

## Frequencies of alleles and genotypes of the PRNP gene in Polish Red, Czech Pied and Czech Black-and-White cattle

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**Abstract.** Frequencies of alleles and genotypes of the *PRNP* gene in 65 Polish Red cows kept under a conservation programme and 52 randomly chosen cows of two Czech breeds: Czech Pied (42) and Black-and-White (10) were studied. All cows were at the age of 4 years or older. It was found that the frequency of allele 5 (5 copies of the octapeptide repeat) ranged from 0.11 (Polish Red) to 0.00 (Czech Black-and-White) whereas the frequency of allele 6 (6 copies of the octapeptide repeat) ranged from 1.00 (Czech Black and White) to 0.89 (Polish Red). The highest frequency was found for the homozygous genotype 6/6 (1.00 in Czech Black-and-White) and the lowest frequency was detected for the heterozygous genotype 6/5 (0.143). In the studied cows the genotype 5/5 was not found. The higher frequency of allele 5 in the native breeds and its lower frequency or lack of this allele in the populations intensively selected for high milk production may suggest that alleles of the *PRNP* gene may be associated with milk production.

**Key words:** allele frequency, BSE, cattle, *PRNP* gene.

The neurodegenerative disease of cattle known as BSE (bovine spongiform encephalopathy) is caused by a form of prion protein (PrP = protease-resistant protein). *PRNP*, a bovine gene encoding PrP, has been mapped to chromosome 13, and its size is estimated at 20 kbp. When transcribing from the 3rd exon of mRNA the gene has 4244 bp. In the open reading frame the polymorphism of

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the coding region of the bovine PrP gene has been found (HUNTER et al. 1994). There are 3 known alleles of the gene *PRNP*, which contain 5, 6 or 7 octapeptide repeats. The 7 octapeptide repeat allele was detected in Brown Swiss cattle (SCHLÄPFER et al. 1999), whereas 5 and 6 octapeptide repeat alleles and both homozygous (5/5 and 6/6) and heterozygous (5/6) genotypes were found in cattle populations in the USA, Belgium and Scotland (GOLDMANN et al. 1991, McKENZIE et al. 1992, ROBINSON et al. 1994, HUNTER et al. 1994, NEIBERGS et al. 1994).

SCHLÄPFER et al. (1998) carried out a study in a herd of 72 Brown Swiss cattle, and estimated the frequency of allele 5 (5 copies of the octapeptide repeat) at 15.3%. According to PREMZL et al. (2000), who studied 4 cattle populations in Croatia, the frequency of allele 6 (6 copies of the octapeptide repeat) was as follows: 97% in a sample of 70 cows of Simental breed, 63% in a group of 12 cows of Slavonian Sarmian breed, and 100% both in a group of 9 cows of Holstein-Friesian breed and in a sample of 22 Istrian cows. A study carried out in the USA (McKENZIE et al. 1992) in a group of 65 cows representing 14 different breeds revealed that 86.1% of studied animals were homozygotes 6/6, 12.3% were heterozygotes 5/6, and only 1.6% were homozygotes 5/5.

GOLDMANN et al. (1991) reported the sequence of different forms of the bovine PrP gene, which contain either 5 or 6 copies of a short G-C-rich element encodes the octapeptide Pro – His – Gly – Gly – Gly – Trp – Gly – Gln or its longer variants Pro – Gln/His – Gly – Gly – Gly – Gly – Trp – Gly – Gln. In this study, among 12 cattle examined, 8 were homozygotes 6/6, 4 were heterozygotes 6/5 and none were homozygotes 5/5. In the group of 8 homozygotes 6/6, two cases of BSE occurred.

Analysis of the octapeptide repeat region of the bovine protease-resistant protein (PrP) gene showed that variations in incubation period, clinical signs, and neurohistological changes were unrelated to the homozygous or heterozygous condition of 6 or 5/6 octapeptide repeats (ROBINSON et al. 1995).

HUNTER et al. (1994) reported that among 370 cattle in Scotland there was no difference in frequencies of genotypes 6/6, 5/6 and 5/5 between healthy cattle and cattle with BSE. However, NEIBERGS et al. (1994) carried out a study in a group of 56 cows affected by BSE, and found that 84% of the examined animals were homozygotes 6/6 and 16% were heterozygotes 5/6. Homozygotes 5/5 were not detected.

The present study aimed at determining the frequency of alleles and genotypes of the *PRNP* gene in samples of 65 cows of the Polish Red breed kept under the conservation programme, and 52 randomly chosen cows of two Czech breeds: Czech Pied (42 animals) and Czech Black-and-White (10 animals). All studied cows were at the age of 4 years or older.

DNA samples were taken from the whole blood or hair bulbs using the method described by DVOŘÁK et al. (1999). Polymerase chain reaction (PCR) primers designed to amplify the octapeptide repeat region of the bovine prion gene were used according to the method described by PREMZL et al. (2000). The PCR products

**Table 1.** PRNP genotype frequency (%) in Polish Red cattle (under a conservation programme), Czech Pied, and Czech Black-and-White cattle (commercial herds)

Breed	Sample size	PRNP genotype		
		6/6	6/5	5/5
Polish Red	65	78.5 % (n=51)	21.5% (n=14)	0
Czech Pied	42	85.7% (n=36)	14.3% (n=6)	0
Czech Black-and-White	10	100% (n=10)	0	0
Total	117	82.9% (n=97)	17.1% (n=18)	0

were electrophoretically analysed on 3% agarose gel. The shorter fragments of DNA (349 bp) were recognised as alleles 5 of the PRNP gene, whereas the longer fragments of DNA (373 bp) were recognised as alleles 6 of the PRNP gene.

Table 1 presents frequencies of PRNP genotypes in Polish Red cattle, Czech Pied and Czech Black-and-White cattle. It was found that all cows representing Czech Black-and-White breed were homozygotes 6/6. This genotype was also the most frequent in Czech Pied cattle (87.5%) and Polish Red cattle (78.5%). Homozygous genotypes 5/5 were not found.

The frequencies of alleles of PRNP gene in the studied breeds and some other examples available in literature are shown in Table 2. In the group of 10 Czech

**Table 2.** PRNP allele and genotype frequency

Breed (Country)	Sample size	PRNP allele		Source
		6	5	
Black-and-White (Czech Republic)	10	1.00	0.00	this study
Holstein (Croatia)	9	1.00	0.00	PREMZL et al. (2000)
Holstein (Great Britain)	94	0.88	0.12	NEIBERGS et al. (1994)
Czech Pied (Czech Republic)	42	0.93	0.07	this study
Simental (Croatia)	70	0.97	0.03	PREMZL et al. (2000)
Brown Swiss (Switzerland)	72	0.85	0.15	SCHLÄPFER et al. (1999)
Herds under conservation				
Polish Red (Poland)	65	0.89	0.11	this study
Czech Red (Czech Republic)	14	0.94	0.06	VRTKOVÁ et al. (2001)
Istrian (Croatia)	22	1.00	0.00	PREMZL et al. (2000)
Slavonian Strymian (Croatia)	12	0.63	0.37	PREMZL et al. (2000)

Black-and-White cows allele 5 was not present. The same situation was found by PREMZL et al. (2000) in Slavonian Syrmian and Holstein breeds bred in Croatia. Relatively high frequencies of allele 5 were detected in Polish Red (0.11) and Czech Pied (0.07) cows. Higher frequencies of this allele were found in Slavonian Syrmian cattle (PREMZL et al. 2000) and Holstein cows kept in Great Britain (NEIBERGS et al. 1994).

The results of the present study as well as the data available in literature indicate that higher frequencies of allele 5 of *PRNP* gene are found in native breeds, whereas the lower frequency of this allele or its lack is typical for the breeds intensively selected towards high milk production. Thus different frequencies of allele 5 and allele 6 of the *PRNP* gene may be brought about by selection of more productive dairy cows.

The higher frequency of allele 5 of the *PRNP* gene in some cattle populations being under a conservation programme (breeds: Slavonian Syrmian, Polish Red, Czech Pied) supports a hypothesis that the allele with the lower number of octapeptide repeats is an older variant as far as evolution is concerned.

The prion gene plays an important role in transmission of BSE. In populations of cattle one of factors determining susceptibility to BSE is polymorphism of the *PRNP* gene. Since many new cases of BSE have been detected in Europe (BSE has not been detected yet in Poland whereas in the Czech Republic the first case of BSE has been registered after submission of the article for publication) the authors are going to undertake further studies on association between the *PRNP* genotype and susceptibility to BSE as well as relation between susceptibility to BSE and other bovine polymorphic genes.

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