

Polymorphism of Prion Protein Gene (PRNP) in Iranian Holstein and two local cattle populations (Golpayegani and Sistani) of Iran

Saber Mohammad Maghsoodi¹, Seyed Reza Miraei-Ashtiani^{1*}, Mohammad Hossein Banabazi², Hassan Mehrabani Yeganeh¹

¹Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, P.O. Box 4111, I.R. Iran ²Department of Biotechnology, Animal Science Research Institute of Iran, Karaj, P.O. Box 1483, I.R. Iran

Abstract

Bovine spongiform encephalopathy (BSE) is a fatal infectious neurodegenerative disease in cattle, characterized by the accumulation of an abnormal, protease-resistant prion protein (PrP^{Sc}) in the brain. BSE is similar to scrapie in sheep and goats and Creutzfeldt-Jakob disease in humans. Susceptibility in cattle has been shown to be under the influence of two polymorphic locations, which are a 23 bp in/del polymorphism and a 12 bp indel within intron 1 of the prion protein gene (PRNP). DNA was extracted from blood samples of three Iranian cattle populations including Sistani (*Bos indicus*) (n=60), Golpayegani (*Bos indicus*) (n=62) and Iranian Holstein (*Bos taurus*) (n=50). In order to identify the putative polymorphisms of the PRNP gene of those breeds. Allele, genotype and haplotype frequencies of the polymorphisms were determined for the three populations. Susceptibility analysis was considered as per literature, upon which, it was suggested that the two *Bos indicus* native populations are more resistant to BSE than the Iranian Holstein (*Bos taurus*), due to higher gene frequency for insertion allele of the intron 1 of the PRNP gene polymorphism.

Keywords: Bovine Spongiform Encephalopathy (BSE); PRNP gene; Iranian Holstein; Golpayegani cattle; Sistani cattle; Genetic susceptibility

INTRODUCTION

Bovine spongiform encephalopathy (BSE) is a fatal

nervous system disease of cattle, caused by the accumulation of abnormally folded protease-resistant prion proteins in the brain (Prusiner, 1998). BSE is similar to scrapie in sheep and goats and Creutzfeldt-Jakob disease in humans (Sander *et al.*, 2004). The prion gene (PRNP) plays a central role in the transmissible spongiform encephalopathies. Therapeutic treatment for BSE in cattle is quite unlikely. However, genetic selection could be used as a potential means for restraining BSE in the cattle population (Ün *et al.*, 2008). In a wide variety of mammalian species, resistance to prion diseases is affected by polymorphisms of PRNP gene. For example, sheep with valine at codon 136 and glutamine at codon 171 of the PRNP coding region are susceptible to scrapie, while sheep with alanine and arginine at the same codons are resistant to this disease (Bossers *et al.*, 1996). The underlying polymorphisms are a 23 bp deletion within the promoter region that removes a binding site for the RP58 repressor protein (located 1.6 kbp upstream of exon 1), and a 12 bp deletion within intron 1 that removes a SP1 transcription factor binding site (Kashkevich *et al.*, 2007; Sander *et al.*, 2004). A transcription factor (sometimes called a sequence-specific DNA-binding factor) is a protein that binds to specific DNA sequences, thereby controlling the movement (or transcription) of genetic information from DNA to mRNA (Latchman, 1997). Individuals having the mentioned two deletions, would lack the binding site for RP58 and the SP1 transcription factor, therefore, they are unable to regulate their respective processes and consequently reported to be more susceptible to BSE (Xue *et al.*, 2008; Kashkevich *et al.*, 2007; Sander *et al.*, 2005).

*Correspondence to: Seyed Reza Miraei Ashtiani, Ph.D.
Tel: +98 261 2248082; Fax: +98 261 2246752
E-mail: ashtiani@ut.ac.ir

This study was designed to identify the in/del polymorphisms of the prion protein gene (PRNP) within the promoter (23 bp) and intron 1 (12 bp) sequences in three cattle populations of Iran, including Golpayegani, Sistani, and Iranian Holstein. Golpayegani and Sistani are indigenous cattle breeds of central Iranian Plateau, both of *Bos indicus* subspecies. While Golpayegani is a dairy breed native to central Iran, Sistani is a typical humped beef cattle of Sistan region in east of Iran, near Afghanistan border (also called Nimruz region, particularly on the Afghan side) (Nassiry *et al.*, 2008). Both breeds have shown great potential to adapt to harsh environmental conditions, such as performance under nutritionally poor ration, and relatively high resistance to endure tropical stresses and diseases (Sadeghi *et al.*, 2008).

MATERIALS AND METHODS

Whole blood samples were collected from Golpayegani and Sistani cattle in the corresponding local breeding stations of the two populations, and from Holstein cows in a number of herds which used semen from Iranian proven bulls. DNA was extracted from 62 Golpayegani, 60 Sistani and 50 Iranian Holstein blood samples, using modified salting out method (Javanrouh *et al.*, 2007).

The polymorphic regions of the PRNP gene promoter (23 bp in/del) and its intron 1 (12 bp in/del) was amplified using the following primers, described by Sander *et al.* (2004).

23-bp in/del: F 5'-GTGCCAGCCATGTAAGTG, and R 5'-TGGACAGGCACAATGGG. 12 bp in/del: F 5'-TTACCCTCCTGGTTAGGAG, and R 5'-CTAGATTCC-TACACACCAC. Polymerase Chain Reaction (PCR) was carried out in 25 μ l reaction volume containing 50-100 ng DNA, 1 unit Taq polymerase (GenNet Bio),

25 pmol of each primer (Metabion), 5 mM dNTPs (GenNet Bio), and 1.5 mM MgCl₂ in the buffer supplied by the manufacturer (GenNet Bio). The amplification was performed using an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 90 s, and extension at 72°C for 90 s, with a final extension at 72°C for 5 min. All PCR products were run on 8% polyacrylamide gel (Fig. 1), and stained using rapid silver staining method (Sanguinetti *et al.*, 1994).

Genotype, allele, and haplotype frequencies of PRNP variants were estimated by direct counting. Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, USA). Fisher's exact test was used to test for differences between allele frequencies, and the chi-square test (χ^2) was used to test for differences between genotype frequencies, as well as between haplotype frequencies.

RESULTS

Allele, genotype and haplotype frequencies for the 23-bp and 12 bp indel polymorphisms were as summarized in Table 1. Based on the p-values reported from Fisher's exact test and the Chi-square test shown in Table 1, the frequencies were different between the three breeds. Allele frequency for the 23 bp locus from Iranian Holstein samples was significantly different from that of Sistani cattle ($P=0.0032$), whereas for the 12 bp locus, Holstein samples were different from both Golpayegani and Sistani cattle ($P<0.0001$). Golpayegani cattle had the highest frequency for insertion allele (0.95) and for insertion/insertion genotype (0.91) of the 12 bp locus.

Genotype frequency for the 23 bp locus was also significantly different between Iranian Holstein and Sistani ($p=0.0092$). For the 12 bp locus, difference in

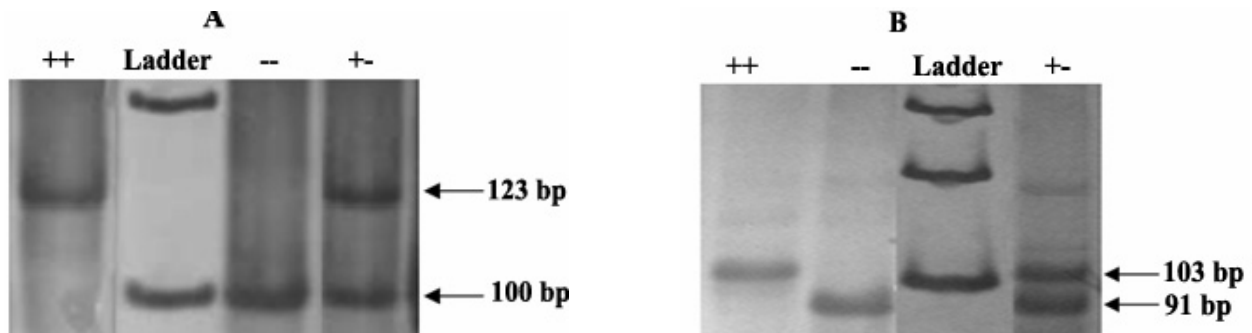


Figure 1. Genotyping of insertion (+) or deletion (-) polymorphisms by polyacrylamide gel electrophoresis. Three different genotypes (++, +-, --) of A: 23 bp and B: 12 bp insertion/ deletion loci.

Table 1. Allele, genotype and haplotype frequencies for the 23-bp and 12-bp insertion/deletion loci.

Allele Frequency								
population	locus	Frequencies			P-value			
		23-bp	n	ins	del	Iranian Holstein	Golpayegani	Sistani
Iranian	23-bp	100	0.37		0.63	-----	0.3148	0.0032
Holstein								
Golpayegani		124	0.30		0.70		-----	0.0644
Sistani	120	0.18		0.82			-----	
population	locus	Frequencies			P-value			
		12-bp	n	ins	del	Iranian Holstein	Golpayegani	Sistani
Iranian	12-bp	100	0.52		0.48	-----	<0.0001	<0.0001
Holstein								
Golpayegani		124	0.95		0.05		-----	0.0523
Sistani	120	0.87		0.13			-----	
Genotype Frequency								
population	locus	Frequencies				P-value		
		23-bp	n	ins/ins	ins/del	del/del	Iranian Holstein	Golpayegani
Iranian	23-bp	50	0.14	0.45	0.41	-----		0.0092
Holstein								
Golpayegani		62	0.08	0.44	0.48		0.5119	0.0450
Sistani	60	0.07	0.23	0.70		-----	-----	
population	locus	Frequencies				P-value		
		12-bp	n	ins/ins	ins/del	del/del	Iranian Holstein	Golpayegani
Iranian	12-bp	50	0.31	0.44	0.25	-----	<0.0001	<0.0001
Holstein								
Golpayegani		62	0.91	0.08	0.01		-----	0.1000
Sistani	60	0.78	0.19	0.03			-----	
Haplotype Frequency								
population	Haplotype	Frequencies				P-value		
		23-bp/12-bp	n	ins/ins	ins/del	del/del	Iranian Holstein	Golpayegani
Iranian	23-bp/12-bp	100	0.37	0.16	0.47	-----	<0.0001	<0.0001
Holstein								
Golpayegani		124	0.31	0.64	0.05		-----	0.0059
Sistani	120	0.17	0.70	0.013			-----	

n= number of total alleles, genotypes or haplotypes correspondingly, ins= insertion, del= deletion, bp= base pairs.

genotype frequency was significant between Iranian Holstein and both native cattle populations ($p < 0.0001$).

Concerning haplotype frequency, difference among three populations was also significant. Haplotype 23ins-12del was obtained for less than 1% of individuals in all three populations, and this small ratio was excluded from further analyses. The most frequent haplotypes in Iranian Holstein cattle were 23del-12del

and 23ins-12ins, whereas 23del-12ins was the most frequent in both Iranian native breeds.

DISCUSSION

Considering both 12 bp and 23 bp loci in Iranian Holstein, our results were very similar to those obtained for German Holstein (Kashkevich *et al.*, 2007

and Juling *et al.*, 2006) and for Polish Holstein (Czarnik *et al.*, 2007). Allele frequency in Golpayegani population for the 23 bp locus was similar to that of German Brown population (Juling *et al.*, 2006).

There are similarly significant differences reported between Holstein populations and local breeds in other studies. The frequency of insertion for 23 bp and 12 bp alleles in Japanese Black cattle (0.43 and 0.41, respectively) were both significantly ($P < 0.01$) higher than the corresponding alleles in Holstein (Nakamitsu *et al.*, 2005). For the 12 bp in/del polymorphism in intron 1, allele and genotype distributions did not show significant differences between Holstein and Hanwoo cattle of Korea (Jeong *et al.*, 2006).

Since we did not have access to infected animals, it was not possible to directly study association between the due polymorphisms and BSE incidence. Therefore, we have discussed about the susceptibility of the Iranian cattle populations, based on associations already reported in the literature. Susceptibility interpretations based on allele, genotype or haplotype frequency differences were considered efficiently (Kashkevich *et al.*, 2007; Juling *et al.*, 2006; Sander *et al.*, 2005; Sander *et al.*, 2004).

The study by Sander *et al.* (2004), considering the 23 bp in/del polymorphism, revealed that allele frequency and genotype distribution show statistically significant differences between healthy and BSE-affected animals. Sander *et al.* (2005) attained more evidence to support their inferences. However, Juling *et al.* (2006) and Kashkevich *et al.* (2007) concluded that the main effect on BSE susceptibility seems to be resulted from the 12 bp in/del polymorphism.

Based on allele and genotype frequencies in the 12 bp locus, both native populations (*Bos indicus*) have higher potential of resistance to BSE than the Iranian Holstein (*Bos taurus*) ($p < 0.0001$). However, based on the results of allele and genotype frequencies of the 23-bp locus, Sistani cattle would show significantly higher susceptibility to BSE than Golpayegani and Holstein ($p = 0.0032$). McCormack *et al.* (2002) and Inoue *et al.* (1997) suggested that polymorphisms in both regions would affect transcription of the PRNP gene. While insertion alleles in these loci decrease the transcription of the PRNP gene, deletion alleles affect that process inversely (Xue *et al.*, 2008; Kashkevich *et al.*, 2007). Based on these findings, Golpayegani cattle should be more resistant to BSE than Sistani and Iranian Holstein, and Sistani should be more resistant than the Iranian Holstein. Thus, altogether it could be suggested

that the native populations have significantly lower susceptibility compared to Iranian Holstein ($p < 0.0001$).

Brunelle *et al.* (2008) indicated that *Bos indicus* purebred cattle had a very low frequency of the 23 bp insertion and a high frequency of the 12 bp insertion, in contrast with *Bos Taurus*. Also the 23del-12ins haplotype was the most frequent haplotype in *Bos indicus*. The current study supports these results, concerning both *Bos indicus* populations, but allele frequency of the 23 bp locus in Golpayegani population (*Bos indicus*) was not significantly different from that of the Iranian Holstein (*Bos taurus*). However, haplotype frequency in Golpayegani was significantly different from that of the Iranian Holstein.

In conclusion, our data indicates that based on allele, genotype and haplotype frequencies and P-values of differences, the two local *Bos indicus* populations (Golpayegani and Sistani) seem to be more resistant to BSE than the *Bos taurus* Iranian Holstein.

Acknowledgements

Authors thank Animal Science Research Institute of Iran for providing the DNA samples and for technical assistance. We extend our appreciations to the University of Tehran for financial support to this research.

References

- Bossers A, Schreuder BE, Muileman, IH, Beltj B, Smits MA (1996). PrP genotype contributes to determining survival times of sheep with natural scrapie. *J Gen Virol.* 77: 2669-2673.
- Brunelle BW, Greenlee JJ, Seabury, CM, Brown CE, Nicholson, EM (2008). Frequencies of polymorphisms associated with BSE resistance differ significantly between *Bos taurus*, *Bos indicus* and composite cattle. *BMC Vet Res.* 4: 36.
- Czarnik U, Zabolewicz T, Strychalski J, Grzybowski G, Bogusz M, Walawski K (2007). Deletion/insertion polymorphism of the prion protein gene (PRNP) in Polish Holstein-Friesian cattle. *J Appl Genet.* 48: 69-71.
- Inoue S, Tanaka M, Horiuchi M, Ishiguro N, Shinagawa M (1997). Characterization of the bovine prion protein gene: the expression requires interaction between the promoter and intron. *J Vet Med Sci.* 59: 175-183.
- Javanrouh A, Banabazi MH, Esmailkhanian S, Amirinia C, Seyedabadi, HR, Emrani H (2006). Optimization of salting out method for DNA extraction from animal and poultry blood cells. The 57th Annual Meeting of the European Association for Animal Production Antalya, Turkey.
- Jeong BH, Sohn HJ, Lee JO, Kim NH, Kim JI, Lee SY, Cho IS, Jooy S, Carp RI, Kim YS (2005). Polymorphisms of the prion protein gene (PRNP) in Hanwoo (*Bos taurus coreanae*) and Holstein cattle Genes. *Genet Syst.* 80: 303-308.
- Juling K, Schwarzenbacher H, Williams JL, Fries R (2006). A

- major genetic component of BSE susceptibility. *BMC Biology*. 4:33.
- Kashkevich K, Humeny A, Ziegler U, Groschup MH, Nicken P, Leeb T, Fischer C, Becker CM, Schiebel K (2007). Functional relevance of DNA polymorphisms within the promoter region of the prion protein gene and their association to BSE infection. *FASEB*. 21: 1547-1555.
- Latchman DS (1997). Transcription factors: an overview. *Int J Biochem Cell Biol*. 29: 1305-1312.
- McCormack JE, Baybutt HN, Everington D, Will RG, Ironside JW, Manson JC (2002). PRNP contains both intronic and upstream regulatory regions that may influence susceptibility to Creutzfeldt-Jakob Disease. *Gene* 288: 139-146.
- Nakamitsu S, Miyazawa T, Horiuchi M, Onose S, Ohoba Y, Kitagawa H, Ishiguo N (2006). Sequence variation of bovine prion protein gene in Japanese cattle (Holstein and Japanese Black). *J Vet Med Sci*. 68: 27-33.
- Nassiry MR, Eftekhari Shahroudi F, Tahmoorespur M, Javadmanesh A (2008). The diversity of BoLA-DRB3 gene in Iranian native cattle. *Asian-Aust J Anim Sci*. 21: 465-470.
- Prusiner SB (1998). Prions. *Proc Natl Acad Sci USA*. 95: 13363-13383.
- Sadeghi B, Nassiry MR, Heydarpour M, Eftekhari Shahroudi F, Mosafer J, Shafagh Motlagh A (2008). Characterization of genetic polymorphism of the Bovine Lymphocyte antigen DRB32 locus in Sistani cattle of Iran (*Bos indicus*). *Biotechnology* 7: 333-337.
- Sander P, Hamann H, Pfeiffer I, Wemheuer W, Brenig B, Groschup MH, Distl O, Leeb T (2004). Analysis of sequence variability of the bovine prion protein gene (PRNP) in German cattle breeds. *Neurogenetics* 5: 19-25.
- Sander P, Hamann H, Drögemüller C, Kashkevich K, Schiebel K, Leeb T (2005). Bovine prion protein gene (PRNP) promoter polymorphisms modulate PRNP expression and may be responsible for differences in bovine spongiform encephalopathy susceptibility. *Biol Chem*. 280: 37408-37414.
- Sanguinetti CJ, Neto ED, Simpson AJG (1994). Rapid silver staining and recovery of PCR product separated on polyacrylamide gels. *Biotechniques* 17: 915-919.
- Ün C, Oztabak K, Özdemir N, Tesfaye D, Mengi A, Schellander K (2008). Detection of bovine spongiform encephalopathy-related prion protein gene promoter polymorphisms in local Turkish cattle. *Biochem Genet*. 46: 820-827.
- Xue G, Sakudo A, Kim C, Onodera T (2008). Coordinate regulation of bovine prion protein gene promoter activity by two Sp1 binding site polymorphisms. *Biochem Biophys Res Comm*. 372: 530-535.