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

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Abstract
Bovine spongiform encephalopathy (BSE) is a fatal infectious neurodegenerative disease in cattle, characterized by the accumulation of an abnormal, protease-resistant prion protein (PrPSc) in the brain. BSE is similar to scrapie in sheep and goats and Creuzfeldt-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 Creuzfeldt-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).

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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′-CTAGATTCCTACACACCAC. Polymerase Chain Reaction (PCR) was carried out in 25 µl 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 $MgCl_2$ in the buffer supplied by the manufacturer (GenNet Bio). The amplification was performed using an initial denaturation step at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 54ºC for 90 s, and extension at 72ºC for 90 s, with a final extension at 72ºC 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

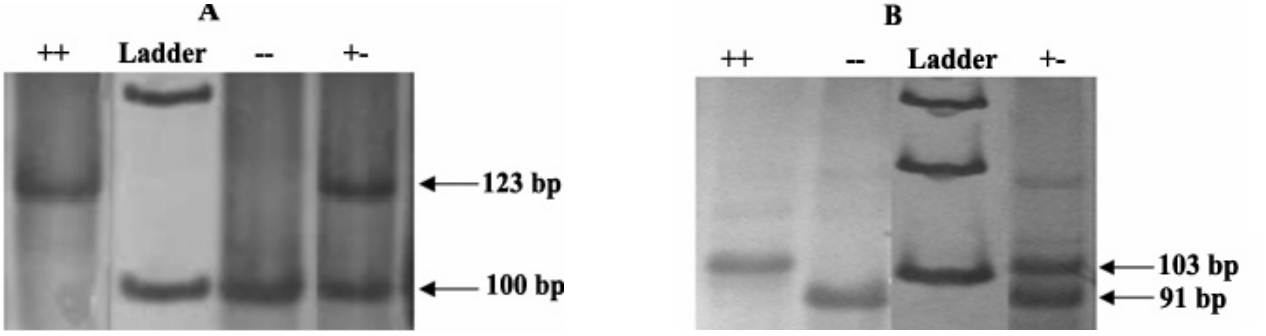

Figure 1. Genotyping of insertion (+) or deletion (–) polymorphisms by polyacrylamide gel electrophoresis. Three different genotypes (++, +-, --) of A: 23 bp and B: 12 bp insertion/ deletion loci.

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 Holstein		100	0.37	0.63	--------	0.3148	0.0032
Golpayegani		124	0.30	0.70		--------	0.0644
Sistani		120	0.18	0.82			--------
	12-bp	n	ins	del	Iranian Holstein	Golpayegani	Sistani
Iranian Holstein		100	0.52	0.48	--------	<0.0001	<0.0001
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	Sistani
Iranian Holstein		50	0.14	0.45	0.41	--------		0.0092
Golpayegani		62	0.08	0.44	0.48		0.5119	0.0450
Sistani		60	0.07	0.23	0.70		--------	--------
	12-bp	n	ins/ins	ins/del	del/del	Iranian Holstein	Golpayegani	Sistani
Iranian Holstein		50	0.31	0.44	0.25	--------	<0.0001	<0.0001
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	Sistani
Iranian Holstein		100	0.37	0.16	0.47	--------	<0.0001	<0.0001
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.

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