Scientific Annals of Polish Society of Animal Production - Vol. 10 (2014), No 4, 25-33

Polymorphism of the prion protein gene *PrP* in Polish Lowland Sheep raised in the Podlasie region

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The study was conducted on ewes and rams of the breed Polish Lowland Sheep from 7 flocks in the Podlasie Voivodeship. The analysis included 349 sheep (326 ♀ and 23 ♂) aged 2 to 11 years, belonging to three varieties of Polish Lowland Sheep: Corriedale (2 herds), Polish Lowland Sheep of Podlasie (3 herds) and Żelaźnieńska Sheep (2 herds). Identification of the *PrP* prion protein gene was performed in all the animals. The variety of Polish Lowland Sheep was found to have a highly significant effect, and sex an insignificant effect, on the frequency of scrapie alleles and genotypes. Five scrapie alleles (ALRR, ALRQ, ALHQ, AFRQ and VLRQ) were found in the Corriedale Sheep. Of these alleles, VLRQ was not found in the Polish Lowland Sheep of Podlasie and VLRQ and AFRQ were not found in the Żelaźnieńska Sheep. Eleven genotypes of scrapie were identified, with the greatest number in the Corriedale Sheep – 11 genotypes (11 in ewes, 3 in rams), 5 genotypes in the Polish Lowland Sheep of Podlasie (5 in ewes, 2 in rams) and 3 genotypes in the Żelaźnieńska Sheep (3 in ewes, 2 in rams). The frequency of ALRR/ALRR and ALRR/ALRQ genotypes in the Polish Lowland Sheep of Podlasie and Żelaźnieńska Sheep was very high compared to the Corriedale Sheep. Genotypes containing the AFRQ allele (ALHQ/AFRQ, AFRQ/AFRQ, VLRQ/AFRQ) and the VLRQ allele (VLRQ/ALRR, VLRQ/ALRQ, VLRQ/ALHQ, VLRQ/AFRQ) were found in the Corriedale Sheep. In the Polish Lowland Sheep of Podlasie the ALRR/AFRQ genotype was found only in one ewe. The very high frequency of alleles and genotypes containing the ALRR allele in the Żelaźnieńska Sheep and Polish Lowland Sheep of Podlasie must be regarded as a very valuable result. Due to the presence in Corriedale Sheep of alleles and genotypes containing VLRQ and AFRQ, with a lower frequency of ALRR, a breeding programme for this breed should be developed to modify the current frequency of scrapie alleles and genotypes.

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

In 2001, the EU Parliament established legal regulations for the prevention, control and eradication of transmissible spongiform encephalopathies [16]. In 2003, by decision 2003/100/EC, the European Commission mandated breeding programmes aimed at increasing genetic resistance to scrapie in all sheep breeds in Europe [1]. In addition, the Commission issued Regulation No 260/2003 on the eradication of BSE (spongiform encephalopathy) in sheep and goats, and regulating trade in live sheep and goats, as well as bovine embryos [15]. The prion protein PrP is responsible for scrapie in sheep. A number of polymorphisms observed in codons 136, 141, 154 and 171 of the *PrP* gene are responsible for genetic resistance or susceptibility to scrapie [2, 5, 12]. In addition, AFRR or ALRR alleles have been found to guarantee the lowest susceptibility to scrapie. In the UK and the Netherlands, the VFRQ allele was observed to be responsible for a high degree of susceptibility to this disease in sheep. The ALRR and AFRR alleles were the least common in sheep with clinical scrapie symptoms, and therefore selection is the primary means of eliminating and controlling the disease in sheep [3, 5, 18].

In Poland, studies have been conducted to monitor the occurrence of scrapie alleles in many breeds [7, 10, 14], as well as an experiment aimed at increasing the frequency of scrapie-resistant genetic determinants [8]. As a result, it was demonstrated that alleles encoding valine were not present in the Polish Heath Sheep [7, 8, 10, 14]. In the remaining domestic breeds this determinant was present with varying frequency. Among the Polish Lowland group of sheep, which occupy an important position in the domestic sheep population [13], such work has not been conducted, apart from the Żelaźnieńska Sheep [10]. The diversity of varieties due to different crossing schemes is considerable. During breeding work, the English breed Leicester Longwool was used in the creation of the Żelaźnieńska Sheep [11] and the Lincoln breed in the production of the Corriedale [9], while Romney Marsh was used to create Polish Lowland Sheep of Podlasie [4]. As all of these sheep varieties are abundant in the Podlaskie Voivodeship, an attempt was made to evaluate the frequency of genes and genotypes of the PrP protein in each of them, in order to learn the genetic susceptibility to scrapie in this group of sheep in north-eastern Poland.

Material and methods

The study included flocks of ewes and rams of different varieties of Polish Lowland Sheep (seven flocks) from the Podlaskie Voivodeship, including two flocks of Corriedale Sheep, two of Żelaźnieńska Sheep and three of Lowland Sheep of Podlasie. Animals aged 2 to 11 years (Table 1) were evaluated.

The flocks from which samples were taken were randomly selected. Blood was drawn from the jugular vein into EDTA tubes for the purpose of isolating genomic DNA for molecular genetic analysis. DNA was isolated from the leukocytes of blood stored with EDTA. In order to obtain high quality DNA that could be used repeatedly after being frozen and thawed, the blood was cleansed of DNA-modifying haem-containing compounds by removing erythrocyte lysis products. DNA was isolated from the leukocytes by chromatography

Table 1
Experimental material used in the study

Breed	Number of ewes and rams		
	total ♀	total ♂	in each herd
Corriedale Sheep	85	4	herd 1: 78 ♀, 4 ♂
			herd 2: 7 ♀
Polish Lowland Sheep of Podlasie	161	14	herd 1: 24 ♀, 4 ♂
			herd 2: 48 ♀, 4 ♂
			herd 3: 89 ♀, 6 ♂
Żelaźnieńska Sheep	80	5	herd 1: 70 ♀, 4 ♂
			herd 2: 10 ♀, 1 ♂
Total within sex	326	23	
Total	349		

on silica minicolumns from A & A Biotechnology (Gdańsk, Poland). The DNA fraction thus obtained served as a template for amplification of the polymorphic fragment of the prion protein gene.

Genotyping of scrapie alleles was carried out by the KASPar® system. This system and the genotyping procedure (www.kbioscience.co.uk) rely on the SNP point polymorphism method using the primers listed in Table 2.

The distribution of frequencies of alleles and genotypes in the ewes and rams, based on reading of the genotyped DNA samples, was presented in preparation for the next stages of the research.

Table 2
Primers and SNP sites of the prion protein locus

Locus	Primers 3'-5'	SNP	Changes	Localization
PrP prion protein	CACAGTCAGTGGAACAAGCC/ CTTTGCCAGGTTGGGG	AY909542:g.385A>G	A/G	exon 3
		AY909542:g.386G>T	G/T	exon 3
		AY909542:g.479C>T	C/T	exon 3
		AY909542:g.493C>T	C/T	exon 3
		AY909542:g.534G>A	G/A	exon 3

Statistical calculations were performed in the SPSS 21.0 software package. The χ^2 test was used to determine the effect of breed and gender on the frequency of alleles and genotypes. In the case of alleles, the threshold values were read at 12 degrees of freedom, and for genotypes at 20 degrees of freedom. The results are presented in Tables 3 and 4.

Results and discussion

Table 3 shows the distribution of alleles in the varieties of Polish Lowland Sheep. Five alleles (ALRR, ALRQ, ALHQ, AFRQ and VLRQ) were noted in the Corriedale variety. Of these, the VLRQ allele was not found in the Polish Lowland Sheep of Podlasie, while the VLRQ and AFRQ alleles were absent from the Żelaźnieńska Sheep. No differences between genders were observed in the frequency of individual alleles, although fewer determinants were found in rams than in ewes. The effect of breed, on the other hand, was highly significant. The very high frequency of the ALRR allele in the Żelaźnieńska Sheep and Polish Lowland Sheep of Podlasie (over 71%) should be considered highly favourable. High frequency of this allele was also noted in the

Table 3
Frequency of PrP alleles in the sheep

Specification	Number and percentage	Alleles					
		ALRR	ALRQ	ALHQ	AFRQ	VLRQ	total
Corriedale Sheep	♀	83	19	25	19	24	170
	♂	6	0	1	1	0	8
	♀♂	89	19	26	20	24	178
	%	50.00	10.67	14.61	11.24	13.48	100.00
Polish Lowland Sheep of Podlasie	♀	241	56	24	1	0	322
	♂	22	6	0	0	0	28
	♀♂	263	62	24	1	0	350
	%	75.14	17.71	6.86	0.29	0.00	100.00
Żelaźnieńska Sheep	♀	116	42	2	0	0	160
	♂	6	4	0	0	0	10
	♀♂	122	46	2	0	0	170
	%	71.76	27.06	1.18	0.00	0,00	100.00
Total	♀	440	117	51	20	24	652
	♂	34	10	1	1	0	46
	♀♂	474	127	52	21	24	698
	%	67.91	18.19	7.45	3.01	3.44	100.00

Corriedale Sheep (50%). The occurrence of the VLRQ allele in Corriedale Sheep and the AFRQ allele in both Corriedale Sheep and Polish Lowland Sheep of Podlasie should be considered unfavourable, despite their very low frequency. The VLRQ allele is considered to be susceptible to classical scrapie and AFRQ to atypical scrapie. In breeding work, animals that carry these alleles should be eliminated from the flock [7, 8, 10, 14].

The results confirm the tendencies reported by other authors in various breeds of sheep [3, 5, 6, 18, 12]. It should be emphasized that the VLRQ allele was lacking in the Zelaznenska Sheep or the Polish Lowland Sheep of Podlasie, as shown in other studies for the Polish Heath Sheep [7, 8, 10, 14].

The distribution of scrapie genotypes is shown in Table 4. We identified 11 scrapie genotypes in the material, with the most genotypes found in the Corriedale sheep – 11 (11 in ewes and 3 in rams), 5 genotypes in Polish Lowland Sheep of Podlasie (5 in ewes and 2 in rams) and 3 genotypes in Żelaźnieńska sheep (3 in ewes and 2 in rams). No genotypes with the VLRQ or AFRQ allele were found in rams, although the effect of gender on their frequency was not significant. The influence of the sheep variety on the frequency of individual scrapie genotypes was statistically highly significant. A very high frequency of the ALRR/ALRR genotype was noted in the Żelaźnieńska Sheep and Polish Lowland Sheep of Podlasie as compared to the Corriedale Sheep. A similar situation was observed for the ALRR/ALRQ genotype. Genotypes containing the AFRQ allele (ALHQ/AFRQ, AFRQ/AFRQ and VLRQ/AFRQ) and the VLRQ allele (VLRQ/ALRR, VLRQ/ALRQ, VLRQ/ALHQ and VLRQ/AFRQ) were found only in Corriedale Sheep and only in ewes, with a very low frequency. The ALRR/AFRQ genotype was found only in one Polish Lowland Sheep of Podlasie ewe. This means that the Żelaźnieńska Sheep and, essentially, the Polish Lowland Sheep of Podlasie have a lack of or marginal frequency of genetic determinants characteristic of genetic susceptibility to both classical and atypical scabies. As regards Corriedale sheep, there is a fairly urgent need to establish a breeding programme aimed at eliminating animals with genetic determinants susceptible to scrapie in order to free this sheep population from them.

The results of the study confirm the distribution of susceptible genotypes in the PrP locus shown in other breeds and indicate their relatively low diversity in the Żelaźnieńska Sheep and the Polish Lowland Sheep of Podlasie, as was the case for Polish Heath Sheep [7, 8, 10, 14], as well as sheep ancestors and their hybrids [3, 5, 6, 12, 18]. The results of research on Corriedale sheep indicate a high degree of variation in both the number of alleles and genotypes, as demonstrated in wool-and-meat breeds and in meat crossbreeds [3, 5, 6, 18]. This indicates that a breeding programme should be developed for this variety of sheep to identify scrapie determinants in order to eliminate the VLRQ and AFRQ alleles from the population. An extremely valuable finding of this study is the very high frequency of alleles and genotypes containing the ALRR allele, especially in the Żelaźnieńska and Polish Lowland Sheep of Podlasie varieties.

Table 4
Frequency of PrP genotypes in the sheep

Specification	Number and percentage	Genotypes											
		ALRR/ ALRR	ALRR/ ALRQ	ALRR/ ALHQ	ALRR/ AFRQ	ALHQ/ ALHQ	ALHQ/ AFRQ	AFRQ/ AFRQ	VLRQ/ ALRR	VLRQ/ ALRQ	VLRQ/ ALHQ	VLRQ /AFRQ	total
Corriedale Sheep	♀	11	17	19	8	1	3	2	17	2	1	4	85
	♂	2	0	1	1	0	0	0	0	0	0	0	4
	♀♂	13	17	20	9	1	3	2	17	2	1	4	89
	%	14.61	19.10	22.47	10.11	1.12	3.37	2.25	19.10	2.25	1.12	4.50	100.00
Polish Lowland Sheep of Podlasie	♀	82	56	20	1	2	0	0	0	0	0	0	161
	♂	8	6	0	0	0	0	0	0	0	0	0	14
	♀♂	90	62	20	1	2	0	0	0	0	0	0	175
	%	51.43	35.43	11.43	0.57	1.14	0.00	0.00	0.00	0.00	0.00	0.00	100.00
Żelaźnieńska Sheep	♀	36	42	2	0	0	0	0	0	0	0	0	80
	♂	1	4	0	0	0	0	0	0	0	0	0	5
	♀♂	37	46	2	0	0	0	0	0	0	0	0	85
	%	43.50	54.10	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Total	♀	129	115	41	9	3	3	2	17	2	1	4	326
	♂	11	10	1	1	0	0	0	0	0	0	0	23
	♀♂	140	125	42	10	3	3	2	17	2	1	4	349
	%	40.11	35.82	12.03	2.87	0.86	0.86	0.57	4.87	0.57	0.29	1.15	100.00

The research carried out on three varieties of Polish Lowland Sheep species raised in the Podlaskie Voivodeship revealed a highly significant influence of the variety and a non-significant effect of gender on the frequency of PrP alleles and genotypes. Five alleles (ALRR, ALRQ, ALHQ, AFRQ and VLRQ) were found in the Corriedale variety; among these alleles VLRQ was not found in the Polish Lowland Sheep of Podlasie and VLRQ and AFRQ were absent from the Żelaźnieńska sheep. We identified 11 genotypes of the PrP protein, with the greatest number in the Corriedale Sheep – 11 genotypes (11 in ewes and 3 in rams), 5 genotypes in Polish Lowland Sheep of Podlasie (5 in ewes and 2 in rams) and 3 genotypes in the Żelaźnieńska sheep (3 in ewes and 2 in rams). There was a very high frequency of the ALRR/ALRR and ALRR/ALRQ genotypes in the Żelaźnieńska Sheep and Polish Lowland Sheep of Podlasie as compared to the Corriedale Sheep. Genotypes containing the AFRQ allele (ALHQ/AFRQ and AFRQ/AFRQ) and VLRQ allele (VLRQ/ALRR, VLRQ/ALRQ, VLRQ/ALHQ and VLRQ/AFRQ) were found only in Corriedale Sheep, while in Polish Lowland Sheep of Podlasie ALRR/AFRQ was found only in ewes, with a very low frequency.

In summary, an extremely valuable finding of this study is the very high frequency of alleles and genotypes containing the ALRR allele in the Żelaźnieńska Sheep and Polish Lowland Sheep of Podlasie. Due to the presence of alleles and genotypes containing VLRQ and AFRQ in Corriedale sheep, with a lower frequency of ALRR, a breeding programme should be developed for this variety of sheep to change the current distribution of alleles and genotypes in the PrP locus.

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