 1 Changes in allele frequencies of *PrP* gene in Improved Valachian bred

Year	ARR	AHQ	ARQ	ARH	VRQ	No. of rams tested
2004	0.377	0.077	0.472	0.006	0.067	2335
2005	0.387	0.097	0.465	0.010	0.040	593
2006	0.390	0.104	0.435	0.012	0.058	3723
2007	0.431	0.106	0.410	0.020	0.033	1564
2008	0.538	0.065	0.349	0.008	0.038	1018
2009	0.551	0.069	0.334	0.010	0.037	766
2010	0.616	0.077	0.274	0.009	0.024	586
2011	0.619	0.060	0.279	0.010	0.032	497
2012	0.644	0.068	0.258	0.002	0.028	471
2013	0.714	0.053	0.209	0.004	0.019	387
2014	0.685	0.054	0.243	0.005	0.013	305
2015	0.745	0.063	0.182	0.005	0.005	280

(IV) and Tsigai (TS)) during the 12-year period. The occurrence of allele frequencies in both breeds was almost the same in 2004. According to Table 1 (only allele frequencies of *PrP* gene in IV are given), the lower frequencies of ARR and VRQ alleles in IV than in TS were found in the beginning of implementation of scrapie eradication programme (0.377 vs. 0.428 and 0.067 vs. 0.094). In 2015, the frequencies of ARR alleles in IV and TS were equal to 0.745 and 0.794, respectively. Moreover, both breeds were of the low frequencies of VRQ allele in 2015 (0.005 in IV and 0.015 in TS). The decrease of (risk) ARQ allele was found (from 0.472 to 0.182 in IV and from 0.446 to 0.187 in TS). At present, the low frequencies of AHQ and ARH alleles can be still found in both populations. As a result of strict positive selection for ARR allele and of strict negative selection for VRQ and ARQ alleles in nucleus flocks, proportions of auction males included in R1 and R2 groups were found 94.7% in IV (proportion of males in R3, R4 and R5 groups was 5.3%) and 95.8% in TS (proportion of males in R3, R4 and R5 groups was 4.2%).

With dairy breeds in Slovakia (Lacaune (LC), East Friesian (EF)), the occurrence of allele frequencies was more desirable in LC breed in the beginning of implementation of the scrapie eradication programme. Although the frequency of ARR allele in LC breed was high (0.616), all five alleles were found (frequencies of ARQ and VRQ alleles were 0.326 and 0.019). At present, frequencies of ARR, ARQ and VRQ are as follows: 0.863, 0.117, 0.003 in LC breed.

The proportion of LC auction males in R1 and R2 groups was 98.6% in 2015. In EF, no auction male included in R1 group was identified in 2004. The frequency of ARR allele was only 0.020, the frequencies of AHQ and ARQ alleles were 0.400 and 0.560 in EF breed. Males with VRQ

allele were not identified. At present, the occurrence of allele frequencies in EF breed is more desirable. The frequencies of ARR and (risk) ARQ alleles were found 0.735 and 0.059.

The frequency of AHQ is still relatively high (0.206), which is the highest in breeds in Slovakia. Nevertheless, animals with this allele are considered to be resistant against classical scrapie. In 2015, all sampled rams were included in R1 and R2 groups. The relatively high proportion of genotyped rams are those of synthetic population of Slovak Dairy sheep (SD). In last three years, the frequencies of ARR, ARQ and VRQ ranged from 0.711 to 0.789, from 0.180 to 0.255 and from 0.005 to 0.010. More than 95% of SD males sampled were included in R1 and R2 groups in 2014 and 2015.

4 Conclusions

Based on changes in allele and genotype frequencies of *PrP* gene or in risk groups during the 12-year period, it can be stated that implementation of eradication programme against classical scrapie in Slovakia was successful. The occurrences of ARR allele and ARR/ARR genotype in all breeds increased. In contrast, the occurrences of VRQ and ARQ alleles decreased to a greater extent. Only animals included in R1 and R2 groups occur in some breeds in Slovakia at present. In future, the analyses of these changes on the occurrence of animals with clinical signs shall be done countrywide. Also, the attention to atypical scrapie shall be given. Breeds with a higher occurrence of undesirable risk groups (mainly IV and TS) shall be predominantly sampled. The genotypes of breeding female lambs and female yearlings shall be also investigated.

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