

Polymorphism of the prion protein gene (*PRNP*) in Polish Merino and Suffolk sheep flocks

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The aim of the study was to investigate the distribution of prion protein *PRNP* alleles in flocks of Polish Merino and Suffolk sheep. The research was conducted in 2012-2017 on ewes and rams kept in the Golina Wielka sheepfold (Greater Poland Voivodeship, Poland). All animals (264 ♀ and 64 ♂ Polish Merino; 98 ♀ and 73 ♂ Suffolk) were up to one year old. *PRNP* gene polymorphism was identified in the sheep. The frequency of scrapie alleles and genotypes was found to be highly significantly influenced by the breed and significantly influenced by the year in the case of Polish Merino sheep. Five alleles (ALRR, ALRQ, ALHQ, AFRQ and VLQR) were detected in Polish Merino sheep, leading to the identification of 10 *PRNP* genotypes. In the Suffolk breed, three alleles (ALRR, ALRQ and ALHQ) and three genotypes were identified. In the Polish Merino breed, the frequency of the ALRR/ALRQ genotype was high and was the highest among all genotypes, followed by ALRR/ALRR. The level of genotypes containing valine at codon 136 was very low. In the Suffolk breed, the frequency of the ALRR/ALRR genotype was very high, and there were no alleles with valine at codon 136. In addition, in Polish Merino sheep, phenylalanine at codon 141 was detected only in the AFRQ allele, which appeared in two genotypes (in combination with ALRR and ALRQ).

KEY WORDS: sheep, *PRNP*, allele and genotype distribution

The EU Parliament has established legal regulations for the prevention, control and eradication of transmissible spongiform encephalopathies (Regulation (EC) 999/2001; Commission decision C/2003/498; Commission Regulation (EC) 260/2003). Two forms of transmissible spongiform encephalopathy have been identified in sheep: classical and atypical scrapie. The prion protein has been identified as responsible for classical scrapie in sheep. A number of polymorphisms at codons 136, 154 and 171 have been observed in the

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gene encoding it and have been recognized as responsible for genetic regulation of scrapie resistance or susceptibility (Lühken et al., 2004; Kaal and Windig, 2005; Van Kaam et al., 2005; Palhiere et al., 2008). The ARR allele (resulting from coding of alanine – A, arginine – R and again arginine – R) is believed to ensure the lowest susceptibility to scrapie, while the valine (V) allele coding at codon 136 is considered responsible for high sensitivity to this disease (Kaal and Windig, 2005; Van Kaam et al., 2005; Palhiere et al., 2008; Rejduch et al., 2009). In the case of atypical scrapie (Nor98), the first case of which was described by Benestad et al. (2003), the alleles located at codon 141 (Goldman, 2008; McIntyre et al., 2008; Mazza et al., 2010) coding for leucine (L) and phenylalanine (F) are suspected, of which the F allele is much more commonly associated with clinical forms of this disease (Goldman, 2008; McIntyre et al., 2008). In addition, classical scrapie has been found to coexist with its atypical form in sheep flocks (Mazza et al., 2010).

In Poland, studies monitoring the occurrence of scrapie alleles have been conducted in many breeds (Rejduch et al., 2009; Wiśniewska and Mroczkowski, 2009; Polak et al., 2010; Niżnikowski et al., 2014). The absence of valine alleles has been demonstrated in Polish Heath sheep, while their frequency is varied in other breeds (Rejduch et al., 2009; Wiśniewska and Mroczkowski, 2009; Niżnikowski et al., 2014). It is therefore worthwhile to carry out breeding work aimed at eliminating the VLRQ allele from sheep populations, while at the same time aiming to increase the frequency of the ALRR allele in order to improve genetic resistance to this disease within individual flocks and breeds. The aim of the present study was to investigate the frequency of alleles of the prion protein gene *PRNP* in flocks of the wool and meat breed Polish Merino and the meat breed Suffolk.

Material and methods

The research was conducted on ewes and rams up to one year of age, belonging to the breeds Polish Merino (264 ♀ and 64 ♂) and Suffolk (98 ♀ and 73 ♂), from the Golina Wielka sheepfold (Greater Poland Voivodeship, Poland), during the years 2012-2017 (Table 1). Animals with the VLRQ allele were eliminated from breeding.

Sample preparation

Blood was collected from the jugular vein into tubes containing K₂EDTA. Genomic DNA was isolated from leukocytes using a commercial kit according to the procedure recommended by the manufacturer (Blood Mini kit, A&A Biotechnology, Poland). Genotyping of scrapie alleles was performed with the KASPar[®] system (www.kbioscience.co.uk), which uses a single nucleotide polymorphism (SNP) method based on the primers given in Table 2. The high reliability of the SNP method compared to the sequencing method has been demonstrated by Green et al. (2006).

Statistical analysis

Statistical calculations were performed using SPSS v. 26.0 software. The χ^2 test was used to assess the effect of breed and of sex within each breed on the allele and genotype frequencies. For the effect of breed, limit values were read at four degrees of freedom in the case of alleles at nine degrees of freedom in the case of genotypes. The effect of sex within

Table 1
Number of sheep used in the study

Year	Breed			
	Polish Merino		Suffolk	
	♀	♂	♀	♂
2012	36	4	15	8
2013	23	3	13	8
2014	81	9	26	21
2015	29	0	7	8
2016	78	0	26	15
2017	17	48	11	13
Total	264	64	98	73
	328		171	

Table 2
Primers and SNPs of the *PRNP* prion protein gene

Locus	Primers	SNP	Changes	Position
<i>PRNP</i> prion protein	CACAGTCAGTGAACAAGCC/ 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

each breed was assessed at four degrees of freedom in Polish Merino and two degrees of freedom in Suffolk for alleles, and at nine degrees of freedom in Polish Merino and two in Suffolk for genotypes. The effect of the year within the breed was assessed at 20 degrees of freedom in Polish Merino and eight degrees in Suffolk for alleles, and at 45 degrees of freedom in Polish Merino and eight degrees in Suffolk for genotypes. The results are presented in Tables 3-6.

Results and discussion

The presence of five alleles (ALRR, ALRQ, AFRQ, ALHQ and VLRQ) was detected in Polish Merino sheep, and only three (ALRR, ALRQ and ALHQ) in the Suffolk breed

(Table 3). Allele distribution was found to be highly significantly influenced by the breed and highly significantly influenced by sex within the Polish Merino breed (Table 3). In Polish Merino, the ALRR allele had the highest frequency in both sexes, followed by ALRQ, with high frequency. The frequency of the AFRQ, ALHQ and VLRQ alleles was similar in ewes and much higher than in rams, in which no AFRQ allele was detected. The AFRQ allele was identified only in Polish Merino ewes, and may indicate susceptibility to atypical scrapie (Goldman, 2008; McIntyre et al., 2008). The VLRQ allele was detected in ewes and in a single ram of the Polish Merino breed. This allele may determine susceptibility to classical scrapie (Rejduch et al., 2009; Wiśniewska and Mroczkowski, 2009; Niżnikowski et al., 2014). It was noteworthy that phenylalanine (F) at codon 141 was identified only in the AFRQ combination. The other genotypes in both breeds had leucine (L) at this site. In the Suffolk breed, three alleles were detected that were present in Polish Merino sheep, except for the AFRQ and VLRQ alleles, with very high frequency of the ALRR allele in both sexes. However, the results for Polish Merino show that the AFRQ allele had a low frequency (1.7%) in this population, very different from that observed in other flocks in Poland (Niżnikowski et al., 2014). The frequency of this allele among all sheep included in the study was only 1.3%, and it was detected only in ewes. In Polish sheep breeds, this allele either has not been found (Żelaźnińska and Podlaska breeds) or has been detected in single flocks with a frequency of 11.24% (Niżnikowski et al., 2014). In these circumstances, there is an urgent need to eliminate animals that have phenylalanine at codon 141.

Table 3
Frequency of *PRNP* alleles in the sheep breeds

Breed	Allele					Total		
	ALRR	ALRQ	AFRQ	ALHQ	VLRQ			
Polish Merino	♀	n	324	180	7	9	8	528
		%	61.4	34.1	1.3	1.7	1.5	100.0
	♂	n	68	57	–	2	1	128
		%	53.1	44.5	–	1.6	0.8	100.0
Suffolk	♀	n	178	17	–	1	–	196
		%	90.8	8.7	–	0.5	–	100.0
	♂	n	135	11	–	–	–	146
		%	92.5	7.5	–	–	–	100.0
Total	n	705	265	7	12	9	998	
	%	70.6	26.6	0.7	1.2	0.9	100.0	

Effect of sex within breed: Polish Merino – $P \leq 0.01$, Suffolk – $P \geq 0.05$

Effect of breed – $P \leq 0.01$

The AFRQ allele has been shown (Benestadt et al., 2003) to be conducive to the occurrence of atypical scrapie in other sheep breeds. Among the alleles determining susceptibility to classical scrapie (Rejduch et al., 2009; Wiśniewska and Mroczkowski, 2009; Niżnikowski et al., 2014), in the present study only the VLRQ allele was detected in Polish Merino (1.5% in ewes and 0.8% in rams). It should be noted that the frequency of alleles determining susceptibility to the classical form of scrapie (with valine at codon 136) in this study is exceptionally low even in comparison to other studies carried out on the Polish Merino breed and the old type of Polish Merino breed (Rejduch et al., 2009; Wiśniewska and Mroczkowski, 2009). To sum up, all carriers of the AFRQ and VLRQ alleles (in Polish Merino sheep) should be removed from flocks, which has not yet been done. Neither of these determinants was detected in the Suffolk breed. The very fact that the AFRQ and VLRQ alleles (suspected of determining susceptibility to the clinical form of atypical and classical scrapie) were detected in Polish Merino sheep fully substantiates the need for genotyping and subsequent elimination of individuals with genetic susceptibility to both forms of this disease. In the flock covered by the study, this was done in accordance with recommendations resulting from other papers (Goldman, 2008; McIntyre et al., 2008; Rejduch et al., 2009; Wiśniewska and Mroczkowski, 2009; Niżnikowski et al., 2014). The occurrence of the scrapie allele according to the year of breeding is presented in Table 4. The assessment of the distribution within breeds was insignificant for the Suffolk breed, whereas in Polish Merino the level of significance was $P < 0.01$. This was because no AFRQ or VLRQ alleles were detected in the Suffolk breed. The AFRQ allele was found in Polish Merino only in the years 2014-2016, while in the case of the VLRQ allele a highly significant decrease in its frequency was detected from 2012 to 2014, after which it was no longer detected in the years 2015-2017. This should be regarded as the result of breeding work aimed at eliminating carriers of this allele from use for breeding. The frequency of the ALRR and ALRQ alleles was high in all the years studied; it was higher for ALRR. While the frequency of the two alleles was varied, it remained close to the mean for the breed. In contrast, the frequency of the ALHQ allele steadily decreased from 2012 to 2017. Eliminating VLRQ individuals and not using them for breeding each year can generally be said to lead to a decrease in the frequency of the ALHQ and VLRQ alleles. The VLRQ allele was completely eliminated in the last three years of the study. As regards the remaining alleles, the methods used in the breeding work were not found to affect their frequency. Similar findings have been reported in other studies (Goldman, 2008; McIntyre et al., 2008; Rejduch et al., 2009; Wiśniewska and Mroczkowski, 2009; Niżnikowski et al., 2014) carried out on various breeds and types of sheep.

Table 5 presents the frequency of scrapie genotypes according to sex. The influence of sex within the breed on genotype frequency was insignificant in both cases, while the influence of the breed on the dependencies proved to be highly significant. There were ten scrapie genotypes in Polish Merino sheep, but only three in the Suf-

Table 4
Frequency of *PRNP* alleles within each breed by year

Breed	Allele						Total	
	ALRR	ALRQ	AFRQ	ALHQ	VLRQ			
Polish Merino	2012	n	45	27	–	3	5	80
		%	56.3	33.8	–	3.8	6.3	100.0
	2013	n	39	9	–	2	2	52
		%	75.0	17.3	–	3.8	3.8	100.0
	2014	n	99	74	2	3	2	180
		%	55.0	41.1	1.1	1.7	1.1	100.0
	2015	n	35	21	1	1	–	58
		%	60.3	36.2	1.7	1.7	–	100.0
	2016	n	102	49	4	1	–	156
		%	65.4	31.4	2.6	0.6	–	100.0
	2017	n	72	57	–	1	–	130
		%	55.4	43.8	–	0.8	–	100.0
	Total Polish Merino	n	392	237	7	11	9	656
		%	59.8	36.1	1.1	1.7	1.4	100.0
Suffolk	2012	n	41	5	–	–	–	46
		%	89.1	10.9	–	–	–	100.0
	2013	n	39	3	–	–	–	42
		%	92.9	7.1	–	–	–	100.0
	2014	n	81	13	–	–	–	94
		%	86.2	13.8	–	–	–	100.0
	2015	n	28	2	–	–	–	30
		%	93.3	6.7	–	–	–	100.0
	2016	n	79	3	–	–	–	82
		%	96.3	3.7	–	–	–	100.0
	2017	n	45	2	–	1	–	48
		%	93.8	4.2	–	2.1	–	100.0
	Total Suffolk	n	313	28	–	1	–	342
		%	91.5	8.2	–	0.3	–	100.0
Total	n	705	265	7	12	9	998	
	%	70.6	26.6	0.7	1.2	0.9	100.0	

Effect of year within breed: Polish Merino – $P \leq 0.01$, Suffolk – $P \geq 0.05$

Effect of breed – $P \leq 0.01$

folk breed (ALRR/ALRR, ALRR/ALRQ and ALRR/ALHQ, with no ALRR/ALHQ genotype detected in rams). The following genotypes were detected in Polish Merino ewes: ALRR/ALRQ, ALRR/ALRR, ALRQ/ALRQ, VLRQ/ALRR, ALRR/ALHQ, ALRQ/AFRQ, ALRQ/ALHQ, ALRR/AFRQ, and VLRQ/ALRQ. The ALRR/AFRQ, ALRQ/AFRQ, ALRQ/ALHQ, VLRQ/ALRR and VLRQ/ALRQ genotypes were not identified in Polish Merino rams. The ALRR/ALRR genotype was clearly dominant in the Suffolk breed (80% in ewes and 62% in rams), while the ALRR/ALRQ genotype was the most common in Polish Merino (45.5% in ewes and 51.6% in rams). No Polish Merino ram had phenylalanine at codon 141, while only one individual had valine at codon 136. The most valuable genotype, ALRR/ALRR, was detected much more frequently in the Suffolk breed than in Polish Merino. The heterozygous genotypes with the ALRR allele (except the combination with AFRQ and VLRQ) were present in 47.4% of ewes and 53.2% of rams of the Polish Merino breed. In the Suffolk breed, apart from ALRR/ALRR, heterozygous genotypes containing ALRR were detected at a rate of 18.3% in ewes and 15.1% in rams. The frequency of favourable genotypes was generally high in both breeds, particularly the ALRR/ALRR genotypes in both sexes of Suffolk sheep. The genotypes in which the AFRQ allele was present are interesting. These were ALRR/AFRQ and ALRQ/AFRQ, occurring only in Polish Merino and only in ewes. The ALRR/AFRQ genotype explains why in other studies the determinant guaranteeing genetic resistance to classical scrapie (the ALRR allele) does not protect against atypical scrapie (combination with the AFRQ allele) (Goldman, 2008; McIntyre et al., 2008). In terms of breeding work, ALRR/AFRQ and VLRQ/ALRR should be considered unfavourable genetic determinants. In both cases, the genotype responsible for genetic resistance to classical scrapie in the heterozygous form is associated with genetic susceptibility to atypical (AFRQ) or classical (VLRQ) scrapie. Both genotypes should be eliminated from sheep flocks, but this course of action may raise doubts in the case of small populations and individuals with outstanding performance traits. In such cases, they should be used for reproduction but the offspring must be genotyped, so that only individuals without valine at codon 136 (Lühken et al., 2004; Rejduch et al., 2009; Niżnikowski et al., 2014; Palhiere et al., 2014) or phenylalanine at codon 141 (Goldman, 2008; McIntyre et al., 2008) remain for further breeding.

The frequency of *PRNP* genotypes within each breed depending on the year is presented in Table 6. The influence of the year within the breed on the frequency of scrapie genotypes was insignificant for the Suffolk breed but significant for the Polish Merino breed. Due to elimination of animals with determinants containing valine at codon 136 from breeding, they were last detected in 2014, while genotypes containing phenylalanine were found in this breed only in the years 2014-2016. This leads to the conclusion that it is advisable to eliminate animals with valine at codon 136 and phenylalanine at codon 141 from the Polish Merino flock. Elimination of genotypes

Table 5
Frequency of *PRNP* genotypes according to sex in the sheep breeds

Breed	Genotype												Total					
	ALRR/ ALRR	ALRR/ ALRQ	ALRR/ AFRQ	ALRR/ ALHQ	5	ALRR/ ALRQ	ALRR/ AFRQ	ALRR/ ALHQ	4	ALRR/ ALRQ	ALRR/ AFRQ	ALRR/ ALHQ		6	VLRQ/ ALRR	VLRQ/ ALRQ	VLRQ/ ALRQ	VLRQ/ ALHQ
Polish Merino	n	95	120	3	5	25	4	4	4	4	4	4	6	2	2	2	2	264
	%	36.0	45.5	1.1	1.9	9.5	1.5	1.5	1.5	1.5	1.5	1.5	2.3	0.8	0.8	0.8	0.8	100.0
	n	17	33	-	1	12	-	-	-	-	-	-	-	-	-	-	-	64
	%	26.6	51.6	-	1.6	18.8	-	-	-	-	-	-	-	-	-	-	-	100.0
Suffolk	n	80	17	-	1	-	-	-	-	-	-	-	-	-	-	-	-	98
	%	81.6	17.3	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	100.0
	n	62	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	73
	%	84.9	15.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0
Total	n	254	181	3	7	37	4	4	4	4	4	4	6	2	2	2	1	499
	%	50.9	36.3	0.6	1.4	7.4	0.8	0.8	0.8	0.8	0.8	0.8	1.2	0.4	0.4	0.4	0.2	100.0

Effect of sex within breed – $P \geq 0.05$

Effect of breed – $P \leq 0.01$

coding for valine at codon 136, which has been recommended in many papers, has been shown to be attainable through breeding work (Lühken et al., 2004; Rejduch et al., 2009; Niznikowski et al., 2014; Palhiere et al., 2014). The breeding programme used has been fully confirmed and should be put into practice in order to eliminate genetic determinants from sheep populations susceptible to classical scrapie. This will make it possible to comply with legal regulations in the European Union (Regulation (EC) 999/2001; Commission decision C/2003/498; Commission Regulation (EC) 260/2003). Yearly restocking with ewes and rams that do not carry determinants of valine at codon 136 and rams with the ALRR/ALRR genotype should quickly result in a sheep population in which all individuals possess alleles resistant to classical scrapie. These recommendations should apply to breeding work carried out on other sheep breeds as well. The absence of such measures may be due to the relatively low frequency of the VLRQ allele compared to its much higher frequency in sheep in other countries (Lühken et al., 2004; Kall and Windig, 2005; Van Kaam et al., 2005; Palhiere et al., 2014). In some flocks, however, where atypical scrapie has not been detected, the incidence of genotypes containing phenylalanine at codon 141 may reach a high level (Niznikowski et al., 2014). In any case, such genotyping for alleles containing phenylalanine at codon 141 is fully justified in the light of research (Goldman, 2008; McIntyre et al., 2008).

Conclusions

The study on Polish Merino and Suffolk sheep found that both the breed and the year significantly affected the frequency of scrapie alleles and genotypes in the Polish Merino breed. Five alleles (ALRR, ALRQ, ALHQ, AFRQ and VLRQ) and ten *PRNP* genotypes were identified in Polish Merino, and three alleles (ALRR, ALRQ and ALHQ) and three genotypes in the Suffolk breed. High frequency was found for the ALRR/ALRQ genotype, followed by ALRR/ALRR, with a very low incidence of genotypes containing valine at codon 136 in Polish Merino. In contrast, the Suffolk sheep had a very high frequency of the ALRR/ALRR genotype and no alleles with valine at codon 136. In Polish Merino sheep, phenylalanine was detected at codon 141 only in the AFRQ allele, which appeared in two genotypes (in combination with ALRR and ALRQ), which according to the literature may determine low genetic resistance to atypical scrapie in these sheep. The breeding work conducted in Polish Merino, involving elimination of sheep with the VLRQ allele, resulted in its complete elimination in the last three years of the study, without affecting the frequencies of other alleles. The results confirm the advisability of such breeding work, which should be recommended for genetic improvement of sheep.

Table 6
Frequency of *PRNP* genotypes within each breed depending on the year

Breed	Genotype														Razem Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
2012	n	12	18	-	-	-	-	3	-	2	3	1	1	40	
	%	30.0	45.0	-	-	-	-	7.5	-	5.0	7.5	2.5	2.5	100.0	
2013	n	13	9	-	-	2	-	-	-	-	2	-	-	26	
	%	50.0	34.6	-	-	7.7	-	-	-	-	7.7	-	-	100.0	
2014	n	27	41	1	1	2	2	15	1	1	1	1	-	90	
	%	30.0	45.6	1.1	1.1	2.2	2.2	16.7	1.1	1.1	1.1	1.1	-	100.0	
2015	n	11	12	-	-	1	1	4	1	-	-	-	-	29	
	%	37.9	41.4	-	-	3.4	3.4	13.8	3.4	-	-	-	-	100.0	
2016	n	30	40	2	-	-	-	3	2	1	-	-	-	78	
	%	38.5	51.3	2.6	-	-	-	3.8	2.6	1.3	-	-	-	100.0	
2017	n	19	33	-	-	1	1	12	-	-	-	-	-	65	
	%	29.2	50.8	-	-	1.5	1.5	18.5	-	-	-	-	-	100.0	
Total Polish Merino	n	112	153	3	3	6	6	37	4	4	6	2	1	328	
	%	34.1	46.6	0.9	0.9	1.8	1.8	11.3	1.2	1.2	1.8	0.6	0.3	100.0	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
	2012	n	18	5	-	-	-	-	-	-	-	-	23
		%	78.3	21.7	-	-	-	-	-	-	-	-	100.0
	2013	n	18	3	-	-	-	-	-	-	-	-	21
		%	85.7	14.3	-	-	-	-	-	-	-	-	100.0
	2014	n	34	13	-	-	-	-	-	-	-	-	47
		%	72.3	27.7	-	-	-	-	-	-	-	-	100.0
	2015	n	13	2	-	-	-	-	-	-	-	-	15
		%	86.7	13.3	-	-	-	-	-	-	-	-	100.0
	2016	n	38	3	-	-	-	-	-	-	-	-	41
		%	92.7	7.3	-	-	-	-	-	-	-	-	100.0
	2017	n	21	2	-	1	-	-	-	-	-	-	24
		%	87.5	8.3	-	4.2	-	-	-	-	-	-	100.0
	Total Suffolk	n	142	28	-	1	-	-	-	-	-	-	171
		%	83.0	16.4	-	0.6	-	-	-	-	-	-	100.0
	Total	n	254	181	3	7	37	4	4	6	2	1	499
		%	50.9	36.3	0.6	1.4	7.4	0.8	0.8	1.2	0.4	0.2	100.0

Effect of year within breed: Suffolk – $P \geq 0.05$; Polish Merino – $P \leq 0.05$

Effect of breed – $P \leq 0.01$

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