

Octapeptide Polymorphism Analysis of Slovak Autochthonous Cattle BreedsL. TKÁČIKOVÁ¹, R. VORÁLEK¹, P. FILIPČÍK², I. MIKULA Sr.¹¹Institute of microbiology and immunology, University of Veterinary Medicine, Košice, Slovakia²Neuroimmunological institute, Slovak Academy of Sciences, Bratislava, Slovakia

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Abstract

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Octapeptide repeats are 24 bp long repeat segments in the PrP gene that may be present in different species in a different number of repetitions. The aim of the present study was to determine the prevalence of this polymorphism in the PrP gene of two Slovak autochthonous cattle breeds, namely Slovak spotted cattle ($n = 89$) and Slovak Pinzgau ($n = 195$). In the study's cohort of animals, repetition numbers 6 and 5 were found for the octapeptide repeats. We found 6/6 homozygous animals to be most prevalent (96.1%); the remaining animals were 5/6 heterozygous (3.9%). We did not observe any 5/5 homozygous animals in the studied group. This analysis included only a small group of animals. However, the results suggest that animals with six octapeptide repeats are dominant in these Slovak cattle breeds.

PrP, octapeptide repeats, polymorphism, Slovak spotted cattle, Slovak Pinzgau

Prion protein (PrP^C) is a glycoprotein expressed by most tissues and is attached to the cell membrane by a glycosyl-phosphatidylinositol anchor. NMR studies revealed that the prion protein has a globular domain and a long amino-terminal tail that contains repeated octapeptide domains that bind metal ions with high affinities (Hornshaw et al. 1995; Brown et al. 1997; Pauly et al. 1998; Burns et al. 2002). These octapeptide domains usually contain either five or six copies of a short, glycine-rich peptide-repeat that encodes for octapeptide Pro-His-Gly-Gly-Gly-Trp-Gly-Gln or its longer variants Pro-Gln/His-Gly-Gly-Gly-Gly-Trp-Gly-Gln (Goldmann et al. 1991).

In cattle, three PrP isoforms are known, arising from variation in the octapeptide repeat units, ranging from five to seven copies (Neibergs et al. 1992; Hunter et al. 1994; Schläpfer et al. 1998; Leone et al. 2002; Walawski et al. 2003). Previous studies showed no association between polymorphisms in the number of octapeptide repeats and bovine spongiform encephalopathy (BSE) susceptibility (Neibergs et al. 1992; Hunter et al. 1994). The first experimental confirmation that an increased number of octapeptide repetitions in the PrP gene may affect the susceptibility to experimental infection with BSE agents is in the study of Castilla et al. (2004, 2005). These authors reported that transgenic mice (boTg) carrying one or four extra octapeptide repeats in the bovine PrP gene (7 or 10 instead of 6) showed an altered course of BSE infection, reflected in reduced incubation times when compared with boTg mice expressing similar levels of the wild-type six-octapeptide protein.

Variations in the number of octapeptide repeat units were also observed in other species. In the human PrP gene 5 octapeptide repetitions are present and mutations resulting in variations of their number are often associated with the inherited prion disease (Goldfarb et al. 1991; Diedrich et al. 1992; Brown 1994; Croes et al. 2004).

In the goat PrP gene presence of 3 or 5 octapeptide repetitions has been detected (Goldmann et al. 1998; Billinis et al. 2002). The shorter allelic variant, containing only three instead of the usual five-octapeptide repeats, was supposed to be associated with an

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increased scrapie incubation period in goats (Goldmann et al. 1998). In the sheep PrP gene 5 copies of the octapeptide repeat are present (Goldmann et al. 1998).

The aim of the present study was to determine the number of octapeptide repeat in the PrP gene of Slovak autochthonous cattle breeds.

Materials and Methods

Samples from healthy cattle of two Slovak autochthonous breeds, the Slovak spotted cattle ($n = 89$) and Slovak Pinzgau ($n = 195$) were included in the study. Genomic DNA was isolated from blood leukocytes (Sambrook et al. 1989). Amplification of octapeptide repeats including a part of the PrP gene was achieved by PCR amplification using primer pairs F: 5'-ACG TGG GCC TCT GCA AGA AGC GAC-3' and R: 5'-GCA CTT CCC AGC ATG TAG CCA CCA-3' (Walawski and Czarnik 2003) with an initial denaturation step at 94 °C for 5 min followed by 35 cycles of one-minute incubations at 95, 65 and 72 °C with final extension 72 °C for 10 min. PCR products were analyzed on 1.8% ethidium-bromide-stained agarose gel.

Results and Discussion

We detected 5 octapeptide repeats (349 bp) or 6 octapeptide repeats (373 bp) (Plate I, Fig. 1) in the studied cattle population. The frequency of 6/6-homozygote animals was higher in the Slovak spotted cattle (97.7%) than in the Slovak Pinzgau (95.4%). Consequently, the frequency of 6/5-heterozygous cattle was higher in the Slovak Pinzgau (4.6%) than in the Slovak spotted cattle (2.3%). Animals homozygous for 5 octapeptide repeats were not recorded in this group (Table 1). Similar distribution of octapeptide repeats was observed previously, where in the cohort of the Holstein-Friesian or Simmental breeds 6 octapeptide repeats were predominant, showing either 6/6 homozygotes or 6/5 heterozygotes, while homozygotes 5/5 were not observed (Brown et al. 1993; McKenzie et al. 1992; Neibergs et al. 1992; Premzl et al. 2000; Leone et al. 2002). On the other hand, Walawski et al. (2003) and Walawski and Czarnik (2003) recorded a relatively high frequency of 6/5 heterozygous animals as well as some 5/5 homozygous animals in the Polish Black and White cattle, which is improved by Holstein-Friesian crossing. These results indicate possibility of relation between octapeptide polymorphism and breed.

Table 1. Octapeptide allele distribution in Slovak spotted cattle and Slovak Pinzgau breeds

	n	6/6	6/5	5/5
Slovak spotted cattle	89	87 (97.7%)	2 (2.3%)	0
Slovak Pinzgau	195	186 (95.4%)	9 (4.6%)	0
Total	284	273 (96.1%)	11 (3.9%)	0 (0%)

Analýza polymorfizmu oktapeptidových repetícií u slovenských autochtónnych plemien hovädzieho dobytká

Oktapeptidové repetície sú 24 bp dlhé opakujúce sa segmenty v PrP géne, ktoré sa môžu u rôznych druhov opakovane vyskytovať v rôznom počte repetícií. Cieľom tejto práce bolo stanoviť výskyt tohto polymorfizmu v PrP géne dvoch slovenských autochtónnych plemien - Slovenský strakatý ($n = 89$) a Pinzgauský dobytok ($n = 195$). Vo vyšetrovanej skupine zvierat boli zaznamenané dve variácie v počte oktapeptidových repetícií pozostávajúce z prítomnosti 5 alebo 6 repetícií. Zistili sme vysokú prevalenciu zvierat homozygotných pre 6/6 oktapeptidových repetícií (96.1%) a nízku prevalenciu 6/5 heterozygotných zvierat (3.9%). V sledovanom súbore zvierat neboli nájdené žiadne zvieratá homozygotné pre 5/5 oktapeptidových repetícií. Toto štúdium zahrnilo iba malú skupinu zvierat, avšak

dosiahnuté výsledky poukazujú na to, že šesť oktapeptidových repetícií má dominantné zastúpenie u týchto slovenských plemien dobytká.

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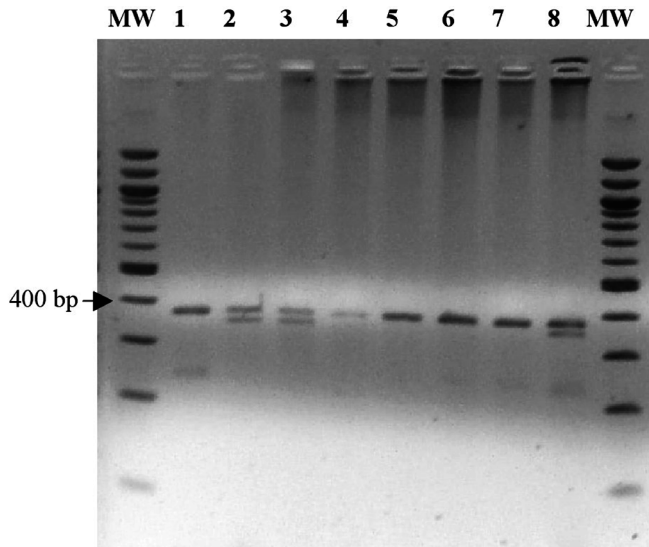


Fig. 1. Ethidium bromide stained PrP fragments amplified from a 6/6 homozygous cattle (lanes 1, 4, 5, 6, 7) and from 6/5 heterozygous cattle (lanes 2, 3, 8)
Standard of molecular weight - 100 bp DNA ladders