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Comparative analysis of *PRNP* 12-bp and 23-bp indels in healthy Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian and Uruguayan Creole cattle[1]

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ABSTRACT.- Artigas R., Vázquez N., Nicolini P., Llambí S. & Armstrong E. 2023. **Comparative analysis of *PRNP* 12-bp and 23-bp indels in healthy Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian and Uruguayan Creole cattle.** *Pesquisa Veterinária Brasileira 43:e07209, 2023*. Unidad Académica de Genética y Mejora Animal, Departamento de Producción Animal y Salud de los Sistemas Productivos, Facultad de Veterinaria, Universidad de la República, Ruta 8 Km 18, Cx. Postal 13000, Villa García, Montevideo, Uruguay. E-mail: rodyartigas@gmail.com

Bovine spongiform encephalopathy (BSE) is a transmissible progressive neurodegenerative disease characterized by the accumulation of a pathological isoform (PrpSC) of the cellular prion protein (PrpC) in the brain of cattle. Two insertion/deletion polymorphisms in the *PRNP* gene (23bp in the promoter and 12bp in intron 1) have been associated with resistance or susceptibility to the disease. The aim of this study was to analyze the distribution of these polymorphisms in 214 healthy bovines belonging to four different breed groups (Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian and Uruguayan Creole cattle). DNA samples were amplified by end-point PCR. A high frequency of the alleles and haplotype associated with susceptibility to BSE (del12 and del23, and del12-del23, respectively) were found in the Aberdeen Angus, Aberdeen Angus x Hereford and Holstein Friesian animals. At the same time, the Uruguayan Creole cattle presented a higher frequency of the alleles and haplotype associated with resistance to BSE (ins12 and ins23, and ins12-ins23, respectively). These data could indicate a greater genetic resistance of the Uruguayan Creole cattle to BSE compared to other analyzed breeds, reinforcing its value as a zoogenetic resource.

INDEX TERMS: Prion, *PRNP* gene, Aberdeen Angus, Hereford, Holstein Friesian, Uruguayan Creole, cattle, indel polymorphism.

RESUMO.- [**Análise comparativa de *PRNP* 12bp e 23bp indels em bovinos Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian e Crioulo Uruguaio saudáveis.**] A encefalopatia espongiforme bovina (EEB) é uma doença neurodegenerativa progressiva transmissível dos bovinos, caracterizada pelo acúmulo no cérebro de uma isoforma patológica (PrpSC) da proteína priônica celular (PrpC). Dois polimorfismos de inserção/deleção no gene *PRNP* (23bp no promotor e 12bp no íntron 1) foram associados à resistência ou suscetibilidade à doença. O objetivo deste trabalho foi analisar a distribuição desses polimorfismos em 214 bovinos sadios, pertencentes a quatro diferentes grupos raciais (Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian e Crioulo Uruguaio). As amostras de DNA foram amplificadas por PCR de tempo final. Uma alta frequência dos alelos e haplótipos associados à suscetibilidade à BSE (del12 e del23 e del12-del23, respectivamente) foram encontrados nos animais Aberdeen Angus, Aberdeen Angus x Hereford e Holstein Friesian, enquanto o gado Crioulo Uruguaio apresentou maior frequência dos alelos e haplótipos associados à resistência à BSE (ins12 e ins23 e ins12-ins23, respectivamente). Esses dados podem indicar uma maior resistência genética do gado

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Crioulo Uruguaio à BSE em comparação com as outras raças analisadas, reforçando seu valor como recurso zoogenético.

TERMOS DE INDEXAÇÃO: príon, gene *PRNP*, Aberdeen Angus, Hereford, Holstein Friesian, Crioulo Uruguaio, bovino, polimorfismo indel.

INTRODUCTION

Bovine spongiform encephalopathy (BSE) is a progressive neurodegenerative disease of the cattle. It belongs to the transmissible spongiform encephalopathies (TSEs) family, with Scrapie in sheep and goats and Creutzfeldt-Jakob disease in humans, among others. It is caused by the accumulation in the central nervous system of a pathological isoform (PrP^{SC}) of the cellular prion protein (PrP^{C}) (Wilesmith et al. 1991).

PrP^{C} is encoded by the *PRNP* gene on the bovine chromosome BTA13 region q17 (Iannuzzi et al. 1998). In recent decades, two insertion/deletion polymorphisms (indel), one of 12bp in intron 1 and another of 23bp in the promoter, have been associated with increased susceptibility to the classic form of the disease (cBSE). It has been observed that the 12del-23del haplotype is more frequent in animals affected by cBSE than in healthy ones (Haase et al. 2007, Kashkevich et al. 2007, Murdoch et al. 2010, Gurgul et al. 2012).

In silico analyses of *PRNP* demonstrated that the 23bp insertion allele acts as a binding site for the RP58 repressor. In contrast, the 12bp insertion allele acts as a specific protein 1 (SP1) binding site (Sander et al. 2005). In this way, when both insertion alleles are present (haplotype 12in-23in), RP58 would bind to the promoter, exerting its repressive effect thanks to the interaction with SP1 (Lee et al. 2002). In contrast, the 12del-23del haplotype would induce failures in gene regulation, producing higher levels of expression (Sander et al. 2005).

The effect of the deletion alleles has been studied independently. Some studies indicate that the del23 allele would have a greater impact on disease susceptibility than the del12 allele (Sander et al. 2004, 2005, Jeong et al. 2006, Vernerova et al. 2014). In this sense, Geldermann et al. (2006) found no association of the del12 allele with disease susceptibility when studying 252 animals with cBSE and 376 healthy animals; however, Juling et al. (2006) and Kashkevich et al. (2007) reported a greater effect of the del12 allele. Nakamitsu et al. (2006) found no differences in the frequencies of the deletion alleles between a group of healthy animals and another group of animals affected by cBSE. However, the number of diseased individuals (n=6) was too small to support this observation. Despite all this, it has been proposed that selection against deletion alleles could be an alternative to reduce the susceptibility of herds to cBSE (Juling et al. 2006), in the event of potential epidemics of the disease.

The 12-bp and 23-bp indels polymorphism distribution has been established in several bovine breeds. However, few populations of the Aberdeen Angus breed have been studied internationally, and there are also few reports for this gene in South American Creole cattle. The Uruguayan Creole is a local breed, a descendant of the animals introduced to the country by Hernando Arias de Saavedra in 1611, of which only a pure nucleus of 600 animals remains in the "Parque Nacional de San Miguel" (Uruguay, department of Rocha; 33° 41' South latitude, 53°27' West longitude) (Armstrong et al. 2021). The Aberdeen Angus and Holstein Friesian breeds are among the most abundant commercial beef and dairy breeds, respectively, in the country. The aim of this study was to determine the population behavior of the 12-bp and 23-bp indels polymorphisms in Aberdeen Angus, Aberdeen Angus x Hereford, Uruguayan Creole and Uruguayan Holstein Friesian cattle.

MATERIALS AND METHODS

Animals. This study included 214 healthy bovines (Aberdeen Angus n=76, Aberdeen Angus x Hereford n=22, Holstein Friesian n=30 and Uruguayan Creole n=86). The Aberdeen Angus and Aberdeen Angus x Hereford animals belonged to establishments in the country's Eastern region, and samples of the cervical spinal cord were obtained in slaughterhouses. The samplings were carried out on different days, selecting animals from different herds and farms. Holstein Friesian and Uruguayan Creole animals' samples belonged to our laboratory's DNA bank. The samples of Holstein Friesian belonged to unrelated animals that came from different farms and regions of the country. In contrast, those of Uruguayan Creole belonged to the purebred nucleus of the "Parque Nacional de San Miguel".

DNA extraction and polymerase chain reaction. DNA samples of the Holstein Friesian and Uruguayan Creole breeds were previously extracted using the method reported by Green & Sambrook (2012) from whole peripheral blood collected from 2014 to 2018. DNA samples of Aberdeen Angus and Aberdeen Angus x Hereford animals were extracted from 30mg of the spinal cord, which were finely cut and treated with 5µL of proteinase K (10mg/mL) and 500µL of digestion buffer (50mM Tris base pH 8.0, 20mM EDTA and 2% SDS) and incubated overnight at 56°C. Subsequently, the samples were centrifuged (16000g/10 min). The supernatant was treated with 250µL of phenol and 250µL of chloroform/isoamyl alcohol (24:1). The samples were centrifuged (16000g/10 min). The supernatant was treated with 50µL of NaCl (2M) and two volumes of absolute ice-cold ethanol. The tubes were kept at -20°C for two hours. The DNA was recovered by centrifugation (21000g/10 min), washed with 70% ethanol and, once dry, re-suspended in TE buffer (10mM Tris HCl pH 8.0, 1mM EDTA).

The 12-bp and 23-bp indels polymorphisms were amplified in two independent PCR reactions using the primers previously designed by Galvão et al. (2012) and Sander et al. (2004), respectively. The reaction was carried out in a Rotor-Gene Q thermal cycler (Corbett Research) in a final volume of 20µL containing: 10µL of QuantiNova SYBR® Green PCR Kit (QIAGEN, Germany), 10pmol of each primer and 50ng of DNA. The amplification consisted of an initial denaturation at 95°C for 10 minutes, followed by 30 cycles of 95°C for 30 s, 56°C (23-bp indel) or 58°C (12-bp indel) for 30 s, 72°C for 30 s and final extension of 72°C for 5 min.

Genotyping and statistical analysis. The amplicons obtained were separated by 4% agarose gel electrophoresis, using GoodViewTM (SBS Genetech Co., Ltd., China) as a coloring agent and a 100bp molecular weight marker (SBS Genetech Co., Ltd., China). Visualization was performed with a BIOTOP-SC805 gel documentation system and BioSens Gel Imaging System V2.0 software (Shanghai Bio-Tech Co., Ltd., China).

For the calculation of allelic and genotypic frequencies, as well as for the Hardy and Weinberg equilibrium analysis (using Fisher's exact test), the free access software Genepop 4.7.5[5] was used (Raymond &

[5] Available at <http://genepop.curtin.edu.au/genepop_op1.html> Accessed on Aug. 10, 2022.

Rousset 1995). Haplotype and diplotype frequencies were calculated by direct counting. Since double heterozygous animals were found, the frequency of the 12ins-23ins/12del-23del diplotype was calculated from the frequency of the accurate haplotypes according to Galvão et al. (2012), using the conditional probability equation:

P(12ins-23ins/12del-23del) = [P(12ins-23ins) x P(12del-23del)] / [P(12ins-23ins) x P(12del-23del)] + [P(12in-23del) x P (12del-23in)]

The comparison between the populations was carried out using the Chi-square test, and the linkage disequilibrium (D, D' and r) was evaluated according to Lewontin (1988) using Microsoft EXCEL spreadsheets. For all comparisons, a *P*-value less than 0.05 was considered significant.

RESULTS

For both markers, amplification fragments were obtained in all the studied animals. For the 12-bp indel polymorphism, 412bp amplicons corresponding to the insertion allele were observed, while for the deletion allele, 400bp amplicons were found. For the 23-bp indel polymorphism, 123bp amplicons were observed corresponding to the insertion allele and 100bp for the deletion allele (Fig.1-2). The observed genotypic frequencies and allele frequencies are shown in Table 1. Deletion alleles were present at a high frequency in Aberdeen Angus, Aberdeen Angus x Hereford, and Holstein Friesian samples. This was not the case for the Uruguayan Creole, where the frequency of the deletion alleles was lower, and the frequency of the insertion alleles was higher than in the other breeds. All the populations analyzed for both markers were in Hardy-Weinberg equilibrium (P>0.05).

When comparing the distribution of the genotypes, the Aberdeen Angus breed and its cross with Hereford did not differ for the indel 12bp marker (P>0.05). However, they differed from the Holstein Friesian and Uruguayan Creole breeds (P<0.05). These last two breeds did not differ from each other for this marker (P>0.05). For the 23-bp indel polymorphism, no differences were observed between Aberdeen Angus, Aberdeen Angus x Hereford and Holstein Friesian. However, they were significantly different from the Uruguayan Creole (P<0.05) (Table 1).

When considering both markers, the four possible haplotypes were detected in Holstein Friesian and Uruguayan Creole, and three were detected in Aberdeen Angus and Aberdeen Angus x Hereford. Its distribution did not differ significantly between the Aberdeen Angus breed and the Aberdeen Angus x Hereford cross (P>0.05). However, it did differ with Holstein Friesian and Uruguayan Creole (P<0.05) (Table 2). The ins12-ins23 haplotype was more abundant in the Uruguayan Creole than in Holstein Friesian and was absent in Aberdeen Angus and Aberdeen Angus x Hereford. Concerning the del12-del23 haplotype, this one was the most frequent in the Aberdeen Angus, Aberdeen Angus x Hereford and Holstein Friesian animals studied (Table 2).

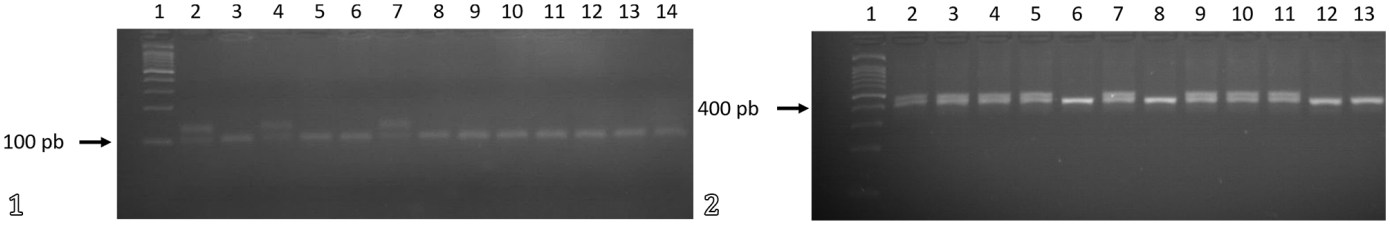

Fig.1-2. Agarose gel electrophoresis of the PCR products for the bovine PRNP gene. (**1**) Genotypes for 23-bp indel. Lane 1 = 100bp molecular weight marker. Lanes 2, 4 and 7 = heterozygous genotypes, fragments of 100 and 123bp. Lanes 3, 5, 6 and 8 to 14 = genotype homozygous for the 23bp deletion allele, 100bp fragments. (**2**) Genotypes for 12-bp indel. Lane 1 = 100bp molecular weight marker. Lanes 6, 8, 12 and 13 = genotypes homozygous for the 12bp deletion allele, 400bp fragments. Lanes 2 to 5, 6 and 9 to 11 = heterozygous genotypes, fragments of 400 and 412bp.

Table 1. Genotypic ratios and allelic frequencies for the indel12bp and indel23bp polymorphisms in Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian and Uruguayan Creole cattle

Breed/indel	% Genotypes			Alleles		*P*-value			
	+/+	+/-	-/-	+	-	AA	AA x H	HF	UC
12bp (400-412)									
AA (n=76)	0	39	61	0.20	0.80	--	0.295	<0.01*	<0.00001*
AA x H (n=22)	0	28	72	0.16	0.84	--	--	<0.01*	<0.00001*
HF(n=30)	17	50	33	0.42	0.58	--	--	--	0.17
UC (n=86)	29	52	19	0.55	0.45	--	--	--	--
23bp (100-123)									
AA (n=76)	5	33	62	0.22	0.78	--	0.195	0.12	<0.00001*
AA x H (n=22)	9	45	45	0.32	0.68	--	--	0.84	<0.01*
H F (n=30)	7	53	40	0.33	0.67	--	--	--	<0.001*
UC (n=86)	27	57	16	0.55	0.45	--	--	--	--

AA = Aberdeen Angus, AA x H = Aberdeen Angus x Hereford, HF = Holstein Friesian, UC = Uruguayan Creole; +/+ homozygous for the insertion, +/- heterozygous, -/- homozygous for the deletion, + insertion, - deletion; *P*-value of the comparison between races, *P<0.05; Fisher's exact test for Hardy-Weinberg equilibrium in all races: 12-bp indel, P>0.05; 23-bp indel, P>0.05.

The haplotypes calculated in this study for the four breeds show linkage disequilibrium (LD) between the markers, 88% for the Uruguayan Creole breed (D`=0.88, *P*<0.05) and 84% for the Holstein Friesian breed (D `=0.84, *P*<0.05). For the Aberdeen Angus breed and the Aberdeen Angus x Hereford cross, the value of D` was -1.0 (Table 3).

Of the ten possible diplotypes for the 12-bp and 23-bp indels polymorphisms, five were detected in Aberdeen Angus and Aberdeen Angus x Hereford animals. At the same time, eight were observed in Uruguayan Creole and Holstein Friesian (Table 4). The 12ins-23ins/12del-23ins diplotype was not observed in any of the animals studied. The most frequent diplotype in Aberdeen Angus and Aberdeen Angus x Hereford animals was 12del-23del/12del-23del, while in Creole and Holstein Friesian, it was 12ins-23ins/12del-23del. Particularly in the Uruguayan Creole breed, the 12ins-23ins/12ins-23ins diplotype was the second most abundant.

DISCUSSION

This study presents the genetic distribution of the 12-bp and 23-bp indels polymorphisms in commercial herds of cattle breeds and, for the first time, in Uruguayan Creole cattle. The amplification fragments obtained for both markers were consistent with those reported by Galvão et al. (2012) for the 12-bp indel polymorphism and Sander et al. (2004) for the 23-bp indel polymorphism.

It has been observed in different studies that the frequencies of the deletion allele in both polymorphisms vary between different bovine breeds. In the Aberdeen Angus and Aberdeen Angus x Hereford animals studied the frequency of the 12bp deletion allele (0.80 and 0.84, respectively) was higher than that reported in the literature for the Aberdeen Angus breed (range 0.46 to 0.63), although lower than the reported in Hereford (0.88). In Holstein Friesian, the frequency was intermediate (0.58) to that reported for the breed (range 0.42 to 0.74) (Table 5).

Table 2. Distribution of haplotypes for the 12-bp indel and 23-bp indel polymorphisms of the *PRNP* gene in Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian and Uruguayan Creole

Haplotype	No. of chromosomes			Freq.	*P*-value			
	n	n*	N		AA	AA x H	HF	UC
AA (n=76)					--	0.37	<0.0001**	<0.0001**
12ins-23ins	0	0	0	0				
12ins-23del	9	21	30	0.20				
12del-23ins	12	21	33	0.22				
12del-23del	89	0	89	0.58				
AA x H (n=22)					--	--	<0.0001**	<0.0001**
12ins-23ins	0	0	0	0				
12ins-23del	1	6	7	0.16				
12del-23ins	8	6	14	0.32				
12del-23del	23	0	23	0.52				
HF (n=30)					--	--	--	0.04**
12ins-23ins	6	12	18	0.3				
12ins-23del	6	1	7	0.12				
12del-23ins	1	1	2	0.03				
12del-23del	21	12	33	0.55				
CU (n=86)					--	--	--	--
12ins-23ins	47	43	90	0.52				
12ins-23del	4	1	5	0.03				
12del-23ins	4	1	5	0.03				
12del-23del	29	43	72	0.42				

n* = Estimated based on the number of double heterozygous animals, Freq. = frequency, AA = Aberdeen Angus, AA x H = Aberdeen Angus x Hereford, HF = Holstein Friesian, UC = Uruguayan Creole; *P*-value of the comparison between races, ** *P*<0.05.

Table 3. Values of D, D` and r2 for the linkage disequilibrium between the 12-bp indel and 23-bp indel polymorphisms for the four breeds studied

Breed	D	D`	r2	*P*-value
Aberdeen Angus	-0.044	-1	0.071	0.001*
Aberdeen Angus x Hereford	-0.051	-1	0.091	0.045*
Holstein Friesian	0.161	0.84	0.49	<0.00001*
Uruguayan Creole	0.217	0.88	0.774	<0.00001*

**P*<0.05.

Regarding the frequency of the 23bp deletion allele in the Aberdeen Angus (0.78) and Aberdeen Angus x Hereford (0.68) animals studied, it was similar to that reported in the literature for the Aberdeen Angus breed (range 0.73 to 0.81), although much lower than that observed in Hereford (0.98). Likewise, the frequency of this allele in the Holstein Friesian animals analyzed (0.67) was found within the range reported for the breed (range 0.55 to 0.79) (Table 5).

The population behavior of the 12-bp and 23-bp indels polymorphisms has been established in a few local South American bovine breeds. Although the frequency of the deletion alleles in the Uruguayan Creole (0.45) is higher than that reported in both markers for the Brazilian Caracú breed (del12=0.30, del23=0.28) and in the 12-bp indel for the Franqueiro breed (del12=0.33, del23=0.64), it is lower than those reported for different populations of Aberdeen Angus, Hereford and Holstein Friesian in different countries (Table 5). The Uruguayan Creole cattle is a local breed with a restricted number of animals in a single purebred population. Therefore, as stated by Kerber et al. (2008) in these types of breeds, it is not possible to establish whether this difference is due to a founder effect, a population bottleneck, or to the direct or indirect effects of natural selection over the centuries of adaptation to unfavorable environmental conditions. However, it has been observed that the frequency of deletion alleles tends to be lower in primitive, less-selected cattle breeds than in modern, highly-selected breeds (Gurgul et al. 2012).

Table 4. Distribution of diplotypes for the 12-bp indel and 23-bp indel polymorphisms of the *PRNP* gene in Aberdeen Angus, Aberdeen Angus x Hereford, Holstein Friesian and Uruguayan Creole

Genotype	Aberdeen Angus (n=76)	Aberdeen Angus x Hereford (n=22)	Uruguayan Creole (n=86)	Holstein Friesian (n=30)
12ins-23ins/12ins-23ins	0	0	0.27 (23)	0.06 (2)
12ins-23ins/12ins-23del	0	0	0.012 (1)	0.06 (2)
12ins-23ins/12del-23ins	0	0	0	0
12ins-23ins/12del-23del*	0*	0*	0.5 (43)*	0.4 (12)*
12del-23del/12del-23del	0.5 (38)	0.41 (9)	0.14 (12)	0.3 (9)
12del-23del/12del-23ins	0.05 (4)	0.1 (4)	0.05 (4)	0.03 (1)
12del-23del/12in-23del	0.12 (9)	0.04 (1)	0.012 (1)	0.06 (2)
12ins-23del/12ins-23del	0	0	0.012 (1)	0.03 (1)
12ins-23del/12del-23ins*	0.28 (21)*	0.27 (6)*	0.012 (1)*	0.03 (1)*
12del-23ins/12del-23ins	0.05 (4)	0.09 (2)	0	0

*Diplotypes were estimated based on double heterozygous animals.

Table 5. Comparison of the allelic frequencies for the 12-bp indel and 23-bp indel polymorphisms in the breeds studied with other populations of the same breeds reported in the consulted bibliography

Breed	Country*	N	12-bp indel		23-bp indel		Reference
			+	-	+	-	
Aberdeen Angus	CN	30	0.37	0.63	0.22	0.78	Zhu et al. (2011)
Aberdeen Angus	BR	99	0.44	0.56	0.27	0.73	Kerber et al. (2008)
Aberdeen Angus	BR	26	0.54	0.46	0.19	0.81	Sanches et al. (2016)
Aberdeen Angus	UY	76	0.2	0.8	0.22	0.78	Present study
Aberdeen Angus x Hereford	UY	22	0.16	0.84	0.32	0.68	Present study
Caracú	BR	40	0.7	0.3	0.72	0.28	Galvâo et al. (2012)
Uruguayan Creole	UY	86	0.55	0.45	0.55	0.45	Present study
Franqueiro	BR	73	0.33	0.67	0.36	0.64	Kerber et al. (2008)
Hereford	CN	30	0.12	0.88	0.02	0.98	Zhu et al. (2008)
Holstein Friesian	UK	276	0.37	0.63	0.29	0.71	Juling et al. (2006)
Holstein Friesian	JP	863	0.26	0.74	0.21	0.79	Nakamitsu et al. (2006)
Holstein Friesian	JP	65	0.58	0.42	0.45	0.55	Msalya et al. (2009)
Holstein Friesian	KR	52	0.38	0.62	0.3	0.7	Jeong et al. (2006)
Holstein Friesian	DE	66	0.47	0.53	0.42	0.58	Kashkevich et al. (2007)
Holstein Friesian	DE	313	0.47	0.53	0.38	0.62	Juling et al (2006)
Holstein Friesian	DE	80	0.34	0.66	0.33	0.67	Haase et al. (2007)
Holstein Friesian	US	690	0.47	0.53	0.43	0.57	Brunelle et al. (2008)
Holstein Friesian	PL	510	0.45	0.55	0.36	0.64	Strychalski et al. (2012)
Holstein Friesian	PL	651	0.48	0.52	0.38	0.62	Gurgul et al. (2012)
Holstein Friesian	UY	30	0.42	0.58	0.33	0.67	Present study

+ Insertion, - deletion; CN = China, BR = Brazil, UY = Uruguay, UK = United Kingdom, JP = Japan, KR = South Korea, DE = Germany, US = United States, PL = Poland.

In local breeds from other continents, a similar trend is observed concerning the distribution of the frequencies of the alleles associated with resistance to the disease. As an example, Ün et al. (2008) observed a high frequency of 12bp insertion alleles for South Anatolian Red, East Anatolian Red and Turkish Gray (in12=0.69, in12=0.72, in12=0.8, respectively). However, the del23 allele was the majority in South Anatolian Red and East Anatolian Red (del23=0.64, del23=0.6), being lower in Turkish Gray (del23=0.38). Teferedegn et al. (2022) recently observed a high frequency of the del23 allele in four local Ethiopian breeds (Afar, Arsi, Fogera, and Ethiopian Borona). This is consistent with previous reports in *Bos indicus* and *Bos taurus x Bos indicus* composite breeds (Brunelle et al. 2008). The frequency of the del12 allele was also high in the studied Ethiopian breeds, contrary to what was reported by Brunelle et al. (2008), placing them in a position of genetic vulnerability to the disease (Teferedegn et al. 2022).

Ins12-ins23 haplotype related to higher genetic resistance to cBSE (Sander et al. 2004, Juling et al. 2006, Haase et al. 2007, Kashkevich et al. 2007, Hreško et al. 2009, Murdoch et al. 2010, Gurgul et al. 2012) was more abundant in Uruguayan Creole Cattle than in the rest of the breeds studied in this research. A high frequency of the ins12-ins23 haplotype, above 50%, has already been observed in ancient breeds such as Brown Swiss, German Brown and Swiss Schwarzfleck (Juling et al. 2006, Haase et al. 2007).

The frequency of the del12-del23 haplotype, related to a higher susceptibility to cBSE, was higher in Aberdeen Angus, Aberdeen Angus x Hereford and Holstein Friesian. Although these breeds indeed have an unfavorable haplotypic distribution with genetic susceptibility to cBSE, this distribution is not different from that reported internationally for the Aberdeen Angus breed (Kerber et al. 2008, Zhu et al. 2011) and Holstein Friesian (Jeong et al. 2006, Juling et al. 2006, Czarnik et al. 2009, Haase et al. 2007, Brunelle et al. 2008, Gurgul et al. 2012). However, it was lower than that reported in Hereford (0.88) (Zhu et al. 2011).

Both polymorphisms proved to be linked in the analyzed populations (P<0.05). Similar results have been reported for these and other breeds by different authors (Sander et al. 2004, Clawson et al. 2006, Juling et al. 2006, Kerber et al. 2008, Zhu et al. 2011, Galvão et al. 2012, Yang et al. 2018), a fact probably related to the distance that separates both polymorphisms within the gene (<2Kbp, Brunelle et al. 2008). Particularly for the Aberdeen Angus breed and the Aberdeen Angus x Hereford cross, the D` value was -1.0, explained by the absence of one of the haplotypes (ins12-ins23). The magnitude of the LD seems to be greater in Uruguayan Creole and Holstein Friesian than in Aberdeen Angus and its cross with Hereford. This may respond to the founder effect in Uruguayan Creole, added to the fact that it is a closed population, with no gene flow from other populations, and to inbreeding in Holstein Friesian, favored by AI with few bulls. Increases in homozygosity limit the effectiveness of recombination and, therefore, maintains haplotypes (Morrell et al. 2005).

The distribution of diplotypes for the polymorphisms studied in this study is important to understand the genetic susceptibility of bovine populations to cBSE. The 12ins-23ins/12del-23ins diplotype was not observed in any of the animals studied, probably due to the absence of the 12in-23in haplotype in Aberdeen Angus and Aberdeen Angus x Hereford animals and the low frequency of the 12del-23in haplotype in Holstein Friesian and Uruguayan Creole animals. Previous studies have shown that in the presence of the infectious agent and favorable environmental conditions, animals with the del12-del23/del12-del23 diplotype have a 1.76 (Juling et al. 2006) to 2.68 (Haase et al. 2007) times higher risk of developing cBSE than animals with the diplotype ins12-ins23/ins12-ins23. However, in the German Brown breed, in which the ins12-in23 haplotype is very frequent, animals with the ins12-ins23/del12-del23 diplotype were the most affected by the disease, thus highlighting the greater susceptibility of the del12-del23 haplotype (Juling et al. 2006). Historically, cBSE has not been diagnosed in Uruguay, and the country has been considered to have a negligible risk of suffering from this disease (WOAH 2022). In this context, the knowledge of the genetic distribution of 12-bp and 23-bp indels polymorphisms in cattle breeds could be a valuable tool in cBSE prevention programs.

CONCLUSIONS

As in other ancient breeds, the Uruguayan Creole has a higher proportion of insertion alleles and haplotypes related to higher genetic resistance to classic bovine spongiform encephalopathy (cBSE), which increases its value as an animal genetic resource.

The commercial breeds studied show an unfavorable distribution of the 12-bp and 23-bp indels markers regarding susceptibility to the disease. However, this is similar to what has been reported internationally.

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