Short communication

Prion octapeptide-repeat polymorphism in Polish Black-and-White cattle

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Abstract. The study was carried out in a Polish Black-and-White cattle population, represented by 167 AI sires, 200 young tested bulls, 190 bull-dams, and 606 randomly chosen cows from commercial herds. The fragment of the bovine prion protein gene (PRNP) coding the octapeptide-repeat sequence, was identified by PCR analysis. Two different gene variants of 349 bp and 373 bp in size, produced three genotypes: PRNP 6/6, PRNP 6/5 and PRNP 5/5, respectively. Allele frequency in all examined populations, on average 0.894 for PRNP 6 and 0.106 for PRNP 5, shows a significant difference between the group of cows from commercial herds, characterised by high frequency of PRNP 5 (q = 0.137) in comparison to AI sires (q = 0.077), young tested bulls (q = 0.052) and bull-dams (q = 0.084). Moreover, both analysed female groups of bull-dams and cows from commercial herds are distinguished by the presence of PRNP 5/5 homozygous animals, which were not recorded in the AI sires and young tested bulls, and had never been recognised in earlier examined Holstein-Friesian populations. Analysis of the genetic equilibrium indicates a very high conformity between observed and expected number of animals in the separate PRNP genotype groups. However, some tendency of difference is observed in highly selected cows, qualified as bull-dams on the basis of very high level of milk performance traits.

Key words: Black-and-White cattle, octapeptide-repeat polymorphism, prion protein.

Conformational changes in the bovine prion protein gene (PRNP) are recognised as the background of Bovine Spongiform Encephalopathy (BSE). A casual origin of the disease is probably due to the interaction effect of prion protein, protein X (probable GP69) and nutritional factors. Nevertheless, it is also noteworthy that
the specific spherical deformations of the molecule are commonly induced by mutation of genes involved in synthesis of the protein.

The bovine PRNP gene was initially localised in the syntenic group U11 (RYAN, WOMACK 1993), which was then mapped to the BTA 13 chromosome (SCHLÄPFER et al. 1998). Three exons of the common size 795 bp of DNA (YOSHIMOTO et al. 1992), subsequently 4 244 bp of cDNA (HORIUCHI et al. 1998), and afterwards 78 056 bp of complete genomic DNA sequence (HILLS et al. 2001) were established. So far, in the coding region of the bovine PRNP gene, two polymorphisms have been found: (1) varying number of octapeptide repeats, resulting from deletion/addition of a DNA fragment located in the ORF region; and (2) transition C → T within the 3’ flanking region of exon 3 (GOLDMANN et al. 1991). Additionally, about 10 mutations in the non-coding region were detected, including a deletion of 12 nucleotides (HERNÁNDEZ-SÁNCHEZ et al. 2002), which were not examined for polymorphism description to date.

Published results of genetic investigations are concerned mainly with the DNA polymorphism associated with the variable number of octapeptide repeats, reflected in the occurrence of three alleles: with five (PRNP 5), six (PRNP 6) and seven (PRNP 7) repeats of the Pro-His/Gln-Gly (Gly)-Gly-Gly-Try-Gly-Gln amino-acid sequence. In the examined cattle breeds, the PRNP 6 allele and PRNP 6/6 homozygous animals were represented most often (BROWN et al. 1993, HUNTER et al. 1994, MCKENZIE et al. 1992, NEIBERGS et al. 1992, PREMZL et al. 2000, LEONE et al. 2002), while PRNP 7 was recognised only in two related breeds of Swiss Brown (SCHLÄPFER et al. 1999) and Bruna Alpina (LEONE et al. 2002). Hitherto reports were subordinate the differences between BSE-affected and BSE-free animals. The specificity of genetic structure of cattle breeds seems to disappear. The aim of our study was to characterise PRNP-octapeptide-repeat polymorphism in the population of Polish Black-and-White cattle.

The investigation involved representative groups of the Polish Black-and-White cattle population, namely 167 AI (artificial insemination) sires, 200 young tested bulls, 190 bull-dams, and 606 randomly selected cows from large commercial herds located in all parts of Poland. The polymorphism of the PRNP gene was determined by application of the PCR procedure according to GROBET et al. (cit. PREMZL et al. 2000). The sources of genome DNA were spermatozoa or leukocytes isolated from blood samples. The PRNP gene fragment, coding the causal region with variable number of octapeptide repeats, was amplified. The following PCR-mixture was used: 100-200 ng DNA, Taq-Buffer 10× (SIGMA), 0.25 mM MgCl₂ solution, 0.2 mM dNTPs (PHARMACIA), 0.1 µM PRNP 1 and PRNP 2 primers, 1U Taq-Polymerase (SIGMA), H₂O ad 25 µl. PCR was performed in 35 cycles (1 min. at 94°C, 1 min. at 65°C, 1 min. at 72°C). PCR products were analysed by electrophoresis in 1.5% Amplisize Agarose (BIO-RAD) ethidium-bromide-stained gels. The following primer pair was used:

PRNP 1 - 5’ACG TGG GCC TCT GCA AGA AGC GAC 3’, and
PRNP 2 – 5’ GCA CT T CCC AGC ATG TAG CCA CCA 3’.
Polymorphism was visualised by Flour STM Multimager System (BIO-RAD). Genotype and allele frequencies were calculated, and then the Hardy-Weinberg equilibrium was verified by application of \( \chi^2 \) test.

In the examined cattle population, two gene variants were detected: 349 bp in the case of five octapeptide repeats and 373 bp in the case of six octapeptide repeats. Three genotypes of PRNP 6/6, PRNP 6/5 and PRNP 5/5 were recognised (Figure 1).

Polish Black-and-White cattle are genetically improved by crossing with Holstein-Friesian cattle. We found that the most selected groups of AI sires, as well as young tested bulls and bull-dams, are characterised by a high genetic similarity to Holstein-Friesian cattle, while the population of cows from commercial herds is distinguished by some remains of native genes. Earlier reports suggested that Holstein-Friesian cattle were characterised by an extremely high prevalence of the PRNP 6 allele and PRNP 6/6 homozygous animals, and the PRNP 5/5 genotype has never been observed in published research (BROWN et al. 1993, MCKENZIE et al. 1992, NEIBERGS et al. 1992, PREMZL et al. 2000, LEONE et al. 2002). By contrast, the Polish Black-and-White cattle population is generally distinguished by a relatively high frequency of PRNP 6/5 genotype and occurrence of PRNP 5/5 homozygous animals (Table 1).

![Figure 1. PRNP octapeptide-repeat polymorphism identified by PCR](image)
Results of our investigations show a great variability in the examined population. Allele frequencies for the whole examined population (0.894 for PRNP 6 and 0.106 for PRNP 5) were very different from those for the group of cows from commercial herds, characterised by a higher frequency of PRNP 5 (q = 0.137), as compared to AI sires (q = 0.077), young bulls (q = 0.052) and bull-dams (q = 0.084). Moreover, both analysed female groups of bull-dams and cows from commercial herds (total number: 796 animals), are distinguished by the presence of PRNP 5/5 homozygous animals, which did not exist in the AI sires and young tested bulls (total number: 367 animals).

Results of the genetic equilibrium analysis indicate a very high conformity between the observed and expected number of PRNP genotypes in the whole examined Black-and-White cattle population as well as in the groups of AI sires, young tested bulls and cows from commercial herds. Some tendency of difference (p < 0.10) was observed only in the group of cows selected as bull-dams.

Hitherto, no association between PRNP octapeptide-repeat polymorphism and BSE was found (HUNTER et al. 1994, NEIBERGS et al. 1992). However, no cases of PRNP 5/5 homozygous animals were reported in the BSE-affected cattle group. The very low frequency of PRNP 5/5 individuals does not enable statistical verification of the relationship between PRNP gene polymorphism and BSE susceptibility. Moreover, this hypothesis cannot be confirmed by the results of genome-wide scanning, which revealed BSE-associated markers in BTA 5, but not in BTA 13 (HERNÁNDEZ-SÁNCHEZ et al. 2002).

Our further research will aim at explanation of effects of PRNP gene polymorphism, not only on BSE susceptibility, but also on other disturbances. Currently conducted studies are concerned with the segregation rate of PRNP 6 and PRNP 5 in the progeny groups related to the different parental mating, and preliminary at-

### Table 1. Distribution of PRNP octapeptide-repeat polymorphism in Polish Black-and-White cattle

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>No. of animals</th>
<th>Genotype frequencies</th>
<th>Allele frequencies</th>
<th>(\chi^2) statistics (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PRNP 6/6</td>
<td>PRNP 6/5</td>
<td>PRNP 5/5</td>
</tr>
<tr>
<td>AI sires</td>
<td>n = 167</td>
<td>0.838</td>
<td>0.162</td>
<td>0.000</td>
</tr>
<tr>
<td>Young tested bulls</td>
<td>n = 200</td>
<td>0.894</td>
<td>0.105</td>
<td>0.000</td>
</tr>
<tr>
<td>Bull-dams</td>
<td>n = 190</td>
<td>0.853</td>
<td>0.126</td>
<td>0.021</td>
</tr>
<tr>
<td>Cows from commercial herds</td>
<td>n = 606</td>
<td>0.741</td>
<td>0.244</td>
<td>0.015</td>
</tr>
<tr>
<td>Total</td>
<td>n = 1163</td>
<td>0.808</td>
<td>0.181</td>
<td>0.011</td>
</tr>
</tbody>
</table>

\(^a\) difference statistically significant at p ≤ 0.10
\(^a\) for Hardy-Weinberg equilibrium
tempts to establish the dependence between PRNP gene polymorphism and variability of important biological and productive traits.

REFERENCES