ANALYSIS OF INDEL POLYMORPHISM OF THE PRNP GENE IN WATER BUFFALO, BUBALUS BUBALIS

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Abstract. The aim of this study was to examine insertion-deletion polymorphisms (one being 12bp-long in intron 1 and second being 23bp-long in the promoter) in water buffalo. The blood samples were collected from two water buffalo herds (40 individuals). DNA was isolated using a commercial Master Pure DNA Purification Kit. After conducting 2 PCRs, and then electrophoreses in 4% agarose gel, it was found that there was no polymorphism in either PRNP 12 ins/del the PRNP 23 ins/del in the analyzed group of animals. All individuals were characterized by the ins/ins genotype for both polymorphisms. Because of the fact that four different genotypes were found in the Anatolian breed and only one genotype was identified in the breed examined in the present study, more breeds of this species should be included in further research.

Key words: PRNP gene, DNA polymorphism, prion disease, water buffalo

INTRODUCTION

Spongiform encephalopathies are fatal diseases that attack the central nervous system of mammals. The greatest influence on their occurrence is exerted by environmental factors, although some individuals will never develop the disease since they exhibit genetic resistance.

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The main role in the pathogenesis of prion diseases is played by the endo-
genus glycosyl prion protein, containing no nucleic acids, but having the ability to multiply and infect. There are two forms of the prions – one not causing disease (PrPC) and the invasive form (PrPSc) [Momcilovic and Rasooly 2000, Brunelle et al. 2007].

These diseases include, among others, bovine spongiform encephalopathy (BSE), which is mainly characterized by irreversible changes in the brain, where the tissue transforms into a spongy structure. The first symptoms occur 4–5 years after infection, and death occurs within the next few months. The prion protein is encoded by the PRNP gene. In humans [Windle et al. 1996], cattle [Juling et al. 2006], mice [Carlson et al. 1988] and sheep [Hunter et al. 1997] susceptibility to prion diseases, and the time in which they develop, depend on the variant of the gene. The PRNP gene is normally expressed in the nervous tissue and tissues of other organs. It is always present in one copy in an organism [Liberksi and Bratosiewicz 1996]. In cattle, it is located in the long arm of chromosome 13 and consists of 3 exons, of which only one (third) is expressed [Yoshimoto et al. 1992, Horiuchi et al. 1998].

Of 60 known mutations in the bovine PRNP gene, two are located in the regulatory region and play important roles. These are insertion-deletion polymorphisms, one 12bp-long in intron 1 [Juling et al. 2006] and second one 23bp-long in the promoter region [Sander et al. 2004, Haase et al. 2007], which determine the occurrence of two homozygous genotypes (ins/ins) and (del/del) and a heterozygous genotype (ins/del). These polymorphisms result in changes in the gene expression. Heterozygotes and homozygotes with deletion in both the first and the second polymorphism are highly susceptible to BSE, although the magnitude of this relationship depends on the breed [Juling et al. 2006, Strychalski et al. 2012, Zhao et al. 2012].

The aim of this study was to examine insertion-deletion polymorphisms (one being 12bp-long in intron 1 and second being 23bp-long in the promoter) in water buffalo.

MATERIAL AND METHODS

The research material comprised the water buffalo blood from two herds. The first herd was kept in Poland, while the second one in Germany. For the first herd, the blood samples were collected from the external jugular vein into tubes with anticoagulant from 29 females (half of the herd) in October 2008. For the German herd (11 individuals), the material was collected from the tail vein in August 2010.

DNA was isolated using a commercial MasterPure DNA Purification Kit. This was followed by the two PCRs with the primers suggested by Juling et al. [2006] –
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F 5’-CCTGTTGAGCGTGCTCGT-3’ and R 5’-ACCTGCGGCTCCTCTACC-3’ for ins/del 23bp (191bp/168bp) as well as F 5’-GGAAGTCACGTGAAGGCACT-3’ and R 5’-CAAAGAGTTGGACAGGCACA-3’ for the ins/del 12bp (215bp/203 bp). Thirty-two cycles were carried out under the following thermal conditions: denaturation at 94°C for 45 s (pre-denaturation for 5 min), primers annealing at 58°C for 45 s, elongation at 72°C for 45 s (in the last cycle for 7 min). The obtained products were separated by electrophoresis on a 4% agarose gel with ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

In the analyzed group of animals, there was no polymorphism in either PRNP 12 ins/del or the PRNP 23 ins/del. All individuals were characterized by the ins/ins genotype for both polymorphisms. Oztabak et al. [2009] examined 106 individuals of river buffalo of the Anatolian breed from various regions of Turkey. They found

Table 1. Comparison of the allele frequencies of the PRNP ins/del polymorphisms in different cattle breeds

Tabela 1. Porównanie częstości alleli z PRNP ins/del polimorfizmów u różnych ras bydła

<table>
<thead>
<tr>
<th>Animal Rasa</th>
<th>Allele frequencies of 12 bp and 23 indel polymorphisms</th>
<th>Reference Pismańewtvo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korean Holstein</td>
<td>0.39 0.61 0.30 0.70</td>
<td>Jeong et al. 2006</td>
</tr>
<tr>
<td>Korean Hanwoo</td>
<td>0.44 0.56 0.40 0.60</td>
<td></td>
</tr>
<tr>
<td>German Holstein</td>
<td>0.57 0.53 0.48 0.62</td>
<td>Juling et al. 2006</td>
</tr>
<tr>
<td>German Brown</td>
<td>0.86 0.14 0.65 0.35</td>
<td></td>
</tr>
<tr>
<td>Fleckvieh</td>
<td>0.38 0.62 0.32 0.68</td>
<td></td>
</tr>
<tr>
<td>USA beef cattle</td>
<td>0.37 0.63 0.26 0.74</td>
<td>Clawson et al. 2006</td>
</tr>
<tr>
<td>USA dairy cattle</td>
<td>0.63 0.47 0.38 0.62</td>
<td></td>
</tr>
<tr>
<td>Polish Holstein</td>
<td>0.46 0.54 0.37 0.63</td>
<td>Czarnik et al. 2007</td>
</tr>
<tr>
<td>Aberdeen Angus</td>
<td>0.44 0.56 0.27 0.73</td>
<td>Kerber et al. 2008</td>
</tr>
<tr>
<td>Charolais</td>
<td>0.42 0.58 0.32 0.68</td>
<td></td>
</tr>
<tr>
<td>Franqueiro</td>
<td>0.67 0.33 0.36 0.64</td>
<td></td>
</tr>
<tr>
<td>Sout Anatolian Red</td>
<td>0.69 0.31 0.36 0.64</td>
<td>Ün et al. 2008</td>
</tr>
<tr>
<td>East Anatolian Red</td>
<td>0.72 0.28 0.40 0.60</td>
<td></td>
</tr>
<tr>
<td>Anatolian Grey</td>
<td>0.80 0.20 0.62 0.38</td>
<td></td>
</tr>
<tr>
<td>Japanese Black</td>
<td>0.42 0.58 0.23 0.77</td>
<td>Msalya et al. 2011</td>
</tr>
<tr>
<td>Japanese Brown</td>
<td>0.47 0.53 0.44 0.77</td>
<td></td>
</tr>
<tr>
<td>Water Buffalo</td>
<td>1.00 – 1.00 –</td>
<td>Our research Badania własne</td>
</tr>
</tbody>
</table>
the presence of four different genotypes (ins12/del23, del12/del23, ins12/ins23, del12/ins23), of which the ins12/ins23 genotype had the highest frequency (0.86). Other genotypes were characterized by much lower frequencies: 0.08, 0.05 and 0.1 for ins12/del23, del12/del23 and ins12/del23, respectively. These results are similar to those obtained in the present study, in which all the individuals were homozygotes, whereas in cattle, disadvantageous alleles predominate, although their frequency depends on the breed. Most of the cattle breeds studied so far are presented in Table 1.

Sander et al. [2004, 2005] demonstrated that the ins/del23 polymorphism is strongly associated with susceptibility/resistance to BSE. However, in later studies Juling et al. [2006] and Kashkevich et al. [2007] showed the same effect of the ins/del12 polymorphism. The results obtained by the above-mentioned authors can also be applied to the water buffalo, because these animals belong to the same family as cattle.

Due to the fact that four different genotypes were found in the Anatolian breed and only one genotype was identified in the breed examined in the present study, more breeds of this species should be included in further research.

REFERENCES


Analysis of indel polymorphism of the PRNP gene


ANALIZA POLIMORFIZMU TYPU INDEL GENU PRNP U BAWOŁÓW, BUBALUS BUBALIS

Streszczenie. Celem niniejszego badania była analiza polimorfizmów typu insercja-delecja (jednego o długości 12 pz w intronie I, drugiego o długości 23 pz w promotorze genu). Próbki krwi zostały pobrane od bawółów z dwóch stad (w sumie od 40 osobników). DNA zostało wyizolowane przy użyciu zestawu Master Pure DNA Purification Kit. Po wykonaniu dwóch reakcji PCR oraz przeprowadzeniu elektroforezy na żelu agarozowym o stężeniu 4%, stwierdzony został brak polimorfizmu zarówno we fragmencie PRNP 12 ins/del, jak i we fragmencie PRNP 23 ins/del u analizowanych osobników. U wszystkich osobników stwierdzono genotypy ins/del dla obydwu polimorfizmów. Ponieważ u rasy Anatolijskiej zidentyfikowano cztery rodzaje genotypów, a w obecnie badanej rasie jedynie jeden rodzaj genotypu, konieczne jest przeanalizowanie obecności polimorfizmu także u innych ras.

Słowa kluczowe: gen PRNP, polimorfizm DNA, choroba prionowa, bawół

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