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Polymorphism of the *PRNP* gene in Polish Merino and old-type Polish Merino in flock with clinical status of atypical scrapie

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Abstract: *Polymorphism of the* PRNP *gene in Polish Merino and old-type Polish Merino in flock with clinical status of atypical scrapie*. The study was conducted in 2014 in flock located in West Pomerania Province, on 378 ewes and 96 rams of Polish Merino and 416 ewes and 58 rams old-type Polish Merino. All animals were subjected to the identification of the *PRNP* gene polymorphism. Based on the studies, there were no effects of breed and sex within breed on frequency of scrapie alleles and genotypes. In Polish Merino were found five alleles (ALRR, ALRQ, ALHQ, AFRQ, VLRQ), and in old-type Polish Merino six alleles (additional – VLRR allele). There were identified nine genotypes of *PRNP* gene in Polish Merino and 11 in old-type Polish Merino. Very high frequencies of ALRR/ALRR, ALRR/ALRQ, ALRR/ALHQ genotypes were stated in both breeds with low level of genotypes with valine amino acid. In both breeds was found only one allele with phenylalanine amino acid at codon 141 – AFRQ, which appeared in three genotypes (in combination with ALRR, ALRQ, ALHQ), which determined low level of resistant to atypical scrapie. Breeding work assumption, which requires elimination individuals with valine amino acid at codon 136 and phenylalanine amino acid at codon 141, and introduce to sheep population rams with ALRR allele would led to higher frequency of ALRR/ALRR genotype and ALRR allele in sheep population. That indicates the advisability of such breeding work, which is worth to recommend for genetic improvement of all sheep breeds in Poland

Key words: sheep, PRNP, alleles, genotypes, polymorphism

INTRODUCTION

The prevention, control, and eradication of transmissible spongiform encephalopathies are regulated by EU legislation (Regulation EC 999/2001, Regulation EC 260/2003, Commission decision C/2003/498). Among sheep there are two forms of scrapie: classic – which is genetically determined, and atypical. Polymorphisms of prion protein gene located at codons 136, 154, 171 were considered as a genetically responsible for occurrence of classic scrapie (Lühken et al. 2004, Kaal and Windig 2005, Kaam et al. 2005, Palhiere et al. 2008). As a guarantee of lowest sensitivity to scrapie ARR allele was regarded (created as a result of encoding alanine – A, arginine – R and also arginine – R), while VRQ allele (created as a result of encoding valine – V, arginine – R, glutamine – Q) was regarded as responsible for susceptibility to that disease (Kaal and Windig 2005, Kaam et al. 2005, Palhiere et al. 2008, Rejduch et al. 2009). In case of atypical

form of scrapie (Nor98), which first case was described by Benestadt et al. (2003), it concerned alleles located at codon 141 (Goldman 2008, Mcintyre 2008, Mazza et al. 2010) which encoding leucine (L) and phenylalanine (F). Allele F more often accompanied to clinical status of atypical scrapie. Moreover, classical and atypical scrapie could coexist in the same sheep flock (Mazza et al. 2010). So far in Poland, studies were performed to monitor the presence of classic scrapie alleles in many breeds (Palhiere et al. 2008, Rejduch et al. 2009, Wiśniewska and Mroczkowski 2009), despite the fact that in 2009 first case of atypical scrapie was found (Polak et al. 2010). The results of previous studies showed the absence of alleles encoding valine at codon 136 in Wrzosówka sheep and various frequency of its presence in other breeds (Rejduch et al. 2009, Wiśniewska and Mroczkowski 2009, Niżnikowski et al. 2014). Relatively not many papers are related to scrapie alleles located at codon 141 (Niżnikowski et al. 2014). It is worth to conduct breeding work which aims to eliminate VRQ allele from sheep population and also eliminate phenylalanine amino acid encoded at codon 141 in order to improve the genetic resistance to both scrapie forms in sheep population. Therefore, direction of the research aimed to determine the frequency of occurrence of scrapie genetic conditions in both breed kept in the same flock, in which clinical status of atypical scrapie was found.

MATERIAL AND METHODS

The study was conducted in 2014 on Polish Merino and old-type Polish Merino kept in one flock located in West Pomerania Province, in which in 2013 clinical status of atypical scrapie was found. The study was performed on foundation stock ewes (Polish Merino – 378; old-type Polish Merino – 416) and stud rams (Polish Merino – 96; old-type Polish Merino – 58) which were born in 2003–2013. Blood was collected from the jugular vein into tubes containing EDTA. The DNA was isolated from blood leukocytes. In order to obtain high quality DNA suitable for multiple use, blood was purified from the heme compounds, which were erythrocyte lysis products. The DNA was isolated by chromatography on mini-columns of silicate (A&A Biotechnology, Poland), and subsequently served as a template DNA for amplification of polymorphic gene allele fragment. Sample genotyping was performed with KASPar® system (www.kbioscience.co.uk), which uses a single nucleotide polymorphism (SNP) based on primers listed in Table 1. A high reliability of SNP genotyping method compared to the sequencing method was proved by Green et al. (2006). Based on the reading of genotyped DNA samples within the ewes and rams, distribution of alleles and genotype was determined. It was preparatory act to the next stages of research. For statistical calculations SPSS ver. 22 software was used. To compare distribution of alleles and genotypes between breeds and sexes within breeds test χ^2 was used.

TABLE 1. The primers and SNP of the *PRNP* prion protein gene

Locus	Primers 5'-3'	SNP	Changes	Position
PRNP prion protein	CACAGTCAGTG-GAACAAGCC/ /CTTTGCCAGGT-TGGGG	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

RESULTS AND DISCUSSION

The distribution of alleles in assessed breeds within sex are presented in Table 2. There were no statistically significant differences in frequencies of alleles and in sexes within breed in both breeds. It was found six alleles (ALRR, ALRQ, ALHQ, AFRQ, VLRQ, VLRR). All alleles were found in old-type Polish Merino whereas in Polish Merino VLRR allele was not observed. Allele with highest frequency was ALRR allele, equally ALRQ allele, whereas allele with the lowest frequency was ALHQ allele. Among alleles, which could cause scrapie, AFRQ allele was found in both

TABLE 2. Frequency of *PRNP* allele occurrence in order to breed and sex

Breed	Sex	Unit	Alleles						Total
			ALRR	ALRQ	ALHQ	AFRQ	VLRR	VLRQ	
Polish Merino	♀	n	194	162	7	6	0	9	378
		%	51.3	42.9	1.9	1.6	0.0	2.4	100.0
	♂	n	51	43	2	0	0	0	96
		%	53.1	44.8	2.1	0.0	0.0	0.0	100.0
Polish Merino Total	×	n	245	205	9	6	0	9	474
		%	51.7	43.2	1.9	1.3	0.0	1.9	100.0
Old-type Polish Merino	♀	n	225	154	4	30	1	2	416
		%	54.1	37.0	1.0	7.2	0.2	0.5	100.0
	♂	n	36	17	0	4	0	1	58
		%	62.1	29.3	0.0	6.9	0.0	1.7	100.0
Old-type Polish Merino Total	×	n	261	171	4	34	1	3	474
		%	55.1	36.1	0.8	7.2	0.2	0.6	100.0
Sex Total	♀	n	419	316	11	36	1	11	794
		%	52.8	39.8	1.4	4.5	0.1	1.4	100.0
	♂	n	87	60	2	4	0	1	154
		%	56.5	39.0	1.3	2.6	0.0	0.6	100.0
Total	×	n	506	376	13	40	1	12	948
		%	53.4	39.7	1.4	4.2	0.1	1.3	100.0

Breed effect – NS; sex effect within breed – NS.

breeds and according to various papers it could indicate susceptibility to atypical scrapie (Goldman 2008, Mcintyre 2008). Moreover VLRQ and VLRR alleles were found which determine genetic susceptibility to classical scrapie (Rejduch et al. 2009, Wiśniewska and Mroczkowski 2009, Niżnikowski et al. 2014). Noticeable is a fact that allele F, which is at codon 141 was state only in AFRQ combination. The rest alleles had leucine amino acid (L) in that place. Frequency of this allele for all animals was 4.2% whereas in each breeds – 1.3% for Polish Merino and 7.2% for old-type Polish Merino. Frequency of scrapie genotypes are presented in Table 3. Similar to alleles, there were no impact of breed and sex within breed on scrapie genotypes distribution. However, it is worth to focus on following results. In old-type Polish Merino 11 genotypes were found and in Polish Merino were found nine genotypes (without ALHQ/AFRQ and VLRR/ALRQ). In both breeds relatively low frequency of the most valuable genotype – ALRR/ALRR was state, whereas heterozygous genotypes of ALRR allele combined with other alleles (with exception AFRQ, VLRQ, VLRR) represented 40% of all. That gives the general opinion that favorable scrapie conditions occurred with a high frequency in both breeds. Interestingly it should be considered configurations in what occurred AFRQ allele. These were ALRR/AFRQ, ALRQ/AFRQ and ALHQ /AFRQ genotypes. ALRQ/AFRQ and ALHQ /AFRQ genotypes occurred only in old-type Polish Merino. AFRQ allele had a high frequency in studied sheep population, completely different from those seen in other herds (Niżnikowski et al. 2014). In national sheep breeds AFRQ allele does not occurred (Żelaźnieńska Sheep and Podlaska Sheep) or occurred in single flocks (Niżnikowski et al. 2014). In this situation there is an urgent need to eliminate animals that has phenylalanine amino acid at codon 141. It could be assumed, that high frequency of this allele favor appearance of clinical forms of atypical scrapie in studied flock (Benestadt 2003). Two alleles which determine genetic susceptibility to classical scrapie were found in tested animals: typical VLRQ allele (Rejduch et al. 2009, Wiśniewska and Mroczkowski 2009, Niżnikowski et al. 2014), and very rare VLRR allele (only in one old-type Polish Merino ewe). Considering alleles frequencies determining susceptibility to classical scrapie, it should be point its extremely low level compared to results from other work carried out on Polish Merino and old-type Polish Merino (Rejduch et al. 2009, Wiśniewska and Mroczkowski 2009). Summing up the results it is need to remove from flock all individuals with AFRQ allele (six Polish Merino ewes and 30 ewes and four trams of old-type Polish Merino) and individuals with VLRR allele (one old-type Polish Merino ewe) and VLRQ allele (nine Polish Merino ewes and two ewes and ram of old-type Polish Merino). Results show to eliminate 53 animals which was 5.59% of all; 3.2% from Polish Merino and 8.0% from old-type Polish Merino. It should be em-

TABLE 3. Frequency of *PRNP* genotypes occurrence in order to breed and sex

Breed	Sex	Unit	Genotypes											Total
			ALRR/ /ALRR	ALRR/ /ALRQ	ALRR/ /ALHQ	ALRR/ /AFRQ	ALRQ/ /ALRQ	ALRQ/ /AFRQ	ALRQ/ /ALHQ	ALHQ/ /AFRQ	VLRR/ /ALRQ	VLRQ/ /ALRR	VLRQ/ /ALRQ	
Polish Merino	♀	n	46	87	6	4	34	2	1	0	0	5	4	189
		%	19.4	36.7	2.5	1.7	14.3	0.8	0.4	0.0	0.0	2.1	1.7	79.7
	♂	n	13	24	1	0	9	0	1	0	0	0	0	48
		%	5.5	10.1	0.4	0.0	3.8	0.0	0.4	0.0	0.0	0.0	0.0	20.3
Polish Merino Total	×	n	59	111	7	4	43	2	2	0	0	5	4	237
		%	24.9	46.8	3.0	1.7	18.1	0.8	0.8	0.0	0.0	2.1	1.7	100.0
Old-type Polish Merino	♀	n	57	92	1	17	23	12	2	1	1	1	1	208
		%	24.1	38.8	0.4	7.2	9.7	5.1	0.8	0.4	0.4	0.4	0.4	87.8
	♂	n	12	9	0	2	3	2	0	0	0	1	0	29
		%	5.1	3.8	0.0	0.8	1.3	0.8	0.0	0.0	0.0	0.4	0.0	12.2
Old-type Polish Merino Total	×	n	69	101	1	19	26	14	2	1	1	2	1	237
		%	29.1	42.6	0.4	8.0	11.0	5.9	0.8	0.4	0.4	0.8	0.4	100.0
Sex Total	♀	n	103	179	7	21	57	14	3	1	1	6	5	397
		%	21.7	37.8	1.5	4.4	12.0	3.0	0.6	0.2	0.2	1.3	1.1	83.8
	♂	n	25	33	1	2	12	2	1	0	0	1	0	77
		%	5.3	7.0	0.2	0.4	2.5	0.4	0.2	0.0	0.0	0.2	0.0	16.2
Total	×	n	128	212	8	23	69	16	4	1	1	7	5	474
		%	27.0	44.7	1.7	4.9	14.6	3.4	0.8	0.2	0.2	1.5	1.1	100.0

Breed effect – NS; Sex effect within breed – NS.

phasized that in old-type Polish Merino was need to eliminate a much larger number of sheep than in Polish Merino. The mere fact of a high frequency of AFRQ allele and reveal clinical status of atypical scrapie fully justified the need for genotyping and elimination animals characterized by a genetic susceptibility to both forms of scrapie, which in the tested flock was done in accordance with the recommendations arising from other research (Goldman 2008, Mcintyre 2008, Rejduch et al. 2009, Wiśniewska and Mroczkowski 2009, Niżnikowski et al. 2014). ALRR/AFRQ genotype is an answer for question, why in various papers genetically resistant conditions for scrapie (ALRR allele) did not protect against atypical form of scrapie (genotypes with AFRQ allele). According to breeding work ALRR/AFRQ and VLRQ/ALRR genotypes should be consider as an unwanted. In both cases resistant conditions for classical scrapie in heterozygous genotypes form was connected with genetically susceptible condition for atypical (AFRQ) and classical (VLRQ) scrapie. Both genotypes should be eliminated from flock, but in case of small populations it may raise doubts. In such cases, all sheep should be used in reproduction and their progeny should be genotype obligatory. That allow to choose animals for further breeding without valine amino acid at codon 136 (Lühken et al. 2004, Palhiere 2008, Rejduch et al. 2009, Niżnikowski et al. 2014) and phenylalanine amino acid at codon 141 (Goldman 2008, Mcintyre 2008). Suggestions appearing in many papers about elimination conditions that encode valine amino acid at codon 136 has been proven to realize through appropriate breeding work (Lühken et al. 2004, Palhiere 2008, Rejduch et al. 2009, Niżnikowski et al. 2014). Applied breeding program has been fully confirmed and worthy to recommend into practice in order to eliminate from the sheep population genetically susceptible to classical scrapie conditions and allow to meet the goals of the EU legislation (Regulation EC 999/2001, Regulation EC 260/2003, Commission decision C/2003/498). The annual introduction to the flock ewes with no valine amino acid at codon 136 and rams with ALRR/ALRR genotype should soon result that whole tested sheep population would have only genetically resistant to classical scrapie alleles. These recommendations should be applied within the breeding work carried out on other sheep breeds. In Poland most cases of atypical form of scrapie were not genotyped; same story was in tested flock. Perhaps this was due to the relatively low frequency of VLRQ and VLRR alleles compared to the much higher frequencies in abroad sheep breeds (Lühken et al. 2004, Kaal and Windig 2005, Kaam et al. 2005, Palhiere et al. 2008). However, in some flocks frequency of genotypes containing the phenylalanine amino acid at codon 141 can reach high level, as it is demonstrated in research

described in other work (Niżnikowski et al. 2014) in which was no atypical scrapie. Moreover, such genotyping in direction to find alleles containing phenylalanine amino acid at codon 141 is justified (Goldman 2008, Mcintyre 2008).

CONCLUSIONS

The obtained results lead up to following statements and conclusions:

1. No statistical significance effect of breed and sex within breed on frequency of occurrence of scrapie alleles and genotypes. In Polish Merino were found five alleles (ALRR, ALRQ, ALHQ, AFRQ, VLRQ), and in old-type Polish Merino six alleles (additional – VLRR allele).
2. Nine genotypes of *PRNP* gene were found in Polish Merino and 11 genotypes were found in old-type Polish Merino.
3. Very high frequency of ALRR/ /ALRR, ALRR/ALRQ, ALRR/ALHQ genotypes were stated in both Merino breeds at low level of genotypes containing valine amino acid.
4. In both breeds, was found only one allele with phenylalanine amino acid at codon 141 – AFRQ, which appeared in three genotypes (in combination with ALRR, ALRQ, ALHQ) and probably determined low level of resistant to atypical scrapie.
5. Breeding work assumption, which requires elimination individuals with valine amino acid at codon 136 and phenylalanine amino acid at codon 141, and introduce to sheep population rams with ALRR allele would led to higher frequency of ALRR/ALRR genotype and ALRR allele in sheep population. That indicates the advisability of such breeding work, which is worth to recommend for genetic improvement of all sheep breeds in Poland. It should also recommend the genotyping of sheep in which clinical status of atypical scrapie was diagnosed.

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Streszczenie: *Polimorfizm genu białka prionowego* PRNP *u owiec ras merynos polski i merynos polski starego typu w gospodarstwie, w którym stwierdzono kliniczny stan trzęsawki atypowej.* Badania przeprowadzono w 2014 roku w jednym gospodarstwie zlokalizowanym w województwie zachodniopomorskim, w którym stwierdzono kliniczną formę trzęsawki atypowej. Badaniami objęto 378 maciorek i 96 tryków rasy merynos polski oraz 416 maciorek i 58 tryków rasy merynos polski starego typu. Wszystkie zwierzęta były poddane identyfikacji polimorfizmu genu białka prionowego *PRNP*. Na podstawie przeprowadzonych prac badawczych stwierdzono nieistotny wpływ rasy owiec oraz płci w obrębie rasy na frekwencje występowania alleli i genotypów trzęsawki. Wykazano występowanie pięciu alleli (ALRR, ALRQ, ALHQ, AFRQ i VLRQ) u merynosa polskiego, sześciu u merynosa polskiego starego typu (dodatkowo VLRR). Zidentyfikowano dziewięć genotypów białka *PRNP* u merynosa polskiego oraz 11 u merynosa polskiego starego typu. Stwierdzono bardzo dużą frekwencję występowania genotypów ALRR/ /ALRR, ALRR/ALRQ i ALRR/ALHQ u ras merynosowych, przy bardzo niskim poziomie występowania genotypów z kodowana waliną u obu ras. Wykazano u obu ras łącznie występowanie uwarunkowania zawierającego w kodonie 141 fenyloalaninę tylko w formie allelu AFRQ, który pojawił się w trzech genotypach (w kombinacji z ALRR, ALRQ i ALHQ), co warunkować może niski poziom oporności genetycznej na trzęsawkę

atypową. Przyjęte założenia pracy hodowlanej polegające na eliminacji nosicieli uwarunkowań kodujących występowanie waliny w kodonie 136 i fenyloalaniny w kodonie 141 oraz wprowadzanie do populacji tryków zawierających w genotypie allel ALRR prowadzić będą do zwiększenia frekwencji występowania w populacji genotypu ALRR/ALRR oraz allelu ALRR. Wskazuje to na zasadność prowadzenia takiej pracy hodowlanej, którą warto zalecić przy doskonaleniu genetycznym innych ras owiec występujących w krajowym pogłowiu.

*Słowa kluczowe***:** owce, *PRNP*, rozkład alleli i genotypów

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