Scientific Annals of Polish Society of Animal Production - Vol. 9 (2013), No 3, 21-29

Evaluation of the frequency of occurrence of the *PRNP* prion protein in old type Polish Merino in the Mazovian region

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The study involved 5 herds of old type of Polish Merino qualified for the conservation of genetic resources of the breed. Ewes and rams were aged 2 to 11 years (total: 181, including 12 rams, and 169 ewes), kept in the indoor system, and fed the fodder produced on the farm. Blood samples were collected from the jugular vein of animals into EDTA-containing tubes for the isolation of genomic DNA for the genetic and molecular analysis. In the study population, the occurrence of five sheep scrapie alleles was revealed: ALRR, ALRQ, AFRQ, ALHQ and VLRQ. In the group of ewes, the high frequencies of ALRR allele and relatively low ALRR/ALRR genotype were found; the range of these latter should be substantially increased. A high frequency of VLRQ allele in both ewes and rams was demonstrated, that should lead to an absolute necessity to remove such animals from the herds. It has been shown that there is an urgent need to bring the rams with genotype ALRR/ALRR to all herds in order to obtain a basis for raising the offspring with resistant prion protein genotypes in the old type of Polish Merino.

KEY WORDS: sheep / prion protein / genotypes

In 2001 the EU Parliament set out legal guidelines concerning prevention, control and eradication of transmissible spongiform encephalopathies (Regulation no. 999/2001/EC) [7]. In 2003 on the power of decision no. 2003/100/EC [8] the European Commission laid down an obligation to establish breeding programmes to increase genetic resistance to scrapie in each sheep breed in Europe. Moreover, Regulation no. 260/2003/EC [9] stipulates eradication of transmissible spongiform encephalopathy (TSE) in sheep and goats and regulates trade in live sheep and goats as well as bovine embryos. Scrapie, similarly as BSE in cattle and Creutzfeldt-Jakob disease in humans, is a naturally appearing TSE

form. It is assumed that prion protein (PrP) is responsible for the incidence of scrapie in sheep. Several polymorphisms were observed in the *PRNP* gene in codons 136, 141, 154 and 171, which seem to be responsible for (genetic) resistance or susceptibility to scrapie [1, 3, 5, 6].

It was found that the AFRR and ALRR alleles guarantee the lowest susceptibility to scrapie. It was observed that in Great Britain and Holland the VFRQ allele is responsible for high susceptibility to this disorder, whereas the AFRR or ALRR alleles were recorded with the lowest frequency in the sheep diagnosed with clinical scrapie symptoms. For this reason selection targeting this allele is the basic tool in the elimination of control of scrapie in sheep [2, 3, 4, 11].

In view of the above it was decided to assess the frequency of genes and genotypes in old type Polish Merino sheep, including in the analyses 5 flocks kept in the Mazowieckie Province (Poland), in accordance with the binding EU regulations and obligations stipulating the need to take respective actions.

Material and methods

Analyses were conducted on flocks of old type Polish Merino ewes from the Mazowieckie Province in 3 counties: the Płock (3 flocks), Ciechanów (1 flock) and the Grójec counties (1 flock), qualified to the genetic conservation programme for this breed. Ewes and rams were aged from 2 to 11 years (a total of 181 animals, including 12 rams and 169 ewes), they were kept in the indoor housing system and fed forage produced on the farms.

Blood samples (from the jugular vein) were collected to EDTA-containing test tubes in order to isolate genomic DNA for molecular and genetic analyses.

DNA was isolated from ovine blood leukocytes stored with EDTA. In order to obtain quality DNA, which might be repeatedly used after several freezing and thawing cycles,

Table 1
The primers and SNP genotyping of the locus of the prion protein

Locus	SNP	Changes	Location
PRNP prion protein	AY909542:g.479C>T	C/T	exon 3
PRNP prion protein	AY909542:g.493C>T	C/T	exon 3
PRNP prion protein	AY909542:g.534G>A	G/A	exon 3
PRNP prion protein	AY909542:g.385A>G	A/G	exon 3
PRNP prion protein	AY909542:g.386G>T	G/T	exon 3

the blood was preliminarily purified by removing erythrocyte lysis products to eliminate haeme compounds causing DNA modification. DNA was isolated from leukocytes by chromatography on silica minicolumns (A&A Biotechnology, Gdańsk, Poland; www.aabiot.com). The obtained DNA fraction was used as a template for the amplification of the prion protein polymorphic gene fragment. Scrapie alleles were genotyped using the KASPar® system.

The KASPar® system and the genotyping protocol (www.kbioscience.co.uk) are based on the application of SNP point polymorphism using primers, which are specified in Table 1.

The distribution of allele and genotype frequencies for ewes and rams is presented based on the readings of genotyped DNA samples. This step was a preparation for the further stages of the analyses.

Statistical calculations were conducted using the SPSS ver. 12.0 software package [10]. The ranges of allele and genotype frequencies in the respective sexes in individual flocks were assessed using the χ^2 test.

Results and discussion

Frequencies of scrapie alleles depending on the flock and sex of animals are presented in Table 2. In ewes the frequency of the ALRR allele turned out to be high, particularly in flock 2 (Fig.). In the other flocks the value of this trait needs to be corrected through selec-

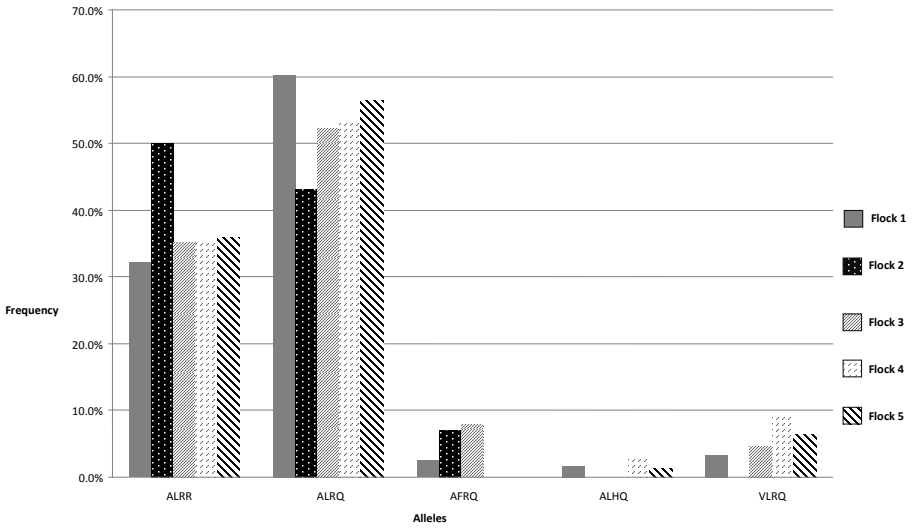

Fig. Frequency distribution of PRNP prion protein alleles in ewes, depending on the flock

tion. The high share of the ALRQ allele is frequently found in sheep populations, which have not been selected towards an increased frequency of scrapie resistance-associated alleles. The other alleles were detected only occasionally. The VLRQ allele, found in four out of the five analysed flocks, should be completely eliminated as scrapie susceptibility-associated [1, 2, 3, 5, 6, 11]. In turn, the ALRQ allele, which is found with the greatest frequency, is considered to be neutral and should be eliminated next, so that its frequency in the flock would gradually decrease and be replaced by the ALRR allele. An interesting finding is connected with the occurrence of the AFRQ allele in three flocks, in a total of 15 ewes. In accordance with the EU directive [9], such animals also need to be eliminated from the flock due to their probable susceptibility to atypical scrapie. However, susceptibility to such a condition is assessed to be much lower than that of alleles containing the prion valine (V) in their genotype.

In the case of rams, due to their low number and their being present in only 4 flocks, it seems that the only rational proposal is to promptly cull the animals carrying the VLRQ allele. Data given in Table 2 show that a total of 2 rams and 22 ewes were carriers of that allele.

In the investigated population among the nine identified genotypes two contained the VLRQ allele (ALRQ/VLRQ and ALRR/VLRQ) – Table 3. In ewes the ALRR/ALRQ and ALRQ/ALRQ genotypes were definitely found the most frequently. In contrast, in rams among the four genotypes which could be identified, ALRR/ALRQ was recorded most frequently. The incidence of the ALRR/ALRR genotype was only reported in ewes, which is not a satisfactory finding. The relatively high share of the other genotypes containing the ALRR allele (except for 5 sheep carrying it in combination with VLRQ) is a promising result, suggesting the potential of producing superior progeny. In turn, the fact that two rams used in the same flock carried the VLRQ allele and there were no sires homozygous for ALRR/ALRR in all the flocks is unacceptable in modern breeding practice [1, 2, 3, 5, 6, 11].

The conditions determining the incidence of scrapie in sheep populations previously not subjected to selection towards an increased incidence of genotypes resistant to that disease [3, 5, 11] indicate a poor condition of the old type Polish Merino population in the analysed region, which may affect breeding of Polish Merinos throughout the country. It is necessary to undertake corrective actions in breeding programmes, as it results from the conclusions given below:

– Five scrapie alleles were identified in the analysed sheep population, i.e. ALRR, ALRQ, AFRQ, ALHQ and VLRQ, along with nine genotypes in ewes and four in rams. The highest frequency was recorded for the ALRQ allele, followed by ALRR in both sexes and the related genotypes;

– In ewes a high frequency of the ALRR allele was recorded and a relatively low frequency of the ALRR/ALRR genotype, which incidence needs to be considerably increased in all the analysed flocks;

Table 2

Frequency of scrapie alleles depending on the flock (n – number)

Specification	Allele					Total	Statistical significance
	ALRR	ALRQ	AFRQ	ALHQ	VLRQ		
				Ewes			
Flock 1							
n	38	71	3	2	4	118	
%	32.2	60.2	2.5	1.7	3.4	100.0	
Flock 2							
n	36	31	5	0	0	72	
%	50.0	43.1	6.9	0.0	0.0	100.0	
Flock 3							
n	31	46	7	0	4	88	
%	35.2	52.3	8.0	0.0	4.5	100.0	XX
Flock 4							
n	35	53	0	3	9	100	
%	35.0	53.0	0.0	3.0	9.0	100.0	
Flock 5							
n	28	44	0	1	5	78	
%	35.9	56.4	0.0	1.3	6.4	100.0	
Total							
n	168	245	15	6	22	456	
%	36.8	53.7	3.3	1.3	4.8	100.0	
				Rams			
Flock 2							
n	0	2	–	–	0	2	
%	0.0	100.0	–	–	0.0	100.0	
Flock 3							
n	3	5	–	–	0	8	
%	37.5	62.5	–	–	0.0	100.0	
Flock 4							
n	4	4	–	–	0	8	
%	50.0	50.0	–	–	0.0	100.0	NS
Flock 5							
n	2	2	–	–	2	6	
%	33.3	33.3	–	–	33.3	100.0	
Total							
n	9	13	–	–	2	24	
%	37.5	54.2	–	–	8.3	100.0	
				Total			
Flock 1							
n	38	71	3	2	4	118	
%	32.2	60.2	2.5	1.7	3.4	100.0	
Flock 2							
n	36	33	5	0	0	74	
%	48.6	44.6	6.8	0.0	0.0	100.0	
Flock 3							
n	34	51	7	0	4	96	
%	35.4	53.1	7.3	0.0	4.2	100.0	
Flock 4							
n	39	57	0	3	9	108	
%	36.1	52.8	0.0	2.8	8.3	100.0	
Flock 5							
n	30	46	0	1	7	84	
%	35.7	54.8	0.0	1.2	8.3	100.0	
Total							
n	177	258	15	6	24	480	
%	36.9	53.8	3.1	1.3	5.0	100.0	

Statistical significance: XX – $P \leq 0.01$; NS – no significant

Table 3

Frequency of scrapie genotypes depending on the flock (n – number)

Specification	Genotype									Total	Statistical signif.
	ALRR/ ALRR	ALRR/ ALRQ	ALRR/ AFRQ	ALRR/ ALHQ	ALRQ/ ALRQ	ALRQ/ AFRQ	ALRQ/ ALHQ	ALRR/ VLRQ	ALRQ/ VLRQ		
1	2	3	4	5	6	7	8	9	10	11	12
Ewes											
Flock 1											
n	8	18	2	1	24	1	1	1	3	59	
%	13.6	30.5	3.4	1.7	40.7	1.7	1.7	1.7	5.1	100.0	
Flock 2											
n	9	15	3	0	7	2	0	0	0	36	
%	25.0	41.7	8.3	0.0	19.4	5.6	0.0	0.0	0.0	100.0	
Flock 3											
n	4	17	4	0	12	3	0	2	2	44	
%	9.1	38.6	9.1	0.0	27.3	6.8	0.0	4.5	4.5	100.0	NS
Flock 4											
n	6	20	0	2	12	0	1	1	8	50	
%	12.0	40.0	0.0	4.0	24.0	0.0	2.0	2.0	16.0	100.0	
Flock 5											
n	6	14	0	1	13	0	0	1	4	39	
%	15.4	35.9	0.0	2.6	33.3	0.0	0.0	2.6	10.3	100.0	
Total											
n	33	84	9	4	68	6	2	5	17	228	
%	14.5	36.8	3.9	1.8	29.8	2.6	0.9	2.2	7.5	100.0	
Rams											
Flock 2											
n	–	0	–	–	1	–	–	0	0	1	
%	–	0.0	–	–	100.0	–	–	0.0	0.0	100.0	NS

1	2	3	4	5	6	7	8	9	10	11	12
Flock 3											
n	–	3	–	–	1	–	–	0	0	4	
%	–	75.0	–	–	25.0	–	–	0.0	0.0	100.0	
Flock 4											
n	–	4	–	–	0	–	–	0	0	4	NS
%	–	100.0	–	–	0.0	–	–	0.0	0.0	100.0	
Flock 5											
n	–	1	–	–	0	–	–	1	1	3	
%	–	33.3	–	–	0.0	–	–	33.3	33.3	100.0	
Total											
n	–	8	–	–	2	–	–	1	1	12	
%	–	66.7	–	–	16.7	–	–	8.3	8.3	100.0	
					Total						
Flock 1											
n	8	18	2	1	24	1	1	1	3	59	
%	13.6	30.5	3.4	1.7	40.7	1.7	1.7	1.7	5.1	100.0	
Flock 2											
n	9	15	3	0	8	2	0	0	0	37	
%	24.3	40.5	8.1	0.0	21.6	5.4	0.0	0.0	0.0	100.0	
Flock 3											
n	4	20	4	0	13	3	0	2	2	48	
%	8.3	41.7	8.3	0.0	27.1	6.3	0.0	4.2	4.2	100.0	
Flock 4											
n	6	24	0	2	12	0	1	1	8	54	
%	11.1	44.4	0.0	3.7	22.2	0.0	1.9	1.9	14.8	100.0	
Flock 5											
n	6	15	0	1	13	0	0	2	5	42	
%	14.3	35.7	0.0	2.4	31.0	0.0	0.0	4.8	11.9	100.0	
Total											
n	33	92	9	4	70	6	2	6	18	240	
%	13.8	38.3	3.8	1.7	29.2	2.5	0.8	2.5	7.5	100.0	

NS – no significant

– the VLRQ allele was identified in ewes and primarily in rams, which should result in the absolute necessity to cull these animals. Only flock 1 turned out to be free of this allele;

– rams carrying the ALRR/ALRR genotype need to be promptly introduced to all the flocks in order to ensure the presence of prion protein resistant genotypes in the resulting progeny;

– This study indicates an absolute necessity to verify the genetic conditions predisposing to scrapie in all sheep flocks, particularly in rams. Sires carrying the VLRQ allele and not carrying the ALRR allele should not be entered in the flock books and incorporated in breeding programmes.

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