

Estimation of genetic variation in Pomeranian sheep population included in the Genetic Resources Conservation Programme

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The aim of the study was to determine genetic diversity of Pomeranian sheep (owca pomorska, POM) based on blood typing and analysis of hemoglobin and transferrin polymorphism. Blood samples collected from 192 sheep from north-eastern Poland were investigated. Calculations were made of the frequency of alleles at the loci of blood groups, transferrin and hemoglobin, the degree of heterozygosity, and the effective number of alleles in different loci. Genetic equilibrium was also evaluated. In the most polymorphic B blood group system 35 alleles were observed, of which B^c, B⁻, B^{sPLB-17} and B^b were most frequent. The HBB^B allele and the BB genotype were most frequent at the hemoglobin locus. Among the transferrin alleles, the highest frequency was found for TF^C and TF^D, with CC being the most frequent genotype at this locus. In the population of Pomeranian sheep, the effective number of alleles (\bar{E}) and the degree of heterozygosity (\bar{H}) averaged to 3.32 and 0.4232, respectively. Of the 7 conserved breeds that have been studied to date, genetic variation in the POM breed is one of the lowest.

KEY WORDS: sheep / blood groups / protein polymorphism / genetic variation

In the recent years around the world, increasing attention has been given to the determination and conservation of biological diversity. FAO's Global Plan of Action for Animal Genetic Resources, adopted at the Interlaken conference in 2007, contains four strategic priority areas. They include actions aimed to assess of the worldwide state of animal genetic resources and monitor rate of erosion of genetic diversity as well as conserve animal diversity [4, 9]. The activities undertaken to preserve the biodiversity of different breeds should also include a comprehensive analysis of their genetic structure, using the greatest possible number of genetic markers.

Blood groups as well as polymorphic variants of proteins have been used since the 1960s to characterize populations, mainly those of farm animals. One example are studies

on genetic characterization of different breeds of sheep [1, 2, 20, 22, 23]. This type of research is of particular significance for protected species. Due to the small populations of these breeds, breeding work should be based not only on phenotypic evaluation and breeding records [17], but also on genetic variation. The latter is most often analysed indirectly, based on polymorphism of blood groups and blood proteins that are class I markers [19, 21] and polymorphism of minisatellite and microsatellite DNA sequences, which are class II markers [18, 26].

To date, the genetic structure of sheep breeds included in the genetic resources conservation programme in Poland has been analysed for Wrzosówka [5, 23], Coloured Merino [24], Olkuska [20], Kamieniecka [6], Corriedale [7], Świniarka [25] and Polish Mountain Sheep [22].

The aim of the study was to determine genetic diversity in Pomeranian sheep based on blood typing and hemoglobin and transferrin polymorphism.

Material and methods

Analysis was made of blood samples collected from 192 Pomeranian sheep (owca pomorska – POM), including 97 lambs (58 ewe lambs and 39 ram lambs), 80 ewes and 18 flock rams. Animals originated from four flocks located in north-eastern Poland. The number of sheep ranged from 45 to 52 in different flocks.

Red blood cell antigens were determined using our own 16 test reagents: anti-Aa, Ab, Bb, Bc, Bd, Be, Bf, Bg, Bi, PLB-17, Ca, Cb, Da, Ma, R and 0 in hemolysis and microplate agglutination tests. These reagents were subjected to standardization in international comparison tests organized by the International Society for Animal Genetics (ISAG). Polymorphic variants of transferrin and hemoglobin were determined by horizontal starch gel electrophoresis according to a modified version of the method described by Smithies [27] and Evans [3].

The genotypes of the analysed animals were determined based on antigenic components of blood and polymorphic variants of blood proteins from the offspring and parents. The frequency of alleles in different loci was calculated by directly summing the number of genes. The degree of heterozygosity was calculated according to the formula of Nei and Roychoudhury [13], whereas the effective number of alleles per locus according to Kimura and Crow [8]. Significant differences between the observed and expected number of HB and TF genotypes was determined by chi-square test, and the state of genetic equilibrium of the analysed population was evaluated following the Hardy-Weinberg law.

The application of the same methods that have been used for many years to characterize sheep breeds raised in Poland enabled comparisons with previous studies.

Results and discussion

Proper management of the world's farm animal genetic resources has been gaining in importance in recent years. In many countries special institutions were established whose statutory tasks include support of biodiversity and protection of endangered breeds and

small populations that can be a source of valuable genes in the future [12, 16]. In Poland, the genetic resources conservation programme covers 13 sheep breeds and varieties, including the Pomeranian sheep. The breed originated from north Poland and is a native variety of Polish Longwool Sheep (polska owca długowłnista – POD) [11].

In our study, we evaluated genetic diversity of the Pomeranian sheep population based on analysis of the polymorphism of erythrocyte antigens from 7 blood group systems (A, B, C, D, M, R, I), blood plasma proteins (transferrins) and erythrocytes (haemoglobin).

Results for the frequency of blood group, haemoglobin and transferrin alleles are given in Table 1. In the analysed sheep group, allele A⁻ was the most frequent (0.8177) and allele A^{ab} the least frequent (0.0365) in the A blood group system. Thirty-five alleles were observed in the most polymorphic B system. Of these, the highest frequency was found for alleles B^c (0.1563), B⁻ (0.1250), B^{cPLB-17} (0.0781) and B^b (0.0625). These findings are similar to those obtained in an earlier study for the population of Polish Longwool Sheep (of which Pomeranian sheep is a variety) and for Friesian rams, which were used to improve the POM population. The previous study showed that the most frequent alleles in the analysed groups were B⁻ (0.2450), B^b (0.0761) and B^c (0.0652) in the POD population [5], and B^c (0.1270), B^b (0.0873) and B⁻ (0.3254) among Friesian rams [19]. In the C system, the frequency of the C^b allele (0.9062) exceeded that of the other alleles, whereas the C^a allele was less frequent (0.0104). In the other systems, the most frequent alleles were D⁻ (0.8438) in the D system, M⁻ (0.4896) in the M system, and I¹ (0.8979) in the I system.

Among hemoglobin alleles (HBB), HBB^B was the most frequent in the analysed flocks (0.8776). At the transferrin locus, the most frequent alleles were TF^C (0.3438) and TF^D (0.3047) (Tab. 1). The same alleles were also found to be most frequent in previous studies in POD sheep (TF^D allele frequency of 0.3200, TF^C allele frequency of 0.2915) [5] and in Friesian rams (TF^C allele frequency of 0.4922) [19].

The frequency of transferrin and hemoglobin genotypes is given in Table 2. BB was the most frequent genotype at the hemoglobin locus (0.7708) and CC at the transferrin locus (0.1719). The CC genotype was also highly frequent in the group of the Friesian rams studied (0.2698) [19] and in the POD population (0.1022) [5].

In order to achieve genetic progress, it is necessary to maintain a certain level of genetic variation, which should be regularly monitored. Genetic variation within breeds is estimated using the effective number of alleles (E), the degree of heterozygosity (h_e) and the total number of alleles. The high values of these parameters are indicative that a given breed shows greater genetic variation.

In the analysed population of Pomeranian sheep, the effective number of alleles (\bar{E}) averaged 3.32. The mean degree of heterozygosity (\bar{H}) in the POM group was 0.4232 (Tab. 3) and was lower than 0.5, which is considered the most favourable value considering the degree of genetic variation in the population. Also compared to the \bar{H} value calculated for the POD population (0.5380) [5] and Friesian rams (0.4799) [19], the value of this parameter in the POM population is lower.

Of the seven native breeds for which genetic variation has been determined based on the mean degree of heterozygosity, the population of Pomeranian sheep shows the lowest variation in addition to Corriedale (\bar{H} =0.414) [7] and Coloured Merino (\bar{H} =0.431) [24].

Low \bar{H} values were also reported for other two native breeds of sheep, i.e. Świniarka

Table 1 – Tabela 1

Frequencies of blood groups (EA), haemoglobin (HB) and transferrin (TF) alleles in investigated population of Pomeranian sheep

Częstość występowania alleli grup krwi (EA), hemoglobiny (HB) i transferyny (TF) w badanej populacji owcy pomorskiej

Locus	Allele Allel	Frequency Częstość	Locus	Allele Allel	Frequency Częstość	
EAA	a	0.0989	dfPLB-17	dfPLB-17	0.0182	
	ab	0.0365		dPLB-17	0.0104	
	b	0.0469		e	0.0156	
	–	0.8177		f	0.0313	
EAB	b	0.0625	PLB-17	fi	0.0078	
	bc	0.0521		fiPLB-17	0.0156	
	bcfiPLB-17	0.0156		fPLB-17	0.0365	
	bcfPLB-17	0.0104		i	0.0313	
	bcPLB-17	0.0313		PLB-17	0.0156	
	bd	0.0104		–	0.1250	
	bdf	0.0078		EAC	a	0.0104
	bdfiPLB-17	0.0078			ab	0.0339
	befiPLB-17	0.0078			b	0.9062
	bf	0.0078			–	0.0495
	bfiPLB-17	0.0078		EAD	a	0.1562
	bfPLB-17	0.0104			–	0.8438
	bg	0.0443		EAM	a	0.5104
	bi	0.0313			–	0.4896
	c	0.1563		EAR	R	0.6250
	cdfiPLB-17	0.0078			0	0.3750
	cf	0.0104		EASI	I	0.8979
	cfi	0.0078			i	0.1021
	cfiPLB-17	0.0260		HBB	A	0.1224
	cfPLB-17	0.0104			B	0.8776
cPLB-17	0.0781	TF	A	0.1484		
d	0.0313		B	0.1536		
df	0.0156		C	0.3438		
dfgPLB-17	0.0104		D	0.3047		
dfiPLB-17	0.0313		E	0.0495		

Table 2 – Tabela 2

Frequencies, observed and expected distributions of haemoglobin (HB) and transferrin (TF) genotypes in investigated group of Pomeranian sheep

Częstość występowania genotypów hemoglobiny (HB) i transferyny (TF) oraz ich obserwowany i oczekiwany rozkład w badanej populacji owcy pomorskiej

Locus	Genotype Genotyp	Frequency Częstość	Observed genotype distribution Obserwowany rozkład genotypów	Expected genotype distribution Oczekiwany rozkład genotypów	Degrees of freedom Stopnie swobody	Chi- squared test Chi ²
HBB	AA	0.0156	3	2.9	2	0.015
	AB	0.2136	41	41.2		
	BB	0.7708	148	147.9		
TF	AA	0.0469	9	4.2	13	23.6*
	AB	0.0313	6	8.8		
	AC	0.0833	16	19.6		
	AD	0.0833	16	17.4		
	AE	0.0052	1	2.8		
	BB	0.0208	4	4.5		
	BC	0.0729	14	20.4		
	BD	0.1406	27	18		
	BE	0.0208	4	2.9		
	CC	0.1719	33	22.7		
	CD	0.1563	30	40.6		
	CE	0.0313	6	6.5		
	DD	0.0937	18	17.8		
	DE	0.0417	8	5.8		

*Statistically significant differences at the level of $P < 0.05$ – Różnice statystycznie istotne przy $P < 0.05$

(0.4608) [25] and Wrzosówka (0.4950) [23]. It should be noted, however, that in the other three analysed breeds included in the genetic resources conservation programme, \bar{H} had high values: 0.5440 for Coloured Polish Mountain Sheep [22], 0.594 for Olkuska sheep [20] and 0.537 for Kamieniecka sheep [6].

The basic objective of breeding work is to improve populations through selection and proper choice of animals for reproduction. These activities may alter the frequency of some genes, considerably reduce their number, or completely eliminate them from certain animal populations [10, 14, 15, 21]. In the analysed POM population, genetic disequilibrium was found in the transferrin system ($P < 0.05$) (Tab. 2), most probably as a result of selection for breed characteristics.

Table 3 – Tabela 3

Number of alleles (N), effective number of alleles (E) and heterozygosity degree in investigated group of Pomeranian sheep (h_k)

Liczba alleli (N), efektywna liczba alleli (E) oraz stopień heterozygotyczności w badanej populacji owcy pomorskiej (h_k)

Locus	Number of alleles Liczba alleli (N)	Effective number of alleles Efektywna liczba alleli (E)	Degree of heterozygosity Stopień heterozygotyczności (h_k)
EAA	4	1.47	0.3181
EAB	35	15.60	0.9361
EAC	4	1.22	0.1748
EAD	2	1.40	0.2636
EAM	2	2.00	0.4998
EAR	2	1.88	0.4688
EASI	2	1.22	0.1834
HBB	2	1.27	0.2148
TF	5	3.86	0.7492
Total Razem	58		
\bar{E}		3.32	
\bar{H}			0.4232

\bar{E} – mean effective number of alleles – średnia efektywna liczba alleli

\bar{H} – mean degree of heterozygosity – średni stopień heterozygotyczności

The results of the present study provided information on the polymorphism of erythrocyte antigens in seven blood group systems and at the transferrin and hemoglobin loci in the population of Pomeranian sheep included in the genetic resources conservation programme. It was found that the most frequent blood group and transferrin alleles in this population are also characteristic of Longwool sheep and Friesian rams. Comparison of the genetic variation based on the degree of heterozygosity revealed a low level of differences in the POM population compared to the POD population and other protected farm animal species.

Because genetic variation is necessary for the success of breeding programmes and for breeding progress, it should be monitored on a regular basis. The present study and the results obtained may provide a starting point for further monitoring of variation in this breed of sheep while providing important information to be used in genetic resources conservation.

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Ocena zmienności genetycznej w populacji owcy pomorskiej objętej programem ochrony zasobów genetycznych

Streszczenie

Celem badań było określenie zróżnicowania genetycznego owcy pomorskiej (POM) na podstawie badań grup krwi oraz polimorfizmu hemoglobiny i transferyny. Materiał badań stanowiły próbki krwi pobrane od 192 owiec pochodzących z północno-wschodniego rejonu Polski. Obliczono częstości występowania alleli w *loci* grup krwi, transferyny i hemoglobiny, stopień heterozygotyczności, efektywną liczbę alleli w poszczególnych *loci* oraz dokonano oceny stanu równowagi genetycznej. W najbardziej polimorficznym układzie grupowym krwi B zaobserwowano 35 alleli, spośród których najwyższą frekwencją charakteryzowały się B^e, B⁻, B^{ePLB-17} i B^b. W *locus* hemoglobiny najczęściej występował allel HBB^B i genotyp BB. Natomiast wśród alleli transferyny z najwyższą frekwencją odnotowano TF^C i TF^D, a najczęstszym genotypem w tym *locus* był CC. W populacji owcy pomorskiej średnie wartości efektywnej liczby alleli (\bar{e}) i stopnia heterozygotyczności (\bar{h}) wyniosły, odpowiednio 3,32 i 0,4232. Spośród 7 przebadanych do tej pory ras chronionych, zmienność genetyczna POM jest jedną z najmniejszych.