Prion protein (PrP) gene polymorphism and simulation study of breeding oriented to scrapie resistance in Polish Merino and Polish Mountain Sheep*

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Genetic susceptibility/resistance of sheep to scrapie is associated with polymorphism particularly in the three codons 136, 154 and 171 of the ovine prion protein (PrP) gene. Scrapie resistance breeding of sheep aims at instant increase of the $A_{136}R_{154}R_{171}$ haplotype frequency in the next generations. The aims of the study were to detect the PrP haplotype and genotype distribution in the Polish Merino and the Polish Mountain Sheep flocks maintained in Poland and to make a forecast of the application of a scrapie resistance breeding programmes for these flocks. In order to determine PrP haplotypes based on the polymorphisms in three codons: 136, 154 and 171 PCR-RFLP analyses were applied. The analysis of 119 sheep revealed dimorphisms only at the codon 171 (A/T). The highest frequency of the ARR haplotype was detected in the Polish Merino flock. A simulation study was done in order to calculate assumed frequencies of the ARR/ARR genotype in the next generations after applying scrapie resistance breeding scheme. In general, according to simulation study, it would be possible to reach ARR/ARR genotype frequency above 97% in F6 generation in the investigated flocks.

KEY WORDS: sheep / prion protein gene (*PRNP*) / polymorphism / PCR-RFLP / PrP genotyping

Scrapie is a prion disease affecting sheep and goats. Scientific research has found that sheep have genetically determined resistance to the classical scrapie. This is connected with the presence of polymorphisms in the prion protein gene (*PRNP*), particularly in the three codons, namely 136, 154, and 171. The most important factors to be considered when carrying out the research on susceptibility of sheep to scrapie include five following haplotypes (further referred to as allels): $A_{136}R_{154}R_{171}$, $A_{136}R_{154}Q_{171,}$ $A_{136}H_{154}Q_{171,}$ $A_{136}R_{154}H_{171,}$ and

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$V_{136}R_{154}Q_{171}$. Generally, the ARR allel increases and the VRQ allel decreases the resistance of sheep to the classical scrapie [2, 6].

Sheep breeding aiming at increasing the resistance of this farm animal species to scrapie, based on DNA tests, is nowadays the European Commission's requirement to be met by the member states. In 2001, the European Parliament established the regulations for prevention, control, and fighting the transmissible spongiform encephalopathies (TSEs) [14]. In 2003, the European Commission decided on compulsory introduction of mating aiming at increasing the genetically controlled resistance to scrapie in each breed of sheep in Europe, which increases the frequency of the ARR/ARR genotype [15].

The purpose of the research was to determine the frequency of prion protein alleles and genotypes in flocks of sheep of the following breeds: Polish Merino and Polish Mountain Sheep as well as carrying out the simulation of mating aiming at increasing the resistance of sheep to classical scrapie.

Material and methods

The research covered 119 sheep from two flocks, where no cases of scrapie had been documented before the day the blood was drawn. The animals were of two breeds of sheep representing different types of purpose. See Table 1 for information on breeds of sheep, types of purpose, sex and size of the groups.

Peripheral blood for testing was drawn from the zygomatic vein to K_2EDTA test tubes. Genomic DNA was isolated from the blood by means of the MasterPure™ DNA Purification Kit for Blood (Epicentre Biotechnologies) reagent kit. To detect polymorphisms in codons 136, 154, and 171 of the *PRNP* gene three PCR-RFLP reactions were carried out, using the following enzymes: 1) BspHI (PagI) by Fermentas; 2) BspHI+BspDI (New England BioLabs) simultaneously; 3) BseLI (Fermentas) as per the methodics of Lühken et al. [10]. The genotype of each particular individual was determined on the basis of the results of 3 reactions of enzymatic hydrolysis. After completion of genotyping, the frequencies of prion protein alleles and genotypes in the tested group of sheep were calculated,

Table 1 – Tabela 1

Information about breed, type of purpose, sex and number of genotyped sheep
Informacje dotyczące rasy, typu użytkowego, płci i liczby zgenotypowanych owiec

Breed Rasa	Type of purpose Typ użytkowy	Number of animals Liczba zwierząt		
		♀	♂	total razem
Polish Merino Merynos polski	wool and meat wełnisto-mięsny	95	4	99
Polish Mountain Sheep Polska owca górska	milk and wool mleczno-wełnisty	20	0	20

depending on breed and sex. On the basis of the genotype the animals were classified into scrapie risk groups as per the DEFRA scheme [3] and the percentage share of each class was calculated, depending on breed and sex.

On the basis of the obtained frequencies of allels of the prion protein gene the mating simulation was carried out, aiming at increasing the genetically controlled resistance of sheep to scrapie, in each flock separately. The theoretical frequencies of the ARR/ARR genotype in generation F1 were calculated on the basis of the detected frequencies of allels of the prion protein gene in a group of sheep having been genotyped in in-house tests. In a Polish Merino flock, at the first stage of simulation only the ARR/ARQ rams could be selected to be the sires of generation F1. In case of Polish Mountain Sheep, where the entire analysed population amounted to 20 ewes, it was assumed that the frequency of genotypes was the same for both sexes. Then, it was assumed that at the first stage of simulation both the homozygotic rams (ARR/ARR) and heterozygotic ones (ARR/ARQ) would be selected for reproduction. Upon calculating the theoretical frequencies of prion protein alleles and genotypes in generation F1, only the homozygotic rams (ARR/ARR) were selected to be the sires of generation F2 in both flocks. Then, the assumed frequency of allels and PrP genotypes in the next generation (F2) was calculated. Following this scheme, the theoretical frequencies of prion protein genotypes and alleles were calculated in generations F3, F4, F5, and F6. Besides, it was assumed that in the subsequent generations the prion protein genotype was determined for all rams and the values of performance traits were randomly distributed in the population of males of various PrP genotypes. No ewes were tested or selected with regard to the prion protein genotype and they were randomly selected for reproduction, making the assumption that the values of performance traits were randomly distributed over the entire tested population, regardless to the ewe's prion protein genotype.

Results and discussion

The in-house research presents polymorphism of the prion protein gene (*PRNP)* in Polish Merino and Polish Mountain Sheep flocks as well as the simulation of mating aiming at increasing the sheep resistance to scrapie. The comparison covered the sheep of two breeds of different type of purpose. Despite such selection of animals, the analysis of polymorphism of the prion protein gene in three codons, namely 136, 154, and 171, showed low variation among genotyped individuals both within a flock and between the flocks. In the tested groups of Polish Merino and Polish Mountain Sheep only polymorphism A/G was detected in codon 171, therefore two allels were identified, namely ARR and ARQ, forming three genotypes. The frequencies of allels and the prion protein genotype, shown in Tables 2 and 3, respectively, were different depending on a breed of sheep. With regard to the fact that three PrP genotypes were detected, the frequencies of the NSP1 group individuals were the same as in the genotype ARR/ARR, NSP2 as in ARR/ARQ, and NSP3 as in ARQ/ARQ [3].

Polish Merino, one of the most popular breeds of sheep in Poland, is bred for its meat and wool. The frequency of sheep most resistant to scrapie (ARR/ARR) in the analysed flock was 35.35%. It is a very good result when compared to other research carried out in

Table 2 –Tabela 2

Frequencies (%) of prion protein (PrP) alleles in the investigated sheep flocks in respect of breed and sex
Frekwencje (%) alleli białka prionowego w objętych badaniami stadach owiec w zależności od rasy i płci

Allele Allel		Polish Merino Merynos polski	Polish Mountain Sheep Polska owca górska
ARR	♀	61.05	50
	♂	50	0
	total razem	60.61	50
ARQ	♀	38.95	50
	♂	50	0
	total razem	39.39	50

Poland by Niżnikowski et al. [11], Wiśniewska et al. [16], Rejduch et al. [12], Wiśniewska and Mroczkowski [17], where significantly lower frequencies of individuals most resistant to classical scrapie were found. Niżnikowski et al. [11], while genotyping 31 sheep from 11 flocks, found more than 20% of the ARR/ARR homozygotes. Whereas the research of Rejduch et al. [12] covered 40 Merino sheep and determined in that group the frequency of sheep of the ARR/ARR genotype as 7.5%. Our previous research covering 4 flocks of Polish Merino the frequencies of the ARR/ARR genotype were different in the flocks and amounted to between 0% and 14.3% [16]. Our other research showed the influence of the flock factor on the frequencies of the PrP genotypes in Polish Merino sheep [17]. The found frequencies of the ARR/ARR genotype ranged from 4.76% to 22.45% [17]. The results obtained in the in-house research appear favourably also when compared to the results of genotyping of the prion protein gene in Germany. Lühken et al. [9] noted higher frequencies of the scrapie-susceptible ARQ allel in two purebred flocks of German Merino (80 and 84%) and found the 1% frequency of sheep of the ARR/ARR genotype in one of the flocks, and the ARR/ARR homozygote was not identified in the other flock. Besides, Drögemüller et al. [5] did not find the ARR/ARR homozygotic system in any of the tested individuals of the German Merino population. The differences in the frequency in the Polish population of Merino sheep in the aforementioned research were influenced by both the flock factor and the size of groups being genotyped. Whereas the differences between the frequency of sheep resistant to scrapie in Poland and Germany may be influenced not only by the size of groups and flock factor but also different history of genetic improvement of Merino sheep in Poland and Germany.

Polish Mountain Sheep is a breed bred for wool and milk production. The presented in-house research showed in a group of Polish Mountain Sheep only two allels of the prion protein gene, namely ARR i ARQ, occurring in equal frequencies. In the analysed group

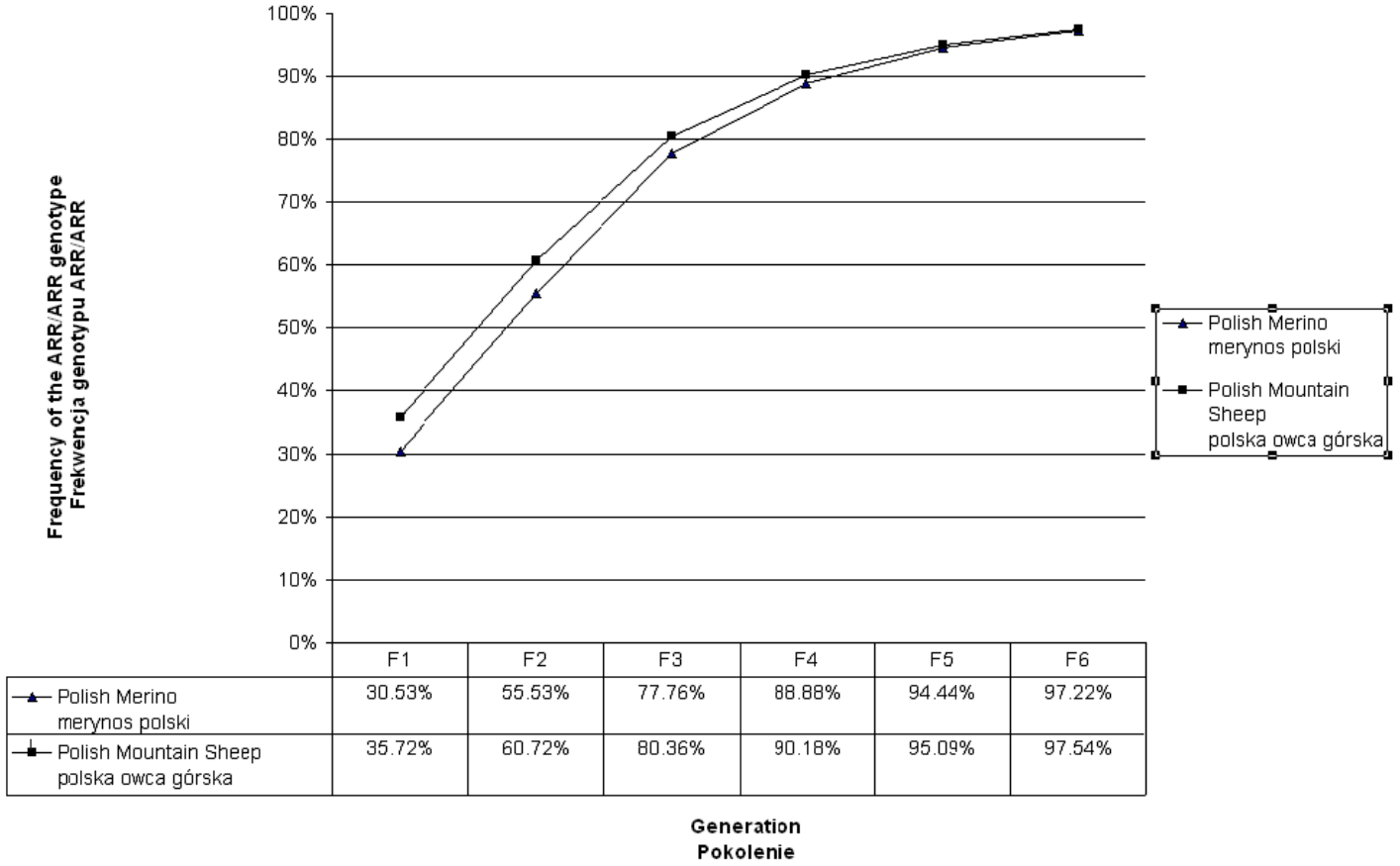
Table 3 – Tabela 3

Frequencies (%) of prion protein (PrP) genotypes in the investigated sheep flocks in respect of breed and sex
Frekwencje (%) genotypów białka prionowego w objętych badaniami stadach owiec w zależności od rasy i płci

Genotype Genotyp		Polish Merino Merynos polski	Polish Mountain Sheep Polska owca górska
ARR/ARR	♀	36.84	30
	♂	0	0
	total razem	35.35	30
ARR/ARQ	♀	48.42	40
	♂	100	0
	total razem	50.51	40
ARQ/ARQ	♀	14.74	30
	♂	0	0
	total razem	14.14	30

of sheep of that breed the ARR/ARQ heterozygotes prevailed (40%), while the ARR/ARR and ARQ/ARQ homozygotes occurred in the same frequencies (30%). About 13% prevalence of the frequency of the ARQ allel over the ARR one and the presence of the AHQ haplotype in a group of Polish Mountain Sheep were found by Niżnikowski et al. [11]. It is quite interesting that Knapik et al. [8] detected 6 allels amongst the Polish Mountain Sheep genotyped, namely ARR, AHQ, ARH, ARQ, AHR, and VRQ. The in-house research showed the 30% share of the ARR/ARR homozygotes in a group of Polish Mountain Sheep genotyped. Both Niżnikowski et al. [11] and Knapik et al. [8] found lower frequency of Polish Mountain Sheep with that genotype. The differences between the populations of Polish Mountain Sheep described above were probably caused by the selection of animal material for the tests as well as the flock factor.

The results of the conducted mating simulation aiming at increasing the genetically determined resistance of sheep in two tested flocks were presented in Figure. Regardless of the flock, a constant increase of the frequency of individuals of the ARR/ARR genotype was found, from generation F1 to generation F6. In the flock of Polish Mountain Sheep the frequency of individuals of the ARR/ARR genotype exceeded 90% in the fourth generation, and in case of Polish Merino sheep as many as five generations were needed to obtain the frequency of 94.44% (Fig.). These forecasts seem to be very optimistic in comparison with analyses of Arnold et al. [1] conducted on the example of population of sheep in Great Britain. The model applied by the researchers demonstrated that achieving 100% of slau-



	F1	F2	F3	F4	F5	F6
Polish Merino merynos polski	30.53%	55.53%	77.76%	88.88%	94.44%	97.22%
Polish Mountain Sheep polska owca górska	35.72%	60.72%	80.36%	90.18%	95.09%	97.54%

Fig. Calculated frequencies (%) of the ARR/ARR genotype in the next generations in two sheep flocks (simulation study)
Rys. Wyliczone frekwencje (%) genotypu ARR/ARR dwóch stad owiec w kolejnych pokoleniach (symulacja)

ghter lambs having even only one ARR allel would take at least 20 years of mating aiming at increasing the genetically controlled resistance of sheep to scrapie.

An approach to achieving the genetically determined resistance to scrapie that is commonly adopted for economic reasons, is genotyping the rams in respect to the prion protein gene. However, Kao et al. [7] indicate that other approach, based on genotyping all rams and ewes in breeds characterized by high susceptibility to scrapie would allow quicker elimination of individuals susceptible to this disease from population of sheep in Great Britain.

To sum up, it should be noted that carrying out the mating aiming at increasing the resistance to scrapie is possible in both flocks covered by the in-house research. For the purposes of comparison, some breeds of sheep, e.g. East Friesian Milk Sheep [4], are characterized by very low frequencies of the ARR allel and ARR/ARR genotype, which significantly hampers carrying out appropriate mating and at the same time achieving the population resistant to scrapie. Besides, in the population of 932 sheep tested in Iceland the codon for arginine was not found in position 171 [13], which precluded carrying out the mating aiming at increasing the resistance of sheep to scrapie with the use of animals having been genotyped in those tests.

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Polimorfizm owczego genu PrP oraz symulacja kojarzeń skierowanych na podwyższenie oporności owiec merynosowych i górskich na trzęsawkę

S t r e s z c z e n i e

Genetycznie regulowana oporność/podatność owiec na trzęsawkę (scrapie) jest związana z polimorfizmami występującymi szczególnie w trzech kodonach: 136, 154 i 171 owczego genu białka prionowego. Kojarzenia skierowane na podwyższenie oporności owiec na trzęsawkę mają na celu stały wzrost frekwencji haplotypu $A_{136}R_{154}R_{171}$ w kolejnych pokoleniach. Celem badań było określenie frekwencji halpotypów i genotypów białka prionowego w stadach owiec rasy merynos polski i polska owca górska utrzymywanych w kraju oraz przeprowadzenie symulacji kojarzeń skierowanych na

podwyższenie oporności owiec na trzęsawkę w tych stadach. Polimorfizmy w trzech kodonach: 136, 154 i 171 wykryto metodą PCR-RFLP. Analiza 119 owiec wykazała zmienność (A/T) tylko w kodonie 171. Najwyższą frekwencję haplotypu ARR wykryto w stadzie owiec merynosowych. W wykonanej symulacji obliczono teoretyczne frekwencje genotypu ARR/ARR w kolejnych pokoleniach owiec po wprowadzeniu kojarzeń skierowanych na podwyższenie oporności na trzęsawkę. Przeprowadzona symulacja wykazała, iż byłoby możliwe osiągnięcie ponad 97% zwierząt o genotypie ARR/ARR w pokoleniu F6 w objętych badaniami stadach owiec.

SŁOWA KLUCZOWE: owce / gen białka prionowego *(PRNP)* / polimorfizm / PCR-RFLP / genotypowanie PrP