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Polymorphism of the prion protein *PrP* in Polish Heath Sheep and Żelaźnieńska Sheep flocks from the Experimental Farm in Żelazna*

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The study was conducted in the Experimental Farm in Żelazna on foundation stocks and stud rams of two breeds: Polish Heath Sheep and Żelaźnieńska Sheep. The research included 538 foundation stock ewes and 29 stud rams of Polish Heath Sheep and 293 foundation stock ewes and 18 stud rams of Żelaźnieńska Sheep. Identification of the *PrP* prion protein gene was carried out in all animals. Genetic diversity in the frequency of scrapie alleles and genotypes was found to be lower in stud rams (3 genotypes and 3 alleles in Polish Heath Sheep and 2 genotypes and 2 alleles in Żelaźnieńska Sheep) than in foundation stock ewes (6 genotypes and 3 alleles in Polish Heath Sheep and 5 genotypes and 3 alleles in Żelaźnieńska Sheep). In the last year of the study, only rams with the ARR/ARR genotype were noted in stud rams of both breeds. The year of research had no significant effect on the frequency of scrapie alleles and genotypes in the foundation stock ewes and stud rams except in Polish Heath Sheep stud rams.

KEY WORDS: sheep / PrP / frequency of alleles and genotypes

Scrapie, like BSE in cows and Creutzfeldt-Jakob disease in humans, is a naturally occurring form of transmissible spongiform encephalopathy (TSE). The prion protein (PrP) is responsible for the occurrence of scrapie in sheep. In the *PrP* gene, which is responsible for genetic resistance or susceptibility to scrapie [1, 3, 5, 9], a number of polymorphisms have been observed in codons 136, 154 and 171. The ARR allele has been shown to guarantee the lowest susceptibility to scrapie. Studies conducted in the United Kingdom

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and the Netherlands have shown that animals carrying the VRQ allele are very highly susceptible to the disease, while the ARR allele is the least common in sheep with clinical symptoms of scrapie. Increased frequencies of this allele are considered the primary means of eliminating and controlling scrapie in sheep [2, 3, 11]. Thus it is unsurprising that determination of prion protein alleles has been included in EU legislation and national regulations [6, 7, 8].

The aim of this study was to determine the frequencies of prion protein alleles and genotypes in animals from breeding flocks of the Polish Heath and Żelaźnieńska Sheep breeds, kept in 2009-2011. Both breeds were covered by a Genetic Resources Conservation Programme.

Material and methods

The study was conducted on a flock of ewes and rams of two species, Polish Heath and Żelaźnieńska, kept at the Professor A. Skoczylas Experimental Sheep and Goat Farm in Żelazna. In the case of the Polish Heath sheep, 538 ewes from the foundation stock and 29 breeding rams were assessed, while in the case of the Żelaźnieńska Sheep, 293 ewes and 18 rams were tested.

DNA was isolated from the leukocytes. In order to obtain high quality DNA that could be used repeatedly after freezing and thawing, the blood was initially cleansed of DNA--modifying haem compounds by removing erythrocyte lysis products. Prion protein alleles were determined using the KASPar® system, by the single nucleotide polymorphism (SNP) method (Table 1).

Table 1
Primers and SNP sites of the prion protein locus

Codon	Primers 3'-5'	SNP	Changes	Localization
171	CACAGTCAGTGGAACAAGCC/ CTTTGCCAGGTTGGGG	AY909542:g.385A>G	A/G	exon 3
171		AY909542:g.386G>T	G/T	exon 3
136		AY909542:g.479C>T;	C/T	exon 3
154		AY909542:g.534G>A	G/A	exon 3

Frequencies of prion protein alleles and genotypes were presented based on the reading of genotypic DNA samples within the breeds in the foundation flock ewes and breeding rams. The SPSS version 21.0 statistical package was used for the statistical computations [10]. The χ^2 test was used to calculate the frequencies of prion protein alleles and genotypes within each breed and sex. The number of degrees of freedom (df) was calculated in each analysis by the formula [(number of years – 1) x (number of genotypes or alleles – 1)].

Results and discussion

A comparison of the frequencies of prion protein genotypes and alleles in the flocks of Polish Heath Sheep and Żelaźnieńska Sheep is presented in Tables 2-9.

Polish Heath sheep

The results of the study conducted in the foundation flock of ewes in 2009-2011 are presented in Table 2. Six prion protein genotypes were identified. Three of them contained the ARR allele, and together they had by far the largest share in the entire flock. The frequency of the most valuable genotype, ARR/ARR, ranged from 16.0% to 20.1%. Although the year of the study was not found to influence the frequency of genotypes, an increasing tendency was shown for the frequency of genotypes containing the ARR allele. The pattern of frequency was similar to that shown in a study by Gombojav et al. [1] conducted on primitive breeds. The frequency of the ARR/ARR genotype, however, was higher than that observed in a study on Polish Heath sheep by Niżnikowski et al. [4].

Table 2

Frequency of prion protein genotypes in foundation stock ewes of Polish Heath Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	175	185	178	538
Genotype				
ARR/ARR	16.00	16.80	20.10	95
ARR/ARQ	40.60	46.50	48.30	243
ARR/AHQ	17.10	15.10	15.20	85
ARQ/ARQ	16.00	14.10	10.70	73
ARQ/AHQ	9.70	7.00	5.60	40
AHQ/AHQ	0.60	0.50	0.00	2
χ^2=7.538; df=10, p=0.674				

The analysis of the results for stud rams is shown in Table 3. A significant influence of the year of the study on the frequency of individual genotypes was observed, indicating that individuals with the ARR/ARR genotype were favoured in selecting individuals for the flock. In 2011, the frequency of this genotype was 100%, so that progeny with only this allele has been obtained since 2012.

At the same time, analogous comparisons were made of the frequency of prion protein alleles in the sheep of the foundation stock in the three years of the study.

Only three alleles, ARR, ARQ and AHQ, were detected in the breeding ewes (Table 4). No influence of the year on the frequency of prion protein alleles was shown. However, a slight increase in the frequency of the ARR allele was observed, accompanied by a decrease in that of the other two.

Table 3

Frequency of prion protein genotypes in stud rams of Polish Heath Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	11	8	10	29
Genotype				
ARR/ARR	45.50	87.50	100.00	22
ARR/ARQ	36.40	12.50	0.00	5
ARR/AHQ	18.20	0.00	0.00	2
χ^2=9.686; df=4, p=0.046				

Table 4

Frequency of prion protein alleles in foundation stock ewes of Polish Heath Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	350	370	356	1076
Alleles				
ARR	44.90	47.60	52.00	518
ARQ	41.10	40.80	37.60	429
AHQ	14.00	11.60	10.40	129
χ^2=0,097; df=4, p=0,999				

The ARQ and AHQ alleles were eliminated in the stud rams, and in 2011 only one allele was observed – ARR (Table 5). The absence of the VRQ allele is a highly beneficial outcome. The elimination of animals with this allele from the flock guarantees that the offspring will be genetically resistant to clinical forms of scrapie.

Table 5

Frequency of prion protein alleles in stud rams of Polish Heath Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	22	16	20	58
Alleles				
ARR	72.70	93.80	100.00	51
ARQ	18.20	6.30	0.00	5
AHQ	9.10	0.00	0.00	2
χ^2=8.405; df=4, p=0.078				

In conclusion, the year of observation was not found to influence the frequency of alleles and genotypes in either group of the Polish Heath flock. An exception was the influence of the year on the frequency of different genotypes in stud rams. The trends shown in the stud rams confirm results reported by other authors, indicating that effective breeding work may be carried out in this area [2, 3, 9, 11].

Żelaźnieńska Sheep

The evaluation of the frequency of prion protein genotypes in breeding ewes and rams is presented in Tables 6 and 7. Five genotypes were found in the ewes and two in the rams. In the ewes, the relatively high proportion of genotypes containing the ARR allele in all years of the study should be regarded as a positive finding. It is much higher than in studies by other authors on native sheep that have not undergone selection in this direction [1, 3, 5, 9].

Table 6

Frequency of prion protein genotypes in foundation stock ewes of Żelaźnieńska Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	92	100	101	293
Genotypes				
ARR/ARR	22.80	28.00	27.70	77
ARR/ARQ	46.70	51.00	52.50	147
ARR/AHQ	5.40	3.00	5.0	13
ARQ/ARQ	20.70	15.00	11.90	46
ARQ/AHQ	4.30	3.00	3.00	10
χ^2=4.429; df=8, p=0.817				

Significantly less variation in the number of genotypes was observed in the breeding rams (Table 7). In 2011, only the ARR/ARR genotype was present, as the genotype with the ARQ allele, which is characteristic for primitive sheep breeds and the European mouflon, had been eliminated [1, 4, 5].

Table 7

Frequency of prion protein genotypes in stud rams of Żelaźnieńska Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	7	6	5	18
Genotypes				
ARR/ARR	42.90	66.70	100.00	12
ARR/ARQ	57.10	33.30	0.00	6
χ^2=4.286; df=2, p=0.117				

The evaluation of the frequencies of individual prion protein alleles in the foundation stock ewes and breeding rams of Żelaźnieńska Sheep is presented in Tables 8 and 9. In the ewes, three alleles were found in all years of the study (Table 8), while in the rams, two alleles were present in 2009 and 2010, but only the ARR allele in 2011 (Table 9). The direction of the changes observed, both in the foundation stock of ewes and in the breeding rams, should be regarded as appropriate, due to the increase in the frequency of the scrapie-resistant ARR allele, which is consistent with the findings of other authors [2, 3, 9, 11].

Table 8

Frequency of prion protein alleles in foundation stock ewes of Żelaźnieńska Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	184	200	202	586
Alleles				
ARR	48.90	55.00	56.40	314
ARQ	46.20	42.00	39.60	249
AHQ	4.90	3.00	4.00	23
χ^2=3.007; df=4, p=0.557				

Table 9

Frequency of prion protein alleles in stud rams of Żelaźnieńska Sheep in 2009-2011

Frequency (%)	Year of study			n
	2009	2010	2011	
n	14	12	10	36
Alleles				
ARR	71.40	83.30	100.00	30
ARQ	28.60	16.70	0.00	6
χ^2=3.429; df=2, p=0.180				

In conclusion, the year of observation was not found to influence the frequency of prion protein alleles and genotypes in either group of sheep of the Żelaźnieńska breed. Fewer *PrP* alleles and genotypes were observed in the breeding rams than in ewes. These results are the effect of breeding work aimed at increasing the frequency of alleles and genotypes that determine genetic resistance to scrapie. The trends demonstrated in adult animals (breeding ewes and rams) support findings by other authors that effective breeding work can be carried out in this area [2, 3, 9, 11].

The research carried out at the Experimental Sheep and Goat Farm in Żelazna on the frequency of prion protein gene (*PrP*) mutations suggests the following conclusions:

– There was less genetic differentiation in the occurrence of prion protein alleles and genotypes in breeding rams (3 genotypes and 3 alleles in Polish Heath Sheep and 2 geno-

types and 2 alleles in Żelaźnieńska Sheep) than in the foundation stock ewes (6 genotypes and 3 alleles in Polish Heath Sheep and 5 genotypes and 3 alleles in Żelaźnieńska Sheep). In 2011, only individuals with the ARR/ARR genotype were present in the breeding rams of both breeds.

– The year of the study was not found to affect the frequencies of prion protein alleles and genotypes in the breeding ewes and rams. A significant influence in this area was found only in breeding rams of the Polish Heath breed, but due to the small number of animals tested, conclusions should be drawn cautiously.

The results obtained for breeding rams indicate that selection aimed at improving the frequency of beneficial prion protein alleles and genotypes is justified and that there is a need to develop a breeding programme.

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