

*SHORT REPORT*

**The polymorphism of a prion protein gene in European bison (*Bison bonasus*, L. 1758) and Polish Red and Polish Whitebacked cattle included in the genetic resources conservation programme**

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The prevention of transmissible spongiform encephalopathy (TSE) in humans and animals has become an important element of safe food production. Part of the studies connected with this problem concentrate on the search for potential markers of the susceptibility or resistance to bovine spongiform encephalopathy (BSE). The present report presents a characteristic of the bovine prion protein (PRNP) gene polymorphism within the population of European bison and two endemic cattle breeds – Polish Red (PR) and Polish Whitebacked (PWb), the latter two included in a genetic resources conservation programme.

European bison appeared monomorphic as regards *PRNP* what was reflected by the presence of only one genotype – *PRNP* 6/6. In the northern (lowland) subpopulation of PR the frequency of *PRNP* 6/6 and *PRNP* 6/5 genotypes was 0.926 and 0.074, respectively. In the PR southern (sub-montane-

ous) subpopulation the share of *PRNP* 6/5 and *PRNP* 5/5 was 0.214 and 0.049. In the PWb cattle the frequency of *PRNP* 6/6 was 0.628, that of *PRNP* 6/5 – 0.321, and of *PRNP* 5/5 – 0.051.

**KEY WORDS:** European bison / Polish Red cattle / *PRNP* polymorphism / Polish Whitebacked cattle

Over the last years the most destructive for cattle and potentially very dangerous for humans has become the transmissible spongiform encephalopathy (TSE), often described as the “mad cow disease”. The pathogenic factor in bovine TSE is the bovine prion protein (PRNP) which, as result of conformation changes, creates a pathogenic molecule [Prusiner 1998]. The occurrence of BSE in Western Europe, and principally in Great Britain, was of a pandemic character, which resulted in considerable economic losses.

The PRNP coding gene (marked as *PrP*) was found to be located on the cattle chromosome BTA13 [Schlapfer *et al.* 1998]. The structure and organization of the cattle *PrP* gene is already known [Yoshimoto *et al.* 1992, Horiushi *et al.* 1998, Hills *et al.* 2001] and about 60 mutations have been identified within its sequence [Sander *et al.* 2004]. The most frequent allele is marked as *PRNP* 6. More rare is *PRNP* 5 while variant *PRNP* 7 was identified only in Brown Swiss cattle [Schlapfer *et al.* 1999, Leone *et al.* 2002]. Results reported by Walawski *et al.* [2003], as well as those obtained by Neibergs *et al.* 1994, Hunter *et al.* 1997, Premzl *et al.* 2000 and Leone *et al.* 2002), indicate that the segregation of alleles *PRNP* 6 and *PRNP* 5 is not regular – genotype *PRNP* 5/5 occurs only in some populations, probably exclusively in females. Studies conducted hitherto concentrated on the search for relations between the PRNP octapeptide-repeat polymorphism and the occurrence of BSE. Susceptibility markers have been identified on chromosomes BTA5 [Hernandez-Sanchez *et al.* 2002] and BTA17 [Zhang *et al.* 2004]. The most recent investigations by Sander *et al.* [2004] demonstrate the existence of statistically significant relations between the polymorphism of varying repeat numbers of the octapeptide PRNP fragment and susceptibility to BSE. In a population comprising animals of eight German breeds the same authors observed that the frequency of allele *PRNP* 5 was significantly higher in cows with BSE than in the group of healthy animals. Family forms of diseases, caused in humans by prions with repeatable sequences (inserts) between *PRNP* codon 51 and 91 form the most heterogeneous group of TSE [Liberski 1999]. Due to the fact that BSE is a disease of animal origin causing in humans a variety of the Creutzfeldt-Jacob disease [Almond and Pattison 1997], studies aiming at elucidating the etiology of prion diseases are continued in numerous research centres. Indicating populations susceptible and resistant is an element of such studies.

This report contains a description of the genetic structure of the gene *PRNP* in a group of European bison and two endemic cattle breeds – Polish Red and Polish Whitebacked both included in a programme for animal genetic resources conservation programme.

### Material and methods

The identification of PRNP genes was conducted on four following groups of animals, three of which were included in the national animal genetic resources conservation programme:

- **European bison** (from *Białowieża* Forest) at various ages, captured and designated for culling (n=51);
- **northern (lowland)** subpopulation of Polish Red (PR) cattle from preserve breeding programme (PR-N, n=95);
- **southern (sub-montaneous)** subpopulation of Polish Red (PR) cattle from preserve breeding programme (PR-S, n=142);
- **Polish Whitebacked** (PWb) cattle comprising a foundation herd in the programme for the restitution of this cattle (n=78).

The PR-N subpopulation group was composed of cows and young animals maintained at the Polish Academy of Sciences, Research Station for Ecological Agriculture and Preserve Animal Breeding, Popielno (northeast of Poland). The PR-S subpopulation group comprised cows and young animals owned by small farmers associated in the *Małopolska* Centre for Preserve Animal Breeding (sub-carpathian region of Poland). The PWb group included cows maintained at the Agricultural University of Lublin Experimental Farm, Uhrusk.

Analytical procedure followed that described by Walawski and Czarnik [2003].

The differentiation of genotype and PRNP allele frequencies as well as the genetic equilibrium were verified by the chi-square test.

### Results and discussion

Table 1 presents the frequency of *PRNP* genotypes and alleles in animals considered. For comparison, earlier results by Walawski and Czarnik [2003] obtained for Polish Black-and-White cattle are included.

The bison appeared monomorphic as regards gene *PRNP*, what was manifested by the occurrence of only one genotype – *PRNP* 6/6 (Tab. 1). It seems that this situation reflects the most recent history connected with the almost total extermination of the species and its restitution on the basis of a very limited number of animals. The whole present world population originates from 12 founders, while that kept in *Białowieża* (Poland) from only 7 founders. This results in a high inbred and genetic uniformity of the population (a bottleneck effect).

Table 1 demonstrates that the frequency of alleles *PRNP* in PR cattle (varieties pooled) was very similar to that found earlier in the local population of Black-and-White cattle by Walawski and Czarnik [2003]. However, an indicator differentiating the PR cattle was the three times higher frequency of homozygotes *PRNP* 5/5. It is also significant, that the PR population analysed was not in a genetic equilibrium as regards

Table 1 The frequency of *MM2* genotypes and alleles in a population of the European brown and Polish Red, Polish  $\mathcal{P}$  hatched and Polish Black-and-White cattle

Animals	n	Genotype			Allele	
		<i>MM2</i> L-L	<i>MM2</i> L-T	<i>MM2</i> T-T	<i>ML2</i>	<i>MT2</i>
European brown	51	1 000	0 000	0 000	1 000	0 000
Polish Red cattle <sup>1</sup>	217	0 810	0 160	0 030	0 850	0 150
Polish $\mathcal{P}$ hatched cattle	78	0 628	0 211	0 061	0 788	0 212
Black-and-White cattle <sup>2</sup>	1167	0 808	0 181	0 011	0 89*	0 106

<sup>1</sup>Subpopulation a pooled<sup>2</sup>after Walawski and Corradi (2001)Table 1 The frequency of *MM2* genotypes and alleles in two subpopulations of Polish Red cattle

Sub-population	n	Genotype			Allele	
		<i>MM2</i> L-L	<i>MM2</i> L-T	<i>MM2</i> T-T	<i>ML2</i>	<i>MT2</i>
Northern (PR-N)	95	LL	T	D	0 961	0 039
Frequency		0 924	0 076	0 000		
Southern (PR-S)	121	LD	TT	T	0 881	0 119
Frequency		0 711	0 289	0 009		

Table 3 *MM2* genetic equilibrium in the European brown, Polish Red and Polish  $\mathcal{P}$  hatched cattle

Animals	Number	Genotype			$\chi^2$
		<i>MM2</i> L-L	<i>MM2</i> L-T	<i>MM2</i> T-T	
European brown	observed	51	0	0	51
	expected	51 00	0 00	0 00	51 00
	$\chi^2$	0 00	0 00	0 00	0 00
Polish Red cattle <sup>1</sup>	observed	192	18	7	217
	expected	187 77	6 60	2 63	217 000
	$\chi^2$	0 097	1 321	3 928**	7 346**
Polish $\mathcal{P}$ hatched cattle	observed	49	13	6	78
	expected	48 47	16 06	7 47	78 000
	$\chi^2$	0 067	0 001	0 060	0 128

<sup>1</sup>Subpopulation a pooled\*\*Significance  $P < 0.05$

*PRNP*, as the number of allele *PRNP 5* homozygotes was greater than that expected, calculated on the basis of the Hardy-Weinberg formula. To preserve the Polish Red cattle from total annihilation two preserve breeding programmes have been created – for a northern (lowland) and southern (sub-montaneous) subpopulation. The results collected in Tables 2 and 3 indicate that the PR population is not uniform. Considerable differences have been observed in the frequency of *PRNP* genotypes and alleles. The PR-N population is characterized by a comparatively large share of homozygotes *PRNP 6/6* (0.926) and small share of heterozygotes *PRNP 6/5* (0.074), while the population PR-S differs by a considerable share of heterozygotes *PRNP 6/5* (0.214) and the presence of homozygotes *PRNP 5/5* (0.049).

Clear specificity of the *PRNP* polymorphism appeared in PWb cattle that was characterized by a low frequency of homozygotes *PRNP 6/6* (0.628), a considerable share of heterozygotes *PRNP 6/5* (0.321) and of *PRNP 5/5* homozygotes (0.051). The PWb cattle was bred over several hundred years in regions lying to the east of the river Vistula [Sławiński 1919]. It is usually assumed that PWb is a lowland type of cattle, moved from the north-east part of Europe and crossed with imported Dutch cattle. In year 1916 PWb cattle, constituted 41% of cows entered into stud books. In 1935/1936 the breed comprised already only 6.1% of the milk-recorded population. In the seventies the breed was considered extinct. Work on its restitution was started in year 2000 and the breed was introduced into the FAO world list of animals covered by the preservation of genetic resources.

At the base of the “National Report on the State of Resources as Regards Animal Genetic Diversity” lies the concept of rational breeding, merging the economic effects of safe food production with the conservation of the biodiversity of wild and domesticated populations. It seems that studies on the *PRNP* polymorphism in Polish Red and Whitebacked cattle, included in the national programme for preserve breeding, are of importance not only due to their cognitive value in determining the genetic characteristic of unique populations, but also due to the potential value of the results obtained for further work on the pathogenesis of neuro-degenerative diseases.

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Polimorfizm białka prionowego u żubra (*Bison bonasus*)  
oraz w populacjach bydła polskiego czerwonego  
i polskiego białogrzbietego objętych krajowym programem  
ochrony zasobów genetycznych

Streszczenie

Zapobieganie gąbczastym encefalopatiom człowieka i zwierząt (TSE) stało się ważnym elementem problematyki produkcji zdrowej żywności. Fragmentem tych badań jest poszukiwanie potencjalnych markerów podatności i odporności na BSE. Przedstawiono charakterystykę polimorfizmu PRNP w obrębie populacji żubra oraz endemicznych ras bydła polskiego czerwonego i polskiego białogrzbietego, objętych programem ochrony zasobów genetycznych. Wyniki wskazują na monomorfizm PRNP w populacji żubra, co przejawia się występowaniem jednego wariantu genotypowego *PRNP 6/6*. Bydło rasy polskiej czerwonej okazuje się pod względem polimorfizmu białka prionowego zróżnicowane. Subpopulacja północna (nizinna) charakteryzuje się dużym udziałem homozygot *PRNP 6/6* (0.926) i małym udziałem heterozygot *PRNP 6/5* (0.074), natomiast populacja południowa (podgórska) charakteryzuje się dużym udziałem heterozygot *PRNP 6/5* (0.214) i niższym występowaniem homozygot *PRNP 5/5* (0.049). Zdecydowaną odrębność polimorfizmu PRNP stwierdzono u bydła białogrzbietego. Przejawia się ona niską frekwencją homozygot *PRNP 6/6* (0.628), dużym udziałem heterozygot *PRNP 6/5* (0.321) oraz stosunkowo dużym udziałem homozygot *PRN 5/5* (0.051).

