Polymorphisms of the prion protein Gene in Iranian Holstein and Najdi and Sarabi cattle breeds

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ABSTRACT: Polymorphisms of the 23-bp in/del in the promoter region and the 12-bp in/del in intron 1 of the prion protein gene, which are shown to be associated with resistance to bovine spongiform encephalopathy (BSE), were assessed in the Nova Scotia Agricultural College at Canada from March to September 2010. We studied polymorphism of the two loci among samples of two Iranian cattle breeds including Najdi, Sarabi and Iranian Holstein. Whole blood samples were collected randomly. Selected cows DNA were extracted by Diatom kit. Holstein breed had significantly different allele, genotypes and haplotype frequency distributions compared with the two native breeds for both polymorphic sites, except for the 23-bp in/del which was comparable with the Sarabi breed. The frequencies of the protective 23-bp insertion allele (23+) were 0.28, 0.15 and 0.33 in the Sarabi, Najdi and Holstein breeds, respectively. The corresponding values for the low susceptibility 12-bp insertion allele (12+) were 0.92, 0.89 and 0.48, respectively. The frequencies of this allele in the two native breeds are at the upper limit of most breeds studied so far. The allele and haplotype frequency distributions of the Najdi breed resembled that of the published data on other B. indicus cattle. It is not possible to predict the degree of susceptibility of the native breeds to BSE based on the frequencies of these two polymorphic sites because of uncertainties regarding the relative contribution of each site to the overall resistance to BSE and the presence of breed-specific background genes affecting this trait.

Key words: Polymorphisms, Bovine, Spongiform encephalopathy

INTRODUCTION

Bovine spongiform encephalopathy (BSE) is a serious animal health issue as well as a human food concern because it is transmittable to humans ¹. BSE is particularly important in countries such as Iran where the cattle brain often enters the human food chain. Protecting the national cattle population against BSE is not only essential for the country’s livestock industry, but also for the human health. The presence of BSE has not been investigated in Iran, and there is no information on the degree of susceptibility of the indigenous cattle breeds to BSE infection².

Recent reports on the significant associations between the 23-bp insertion/deletion (in/del) polymorphism in the promoter region and the 12-bp in/del within intron 1 of the bovine prion protein gene (PRNP) with BSE resistance in cattle breeds of Germany, Switzerland and Britain generated a considerable interest among scientists globally ³,⁴,⁵,⁶,⁷. Although differences between healthy and infected cattle for allele, haplotype and/or genotype frequencies were statistically significant in those studies, and animals carrying the insertion alleles at both sites (23+ and 12+) were more resistant to BSE, there is no agreement on the relative influence of each allele on resistance to BSE infection. The favorable alleles decrease the odds of the establishment of infection when an animal is exposed to the BSE agent, but do not confer complete protection as indicated by the presence of all genotypes in both healthy and infected cattle ³,⁵,⁶,⁸. Associations between these two polymorphisms and the incidence of BSE were not detected for the German and Swiss Brown breed ⁴,⁷ or the Holstein breed in Japan ⁹. Furthermore, the association between these polymorphisms and infection by non-classical BSE agents has not been established yet ¹⁰.

The picture that emerges from the published information on the allele frequency distributions of various breeds is, nevertheless, interesting. The B. indicus cattle have significantly higher frequencies of the 23- and the 12+ alleles as compared to B. taurus, resulting in the 23-/-12+ to be the major haplotype in B. indicus ¹¹.
Significant differences among cattle breeds for allele and haplotype frequencies may arise because of I-founder effect and random genetic drift, particularly if the population has gone through a bottleneck. Such phenomenon should cause parallel changes in many loci. II - natural selection for resistance to BSE, which requires that BSE has been epidemic in the region, and iii-linkage with other genes that influence production performance or adaptability to certain environmental conditions. Genotyping of cattle breeds across the globe with different breeding histories and production performances may shed some light on the relative importance of this gene on BSE resistance.

The objective of this study was to determine the allele, genotype and haplotype frequencies of two indigenous cattle breeds of Iran; Sarabi and Najdi. A sample of Holstein cattle was used for comparison. The information is useful in assessing the natural selection pressure imposed on this gene as well as the relative level of resistance of indigenous breeds to classical BSE.

**MATERIALS AND METHODS**

**Breeds**

Najdi is a Bos indicus breed of Iran whose origin is not clearly known. The majority of estimated 380,000 heads of the indigenous cattle of the Khuzestan province in south-western Iran comprised of the Najdi breed with the average daily mink yield of 6-8 kg during 125-day lactation period with 5.8% milk fat. This breed is adapted to the high temperatures of the region, which often reached 50°C in the summer. Sarabi is the most famous indigenous cattle breed, which is mainly distributed in north-western part of Iran (Azerbaijan Province). Daily milk production of this breed ranges between 6 and 14 liters during 250 days lactation period with 5.4% milk fat. Sarabi is also adapted to local conditions, and is particularly known to be resistant to ticks.

The Holstein breed was imported to Iran in late 1950, and has become the most popular exotic dairy breed of the country. The breed comprises approximately 10% of the 7.9 million cattle population of Iran. Artificial insemination with semen from North America and Europe is a common practice.

Source of animals: Blood samples were collected into 3 mL EDTA-treated vacutainers from 55 Holstein, 56 Sarabi and 55 Najdi cattle breeds. Samples of the Sarabi breed were collected from Azerbaijan Agricultural Research Centers in Ardabil (16 of 597 heads), Sarab (15 of 580 heads) and Moghan (25 of 650 heads). Samples of the Najdi breed were collected from Khuzestan Agricultural Research Centers in Ahvaz (26 of 675 head), Shooshtar (15 of 598 heads) and Hendijan (14 of 527 heads). Samples of the Holstein breed were collected from Research Stations in Yasouj (21 of 909 heads), Karaj (18 of 781 heads) and Kermanshah (16 of 526 heads), which are located in different provinces. Animals were randomly selected for sampling.

**Laboratory procedures**

The samples were analyzed in the laboratory of Nova Scotia Agricultural College at Canada from March to September 2010. Genomic DNA was extracted from whole blood using the salting-out procedure of Miller et al. Two regions of the bovine PRNP gene, namely the 12-bp indel in intron 1 and the 23-bp indel in the promoter were amplified by the polymerase chain reaction (PCR). The 100 or 123-bp fragment containing the 23-bp indel was amplified by the primers PRNP47784-F (5'-GGACAGGCACAATGGG) and PRNP47883-R (5'-TGACAGGCACAATGGG), and the 91 or 103-bp fragment containing the 12-bp indel was amplified by the primers PRNP49686-F (5'-TTACCTCTTGTTAGGAG) and PRNP49777-R (5'-CTAGATTCCCTACACCCAC) that were described by Sander et al.

PCR was carried out in a 25 μl reaction volume containing 50-100 ng DNA, 1 unit Taq polymerase (Gen Net Bio, Tehran), 20 pM of each primer, 5 mM dNTPs (Gen Net Bio) and 2.5 μl 10X PCR buffer supplied by the manufacturer and contained 1.5 mM MgCl2. The thermal cycler (Corbett Life Sciences, Australia) was programmed for 5 min initial denaturation at 94°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 90 s, with a final extension step at 72°C for 5 min. The PCR products were size differentiated on 8% polyacrylamide gels and fragments were visualized by silver nitrate.

Allele and genotype frequencies were determined by direct count. Haplotypes were subjectively inferred from genotype information using the fact that homozygous individuals carry a single haplotype, those heterozygous at a single site carry two haplotypes, and the haplotype 23+/12- is very rare in cattle.

Statistical analysis: Differences among breeds for allele frequencies were tested by the Fisher’s exact test, and genotype and haplotype frequency distributions were compared with Chi-square using the SAS statistical software (2005). One rare 23+/12- haplotype was detected in the Holstein breed and was not included in the analysis.
RESULTS

Genotype and allele frequencies of the 23-bp and 12-bp in/dels in Holstein and the native Sarabi and Najdi breeds are shown in Tables 1 and 2. Differences between the two native breeds were significant (P<0.05) or approached significance (P=0.053) for allele, genotype and haplotype frequency distributions. The frequencies of the protective 23+ (0.28) and 12+ alleles (0.92) in the Sarabi breed were higher than those in the Najdi breed (0.15 and 0.89, respectively). The Sarabi breed had a higher frequency of the 23+/12+ haplotype and a lower frequency of the 23/-12- haplotype compared with the Najdi breed (Table 2).

All comparisons involving Holstein and Najdi breeds were highly significant. Holstein had greater values compared with Najdi for the 23+ and 12- alleles and the 23+/12+ and 23/-12- haplotypes. Holstein and Sarabi had comparable allele and genotype frequencies for the 23-bp indel, but Sarabi had a greater frequency of the 12+ allele, a lower frequency of the 23/-12- haplotype and a greater frequency of the 23/-12+ haplotype than the Holstein breed.

Differences among the breeds for haplotype frequencies were highly significant (Table 3). While the 23/-12+ was the most frequent haplotype in the two native breeds (0.62 for Sarabi and 0.72 for Najdi), it had the lowest frequency in the Holstein breed. In contrast, the 23/-12- haplotype had the highest frequency in Holstein and the lowest frequency in the native breeds. Genotyping map is presented in figure as followed.

DISCUSSION

Significant differences were detected among the breeds for the frequencies of the two polymorphisms associated with differential susceptibility to BSE. Allele frequencies for the 23+ and 12+ alleles and the haplotypes for the Holstein breed were comparable with the published information on this breed.

The observation that the 12+ allele was present in a high frequency in the native Sarabi breed deserves attention. First, this breed may possess an important genetic merit for selection for resistance for BSE, particularly because this polymorphism is shown to be the major determinant of resistant against BSE infection, at least in some breeds. Second, the 12+ has a high frequency in several other unimproved breeds, such as Franqueiro, which is an unimproved old breed adapted to unfavorable environmental conditions of southern Brazil, Korean Hanwoo and three Anatolian cattle breed.

This phenomenon is too common in unimproved breeds to be attributed to a bottleneck or founder effect. It may be hypothesized that 12+ is the ancestral allele, and intense selection for production traits has resulted in the reduced frequency of this allele. Alternatively, natural selection under harsh environmental condition may favor this allele. Brunell and his coulages indicated the 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 23del-12ins was the most frequent haplotype in Bos indicus.

The current study supports these results especially concerning Najdi cattle as a Bos indicus population. However, allele frequencies of 23-bp locus in Sarabi breed was not significantly different from that of Iranian Holstein. There is certainly a need for a national BSE monitoring program and tight import regulations to protect the native breeds against the BSE agents.

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