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PRNP haplotype and genotype frequencies in Brazilian sheep: Issues for conservation and breeding programs

P. Ianella [a], C.M. McManus [b], A.R. Caetano [c], S.R. Paiva [a,*]

[a] Prédio de Conservação Germoplasma, Laboratório de Genética Animal, Embrapa Recursos Genéticos e Biotecnologia, Brasilia-DF 70770-917, Brazil
[b] Departamento Produção Animal, Universidade Federal do Rio Grande do Sul, INCT-IGSPB, Porto Alegre, RS, Brazil
[c] Prédio de Biotecnologia, Embrapa Recursos Genéticos e Biotecnologia, Brasilia-DF 70770-917, Brazil

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ABSTRACT

Polymorphisms of PRNP gene have been strongly correlated to the susceptibility/resistance to scrapie in sheep. Variants at the coding positions 136, 154 and 171 have been the most frequently associated to susceptibility to classical scrapie. The aim of this study was to estimate PRNP haplotype and genotype frequencies in a sample of 1400 sheep from 13 different breeds that are representative of the main production regions in Brazil. A total of four different alleles (ARR, ARQ, AHQ and VRQ) and nine genotypes were observed at different frequencies among the investigated breeds. There were distinct patterns of allelic distribution between naturalized and commercial/specialized breeds and different geographic regions. These results will influence the development and management of breeding and conservation programs and will help to develop Brazilian efforts to avoid scrapie epidemics.

1. Introduction

Scrapie is a disease that affects sheep and goats. It belongs to a group of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs) or prion diseases. TSEs are associated with accumulation of an abnormal isoform of a host-encoded protein called *prion* (Prusiner, 1982, 1998). The *prion* protein is coded by the PRNP gene and specific polymorphisms of this gene are strongly associated with resistance and susceptibility to TSEs in many species. Over 40 polymorphisms have been reported so far for the ovine PRNP gene (Goldmann, 2008). However, polymorphisms at codons 136 (A/V – substitution of alanine by valine), 154 (R/H – arginine by histidine) and 171 (Q/R/H – glutamine by arginine or histidine) have been frequently correlated to host susceptibility to natural and experimentally induced classic scrapie (Benkel et al., 2007; Goldmann et al., 1990; Hunter and Cairns, 1998). These polymorphisms have been observed in four main haplotype variants of the wild type $A_{136}R_{154}Q_{171}$ (ARQ): ARR, ARH, AHQ and VRQ (Belt et al., 1995). The ARR allele is associated with strong resistance to classical scrapie whereas VRQ is associated with susceptibility (Elsen et al., 1999).

Several countries have implemented routine genotyping of the PRNP gene as an important step in their breeding programs to reduce classical scrapie. The selection of homozygous ARR rams for reproduction would increase the frequency of this allele in sheep populations. In Brazil, PRNP genotyping has not been incorporated in evaluation and breeding programs and only a few studies have been performed to evaluate PRNP variant frequencies in commercial sheep flocks (Lima et al., 2007; Pacheco et al., 2007; Sotomaior et al., 2008).

The first report of a case of clinical scrapie in Brazil was before 1978 (Driemeier, 1998). More recently, a few cases of classical scrapie were notified to competent organs of sanitary defense, but all occurred in imported rams (Driemeier, 1998; Felicio, 2001). The use of bone meal and other animal-derived feed ingredients, which has been identified as an important mode of scrapie transmission, has never been a common practice in Brazilian goat and sheep production systems and since 1996 its use has been banned through federal legislation, minimizing the risks of scrapie dissemination in the country. However, the lack of solid information about the genetic composition for scrapie resistance/susceptibility of Brazilian flocks based on the PRNP gene, and further incorporation of this information into the national breeding programs needs to be addressed to further minimize risks of dissemination of the disease, since there are additional means of transmission, including contact with animal tissues (i.e. placenta) or secretions (i.e. milk), as well as exposure to environmental prion contamination (Haybaeck et al., 2011), which cannot be easily controlled.

The present study was conducted to investigate four PRNP polymorphisms previously correlated with scrapie resistance/susceptibility, and estimate allelic and haplotype frequencies in Brazilian

* Corresponding author. Tel.: +55 61 3448 4669; fax: +55 61 3340 3624.
E-mail address: samuel@cenargen.embrapa.br (S.R. Paiva).

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sheep flocks from thirteen different breeds. Our results provide information about scrapie susceptibility in different production regions in Brazil and will subsidize development of National Policies to prevent outbreaks of the disease.

2. Materials and methods

2.1. Samples

A total of 1400 sheep DNA samples from Embrapa Genetic Resources and Biotechnology Animal Genetics Laboratory repository were used in the study (Table 1). Animals sampled were from commercial/specialized breeds (Corridale, Dâmara, Dorper, Hampshire Down, Ile de France and Suffolk) and local adapted breeds (Brazilian Bergamasca, Brazilian Creole, Morada Nova, Rabo Largo, Pantanal Creole, Santa Inês and Brazilian Somali) from at least 30 flocks and cover all the main breeds used in Brazilian production systems/regions.

2.2. PCR amplification and fragment purification

A fragment of the PRNP coding region from nucleotide 287 to 613 (GenBank Accession No. AJ223072) was amplified with primers 5′-GGTAGCCACAGTCAGTGG-3′ and 5′-CAGTTTCGGTGAAGTT CTCC-3′. PCR was performed in 10 μl reactions containing 5 ng of genomic DNA, 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.25 mM $MgCl_2$, 200 mM dNTPs, 0.125 μM each primer and 1 unit of Taq polymerase. The following cycling protocol was used: 96 °C (5 min) followed by 30 cycles of 96 °C (30 s), 64 °C (45 s) and 72 °C (1 min) and a final step at 72 °C (7 min). PCR products were checked by electrophoresis in 2% ethidium bromide-stained agarose gels and visualized in a UV transilluminator. Post-PCR clean-up was performed by adding 2 μl of ExoSAP-IT (GE Healthcare) mix (0.2 μl of ExoSAP-IT and 1.8 μl of ddH_2O as described by Ianella et al. (2011)) to 3 μl of PCR product, with an incubation of 45 min at 37 °C followed by 15 min at 80 °C for enzyme inactivation.

2.3. SNP genotyping, allele and haplotype frequency estimation

SNP interrogation was carried out using a primer extension method (SNaPshot, Applied Biosystems) using a protocol adapted by Ianella et al. (2011). Primers previously described by Vaccari et al. (2004) were used to identify the polymorphisms at codons 136 (SNP p407), 154 (SNP p461) and 171 (SNP p512 and p513). Multiplex primer extension reactions were performed in a 10 μl final volume containing 3 μl of purified PCR product, 0.6 μl of SNaPshot Multiplex mix (Applied Biosystens), 0.65 pmol of primer p407, 0.94 pmol of p461, 0.095 pmol of p512 e 0.94 pmol de p513. The reaction was submitted to 25 cycles of 96 °C (10 s), 58 °C (5 s) and 60 °C (30 s). Unincorporated ddNTPs were removed by treatment with shrimp alkaline phosphatase (SAP-IT, Amershan) according to Ianella et al. (2011).

Following the SAP-IT treatment, 1.5 μl of the reaction mixture was incubated for 5 min at 95 °C with 8.1 μl of HiDi formamide and 0.4 μl of GeneScan120 LIZ size standard (Applied Biosystems). Capillary electrophoresis was carried out in an ABI3100 automated sequencer (Applied Biosystems), and the electropherograms were analyzed with GeneMapper software (Applied Biosystems).

Haplotype estimation was carried out with Haploview (Barret et al., 2005). Allelic and genotypic frequencies were estimated using Fstat 2.9.3 software (Goudet, 2002).

2.4. Validation study

A cross-validation study was carried out to evaluate the accuracy of the primer extension reaction. A total of 96 samples covering all genotypes found in the thirteen breeds were regenotyped by direct sequencing of PCR products. Sequencing reactions were performed with BigDye terminator chemistry (Applied Biosystems) using 3–10 ng of ExoSAP-IT-purified (Amershan) PCR products according to manufacturer instructions. Capillary electrophoresis was carried out in an ABI3100 automated sequencer and the sequences were analyzed and aligned using Phred/Phrap/Consed (Ewing and Green, 1998; Ewing et al., 1998).

Table 1
Haplotype frequencies for PRNP gene in 13 Brazilian sheep breeds (n = 1400).

Breeds	Haplotypic frequencies				*N*
	ARR	ARQ	AHQ	VRQ	
Local adapted breeds					
OB	20.65	71.74	7.61	–	46
OCL	38.83	59.50	1.67	–	300
OMN	20.06	77.47	1.85	0.62	162
OPT	33.85	54.17	10.68	1.30	192
ORL	12.07	85.78	2.16	–	116
OSI	24.88	54.00	17.35	3.76	412
OSO	8.51	91.49	–	–	47
Commercial or breeds					
OC	45.00	25.00	20.00	10.00	10
ODA	10.00	90.00	–	–	10
ODO	15.00	75.00	–	10.00	30
OH	47.92	50.00	–	2.08	24
OIF	64.58	18.75	–	16.67	24
OSU	33.33	62.96	–	3.70	27
Total	28.82	62.76	4.72	3.70	1400

OB, Brazilian Bergamasca; OC, Corriedale; OCL, Brazilian Creole; ODA, Dâmara; ODO, Dorper; OH, Hampshire; OIF, Ile de France; OMN, Morada Nova; OPT, Pantanal Creole; ORL, Brazilian Fat Tail; OSI, Santa Inês; OSO, Brazilian Somali; OSU, Suffolk.

3. Results

Considering the 136, 154, and 171 polymorphisms, a total of four of the five previously reported haplotypes were found in the studied samples (ARR, ARQ, AHQ and VRQ) (Table 1). Haplotypes ARR and VRQ were found to be the most frequent in commercial breeds while ARQ and AHQ were found to be the most frequent in naturalized breeds. Corriedale, Morada Nova, Pantanal Creole and Santa Inês breeds show all the allelic variants found in this study. The Damara and Brazilian Somali breeds only showed alleles ARR and ARQ, indicating low variability for this locus in these breeds.

Codon 171 was polymorphic in all breeds. However, the H variant was not found in any of the tested samples and the rare *K* variant (Pongolini et al., 2009) could not be detected with the assay used. Codon 154 was polymorphic in all breeds except for Dâmara, Dorper, Hampshire Down, Ile de France, Brazilian Somali and Suffolk, where the H variant was not observed. Brazilian Bergamasca, Brazilian Creole, Dâmara, Brazilian Fat Tail and Brazilian Somali did not show the *V* variant at codon 136.

Frequencies for the haplotype correlated with the highest level of resistance to scrapie (ARR) ranged from 8.51% (Brazilian Somali) to 64.68% (Ile de France). The VRQ haplotype, which has been correlated with the highest level of susceptibility to scrapie, was found in frequencies lower than 10% in most breeds (except for Ile de France – 16.67%) and was absent in the Brazilian Bergamasca, Brazilian Creole, Dâmara, Brazilian Fat Tail and Brazilian Somali. The ARQ haplotype was present in all breeds. Altogether, ARQ was the most frequent haplotype in Brazilian sheep (62.76%), followed by ARR (28.82%).

A total of nine out of 15 possible genotypes for the four observed PRNP haplotypes were identified in the analyzed samples

Table 2
PRNP genotype frequencies (%) in 13 sheep breeds from Brazil (n = 1400).

Breeds	N	Genotypic frequencies (%)								
		ARR/ARR	ARR/ARQ	ARR/AHQ	ARR/VRQ	ARQ/ARQ	ARQ/AHQ	ARQ/VRQ	AHQ/AHQ	AHQ/VRQ
OB	46	2.2	32.6	4.3	–	50.0	10.9	–	–	–
OC	10	10.0	40.0	10.0	20.0	–	10.0	–	10.0	–
OCL	300	15.4	44.7	2.3	–	36.3	1.3	–	–	–
ODA	10	–	20.0	–	–	80.0	–	–	–	–
ODO	30	–	30.0	–	–	50.0	–	20.0	–	–
OH	24	20.8	50.0	–	4.2	25.0	–	–	–	–
OIF	24	41.7	20.8	–	25.0	4.2	–	8.3	–	–
OMN	162	6.2	27.3	–	0.6	62.0	3.7	0.6	–	–
OPT	192	8.4	38.0	12.0	1.0	30.8	7.8	1.0	0.5	0.5
ORL	116	–	24.2	–	–	71.5	4.3	–	–	–
OSI	412	2.7	32.3	9.2	2.9	26.0	19.7	4.1	2.6	0.5
OSO	47	–	17.0	–	–	83.0	–	–	–	–
OSU	27	3.7	51.9	–	7.4	37.0	–	–	–	–
Total	1400	8.5	33.0	2.9	4.7	42.7	4.4	2.6	1.1	0.1

OB, Brazilian Bergamasca; OC, Corriedale; OCL, Brazilia Creole; ODA, Dâmara; ODO, Dorper; OH, Hampshire; OIF, Ile de France; OMN, Morada Nova; OPT, Pantanal Creole; ORL, Brazilian Fat Tail; OSI, Santa Inês; OSO, Brazilian Somali; OSU, Suffolk.

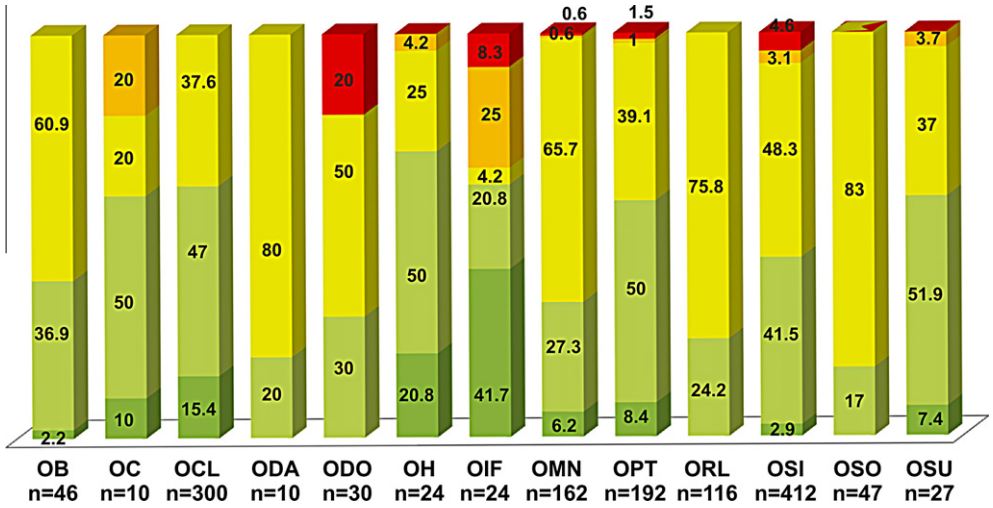

Fig. 1. Genotype frequencies in the different breeds according to NSP classical scrapie susceptibility classification ■, type 1; ■, type 2; ■, type 3; ■, type 4; ■, type 5; NSP, National Scrapie Plan – Great Britain. OB, Brazilian Bergamasca; OC, Corriedale; OCL, Brazilian Creole; ODA, Dâmara; ODO, Dorper; OH, Hampshire; OIF, Ile de France; OMN, Morada Nova; OPT, Pantanal Creole; ORL, Brazilian Fat Tail; OSI, Santa Inês; OSO, Brazilian Somali; OSU, Suffolk.

(Table 2) and all of them were observed in the Pantanal Creole region and Santa Inês breeds, evidencing a higher genetic variability for this locus compared with the other breeds. The VRQ/VRQ genotype, which is considered to be the most susceptible for the onset of scrapie, was not observed in any of the samples tested.

The ARR/VRQ genotype was observed in frequencies between 1% and 20% in the Pantanal Creole and Corriedale respectively, and was not found in Brazilian Creole, Dâmara, Dorper, Brazilian Fat Tail and Brazilian Somali. ARQ/ARQ animals were not observed in Corriedale breed. The susceptible genotypes (ARQ/VRQ and AHQ/VRQ) were not present in Brazilian Bergamasca, Corriedale, Brazilian Creole, Dâmara, Hampshire, Brazilian Somali and Suffolk. The AHQ/VRQ genotype was only observed in Pantanal Creole and Santa Inês. All of the breeds tested, except for Santa Inês and Pantanal Creole were found to be in Hardy–Weinberg Equilibrium (HWE) at this locus (Supplementary Table S1). However, when individual flocks of Santa Inês and Pantanal Creole genetic groups were analyzed separately, all flocks were found to be in HWE.

Fig. 1 shows the distribution of breed genotypes according to scrapie infection risk, using the NSP classification system (DEFRA, 2001). NSP Type 1 (ARR/ARR) and Type 2 (ARR/ARQ e ARR/AHQ), which are all considered to confer resistance to scrapie, have cumulative frequencies higher than 60% in Corriedale (60%), Brazilian Creole (62.4%), Hampshire (70.8%) and Ile de France (62.5%). NSP Type 5 genotypes (ARQ/VRQ, AHQ/VRQ and VRQ/VRQ), the most susceptible, were found only in Ile de France (8.3%), Morada Nova (0.6%), Pantanal Creole (1.5%), Santa Inês (4.6%) and Dorper (20%).

Table 3
PRNP Haplotypic frequencies for different sheep production regions in Brazil (n = 1400).

Region	Haplotypic frequencies (%)			
	ARR	ARQ	AHQ	VRQ
CW (n = 285)	31.1	58.0	9.8	1.1
NE (n = 740)	19.8	67.6	10	2.6
SE (n = 56)	55.4	31.2	3.6	9.8
S (n = 327)	38.5	59.7	1.5	0.3

CW, Centerwest region; NE, Northeast region; SE, Southeast region; S, Southern region.

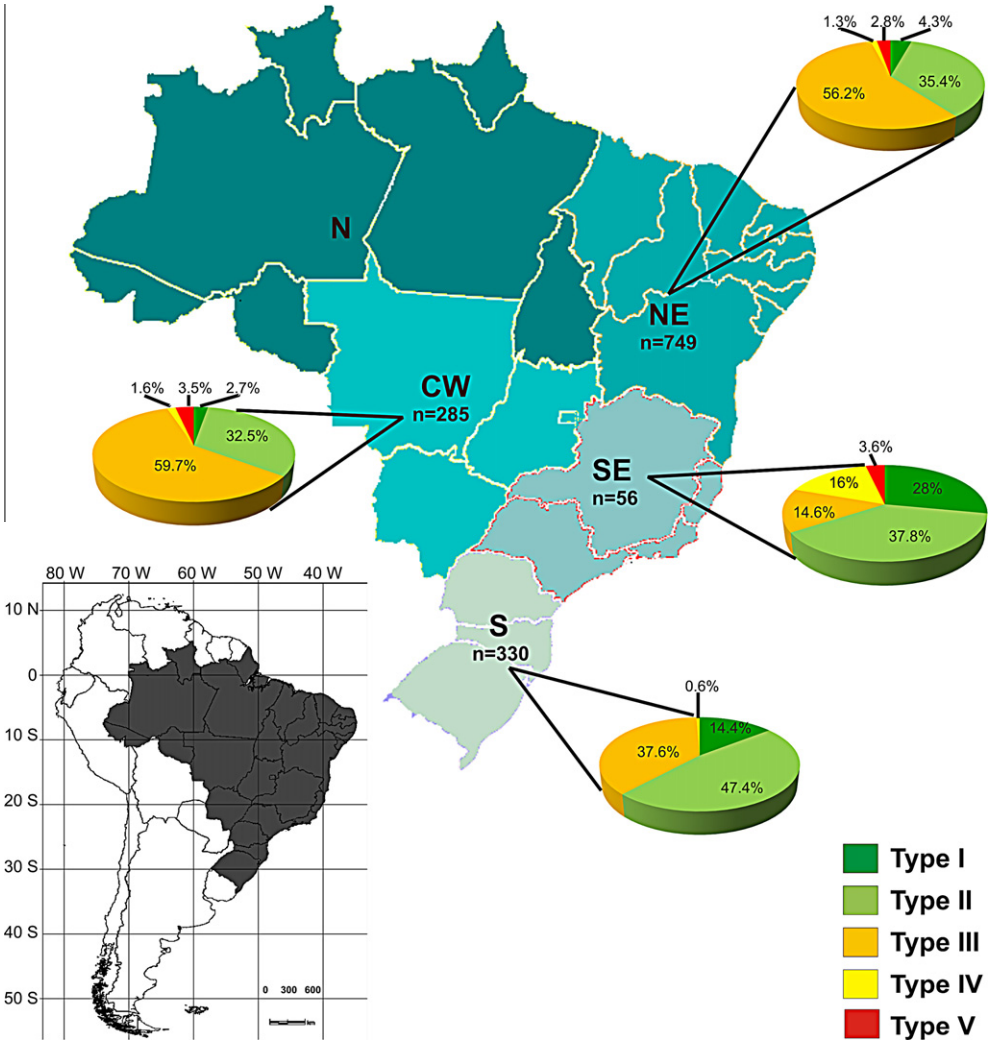

Fig. 2. Haplotype frequencies in different producing regions of Brazil following the NSP classification: WC = Centerwest, three breeds; NE = Northeast, six breeds; S = South, two breeds; SE = Southeast, three breeds.

To evaluate trends in PRNP allele frequencies in major Brazilian production regions a geographic analysis was employed (Table 3). Breeds sampled in each region were the following: Centerwest – Brazilian Bergamasca, Pantanal Creole and Santa Inês; Northeast – Damara, Dorper, Morada Nova, Brazilian Fat Tail, Santa Inês and Brazilian Somali breeds; Southeast – Corriedale, Hampshire and Ile de France; South – Brazilian Creole and Suffolk. ARR and VRQ alleles were found to be the most frequent in Southeast region and ARQ and AHQ in the regions Centerwest and Northeast. In all regions, the most frequent alleles were ARR and ARQ and the lowest VRQ frequency was found in the South region.

When genotypic frequencies were considered by region (Supplementary Table S2), ARR/ARQ was the most frequent in South and Southeast and ARQ/ARQ genotype was the most frequent in Centerwest and Northeast. In Centerwest and Northeast regions all nine genotypes were found to be present. Fig. 2 illustrates genotypic frequencies classified according to their resistance to classical scrapie (DEFRA, 2001). Animals with type 1 and type 2 genotypes show cumulative frequencies higher than 55% in Centerwest, Southeast and South region.

Direct sequencing of 96 PRNP amplicons confirmed all the genotypes obtained with the SNaPshot assay. Additionally, it was possible to obtain data for a SNP at position 141 that results in a change of a Leucine by a Phenylalanine and has recently been associated with atypical cases of scrapie (Moum et al., 2005). Haplotype analysis of these 96 animal genotypes for SNPs 136, 141, 154 and 171 revealed that the F variant was always in phase with the ARQ haplotype (i.e. AFRQ), A total of four L/F_{141} heterozygote samples were observed: two Santa Inês (ARLR/ARFQ), one Ile de France (ARFQ/VRLQ) and one Brazilian Somali (ARFQ/ARLQ).

4. Discussion

A total of 1400 samples from 13 different sheep breeds sampled in Brazil were genotyped for the polymorphisms at PRNP gene related to susceptibility/resistance to scrapie, representing the first robust PRNP genotyping study in this country. In addition this study contributes at International level as giving robust genotype information from South America sheep for global policies, for example FAO, considering that this disease is of major importance in other continents, such as Europe.

Several studies revealed that the ovine PRNP gene is highly polymorphic (Goldmann, 2008). However, only the polymorphisms at positions 136, 154 and 171 have been clearly correlated with the onset of classic scrapie (Belt et al., 1995; Hunter et al., 1996). The haplotypic variants most commonly found in different breeds worldwide for these three polymorphisms are ARR, ARQ, AHQ

and VRQ (Bossers et al., 1996; Hunter et al., 1996; Laplanche et al., 1993). All these PRNP variants were found in the samples tested in this study. The ARH variant was not found, as previously described by Sotomaior et al. (2008). This variant is usually found at low frequencies in few breeds such as Texel and Suffolk (Drogemuller et al., 2001; Sild et al., 2006).

ARR was found to be the most frequent allele in Corriedale and Ile de France (Table 1). These breeds are part of European breeding programs that have been heavily selected for the ARR allele over the last two decades (Dawson et al., 2008; Hunter et al., 1997), and have only been recently introduced in Brazil by means of European imports. Therefore, the observed high frequencies of ARR in Brazilian Corriedale and Ile de France animals can be considered a result of high frequencies of this allele in the imported founders of the Brazilian herds. Conversely, ARQ was found to be the most frequent allele in all other analyzed breeds as expected, since it is regarded as the archetypal allele (Goldmann, 2008).

Allele frequencies estimated for Dorper, Hampshire, Ile de France, Santa Inês and Suffolk breeds were very similar with the frequencies reported by Sotomaior et al. (2008), with exception that they did not find the VRQ allele in Dorper, Ile de France and Suffolk breeds. Lima et al. (2007) described ARR as the most frequent allele in Santa Inês ($N = 29$ sampled animals) but the present study found ARQ to be the most frequent ($N = 412$ analyzed animals). This is important as Santa Inês is the largest breed in the country (McManus et al., 2010) and the presence of susceptible genotypes in this breed may have a large impact on the national sheep production system.

Traditional breeding programs aimed at increasing scrapie resistance in sheep flocks have simultaneously selected for alleles considered to be resistant (ARR) and against susceptible (VRQ) alleles, respectively (Dawson et al., 2008). However, with the occurrence of atypical scrapie cases, with different patterns of relation with PRNP genotype, this strategy has been questioned. Since initial reports in 1998, atypical scrapie cases have been occurring in small frequencies, especially in European countries (Goldmann, 2008) and have been strongly correlated to the presence of alleles $AF_{141}RQ$ and AHQ (Benestad et al., 2008; Moum et al., 2005; Saunders et al., 2006), while resistant animals carrying the susceptibility allele (VRQ) have also been found. The polymorphism at the 141th codon causes a substitution of a lysine (L) by a phenylalanine (F) and is frequently found in several breeds in Europe. In the evaluated samples, the F_{141} variant was found in phase with ARQ allele, as previously reported (Bossers et al., 1996; Hunter et al., 1996; Moum et al., 2005), at low frequencies (4%), similar to the observations reported by Lima et al. (2007).

Selection programs based on scrapie genotypes have not been implemented in Brazil mainly because significant numbers of scrapie cases have not been reported in the country. Brazil is free of scrapie, with the exception of a few sporadic registered cases detected in animals imported from 1930s through 1970s from the UK (Smith and Sherman, 2009). Nonetheless, starting in 1986 the Brazilian Ministry of Agriculture, Livestock and Supply began implementing National policies to increase monitoring for this disease. As a consequence, scrapie and Bovine Spongiform Encephalopathy (BSE) have been included in the National Surveillance System for Animal Diseases since 1997. However, our findings have provided a strong knowledge base to support the elaboration and establishment of future government policies.

The National Scrapie Plan of Great Britain (DEFRA, 2009) classified the 15 possible genotypes according to their resistance/susceptibility to scrapie. NSP type 1 (ARR/ARR), the genotype with highest resistance, was found at an average frequency of 8.5% for all breeds and at 41.7% in Ile de France (Fig. 1). The ARR/ARQ genotype, classified as NSP type 2, was found in all breeds as described by Sotomaior et al. (2008) with an average frequency of 33%. The ancestral ARQ/ARQ genotype (NSP type 3) was the most frequent genotype with an average frequency of 42.7%. The ARR/ARQ and ARQ/ARQ genotypes are commonly the most frequent in sheep breeds worldwide (Babar et al., 2009; L'Homme et al., 2008; Luhken et al., 2007).

VRQ carrying animals (NSP type 4 and 5), the most susceptible genotypes to classical scrapie, were found at high frequencies only in the commercial/specialized Dorper and Ile de France breeds. This result is very important for the Brazilian lamb industry, as Dorper rams are commonly used in crossbreeding schemes with ewes from local adapted breeds. In the medium term, this practice could raise frequencies of susceptible alleles in local adapted breeds as frequently crossbred ewe lambs are used for crossing with local rams. This trend can already be observed in the Santa Inês breed which has probably suffered recent introgression from commercial and recent imported flocks as was showed by McManus et al. (2010) with nuclear and mitochondrial markers. To refute the hypothesis of low sampling size from our results, Cervelatti et al. (2010) and Sotomaior et al. (2008) had found very similar frequencies of ARQ/VRQ genotype in Dorper breed sampled in other States from Brazil. The same results were observed in Europe for Ile de France breed (François et al., 2003; Wisniewska and Mroczkowski, 2009). It should be noted that the commercial breeds sampled in this study probably have a lower number of animals than the local adapted ones and only registered animals were sampled in the States responsible for the main importations in the country. When the sampled animals were grouped in commercial/specialized and local adapted breeds, ARR and VRQ alleles were the most frequent in commercial/specialized breeds, whereas ARQ and AHQ were the most frequent in local adapted breeds. Local adapted breeds have not been selected for resistance since they have been historically raised in a production system that does not offer high risks of scrapie infection, therefore allowing the wild type allele (ARQ) to remain at high frequencies.

The contrasting patterns revealed by geographic analysis of allele frequencies (Table 3) can be attributed to differences in breed composition of the regional flocks. The high frequencies of ARR and VRQ alleles observed in Southeast region can be attributed to flock being mainly made up of Corriedale, Hampshire Down and Ile de France breeds that has a historical background of major incidence of those alleles (Gama et al., 2006; Wisniewska and Mroczkowski, 2009). The ARQ allele is the most frequent in all other regions. In the Southern region, where the sampled animals belong to the local adapted breed Brazilian Creole and the commercial Suffolk breed, the ARR and ARQ frequencies were approximately the same. In Centerwest and Northeast regions, where greater part of the sampled animals belongs to local adapted breeds, ARQ frequencies are significantly higher than other alleles. These findings can be explained by the absence of artificial selection pressures on local adapted breeds, reducing the probability of fixation of the ARR allele. The prevalence of ARQ allele was observed in other studies with flocks from countries where scrapie is not endemic and where this gene is also not under selection pressure (Babar et al., 2009; Bossers et al., 1999; Cooper, 1973; Hunter and Cairns, 1998). The allelic frequencies of the introduced commercial breeds show the results of previous selection for the PRNP gene.

These conclusions can be corroborated by the genotype frequencies. In spite of all the alleles being present in all regions, all the genotypes were found only in Northeast and Centerwest, evidencing a higher variability in these regions. In the Southern region NSP type 5 genotype was not found, while in the Southeast the extreme NSP type genotypes were present at their highest frequencies. The South and Centerwest show the highest frequencies of NSP type 2 and 3 respectively, showing a high proportion of intermediate risk genotypes. In this way, for conservation (e.g., ram selection for repositories) and breeding purposes, Brazilian local

adapted breeds have sufficient genetic diversity for PRNP to permit a degree of selection for national or international markets.

A few previous studies estimating ovine PRNP genotype frequencies (Lima et al., 2007; Pacheco et al., 2007; Passos et al., 2008; Sotomaior et al., 2008) had been carried out with samples from Brazil. However, these studies were performed with small sample numbers and therefore none were able to estimate PRNP haplotype frequencies with a high degree of certainty. With the present study we were able to robustly estimate PRNP haplotypes and evaluate the haplotype and genotype frequencies among commercial and local adapted sheep breeds from different production regions in Brazil. Our results show scrapie resistance alleles are at high frequencies in all flocks from different production regions and therefore, establish the basis for development and implementation of a national breeding program aimed at lowering the frequency of susceptible.

The wild type ARQ allele is the most frequent followed by ARR. The genotypes that confer intermediate risk for classical scrapie infection (ARR/ARQ and ARQ/ARQ) are the most frequent. These findings can be explained by the absence of scrapie Brazilian production systems and the lack of strong selection pressure that make the presence of the wild type allele more favorable. This study was significant in offering information on PRNP genetics in Brazilian sheep countrywide. It reports a first haplotype study in national flocks and the PRNP genotype and haplotype frequencies reported can be considered as a starting point to increase genotyping of animals/ breeds to support either a National policy for scrapie prevention as the basis for policies that might regulate future exportation of meat from Brazilian sheep flocks.

Conflict of interest statement

All authors of this paper are disclosed any financial and personal relationships with other people or organizations that could inappropriately influence this work.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rvsc.2011.06.025.

References

Babar, M.E., Farid, A., Benkel, B.F., Ahmad, J., Nadeem, A., Imran, M., 2009. Frequencies of PrP genotypes and their implication for breeding against scrapie susceptibility in nine Pakistani sheep breeds. Molecular Biology Reports 36, 561–565.

Barret, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotypes maps. Bioinformatics 21, 263–265.

Belt, P.B.G., Muileman, I.H., Schreuder, B.E.C., Bos-de Ruijter, J., Gielkens, A.L.J., Smits, A.A., 1995. Identification of the five allelic variants of the sheep PrP gene and their association with natural scrapie. Journal of General Virology 76, 509–517.

Benestad, S.L., Arsac, J.-N., Goldmann, M.N., 2008. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. Veterinary Research 39, 19.

Benkel, B.F., Issonnette, N., Hossain Farid, A., 2007. Simultaneous detection of eight single nucleotide polymorphisms in the ovine prion protein gene. Molecular and Cellular Probes 21, 363–367.

Bossers, A., Schreuder, E.C., Muileman, I.H., Belt, P.B.G.M., Smits, M.A., 1996. PrP genotypes contributes to determining survival times of sheep with natural scrapie. Journal of General Virology 77, 2669–2673.

Bossers, A., Harders, F.L., Smits, M.A., 1999. Prp genotypes frequencies of the most dominant sheep breed in a country free from scrapie. Archives of Virology 144, 829–834.

Cervelatti, E., Ribeiro, E., Passos, D.T., Nunes, T., Heaton, M. P., Colussi, S., Acutis, P.L., Pelleto, S. Garcia, F. J. 2010. Allelic variants of ovine prion protein gene (PRNP) in Brazilian local sheep breed Santa Ines. In: Proceedings of 32 Conference for the International Society of Animal Genetics (ISAG), p.143.

Cooper, J.E., 1973. A report of scrapie in sheep in Kenya. British Veterinary Journal 129, 2.

Dawson, M., Moore, R.C., Bishop, S.C., 2008. Progress and limits of PrP gene selection policy. Veterinary Research 39, 25.

DEFRA, 2001. National Scrapie Plan for Great Bretain. Department of Environment, Food and Rural Affairs, UK, London.

Driemeier, D., 1998. Scrapie. In: Riet-Correa, Franklin., Schild, Ana Lucia., Méndez, Maria C. (Eds.), Doenças em Ruminantes e Equinos, first ed. Pelotas Editorae Gráfica Universitária, pp. 305–311.

Drogemuller, C., Leb, T., Distl, O., 2001. Prp genotypes frequencies in German breeding sheep and the potential to breed for resistance to scrapie. Veterinary Research 149, 349–542.

Elsen, J.M., Amigues, Y., Schelder, F., Ducrocq, V., Andreoletti, O., Eychene, F., Khang, J.V., Poivey, J.P., Lantier, F., Laplanche, J.L., 1999. Genetic susceptibility and transmission factors in scrapie: detailed analysis of a closed flock of Romanov. Archives of Virology 144, 43–445.

Ewing, B., Hillier, L., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Research 8, 175–185.

Ewing, B., Green, P., 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Research 8, 186–194.

Felicio, P.E., 2001. Em Guerra por engano. Agroanalysis 21, 14–18.

François, D., Elsen, J.M., Barillet, F., Lajous, D., Eychenne, F., Palhière, I., 2003. Breeding programmes for improving the quality and safety of products (Options Mediterrane'ennes: Se'rie A). In: Gabina, D., Sanna, S. (Eds.), New Traits, Tools, Rules and Organization? CIHEAM-IAMZ, Zaragoza, Spain, pp. 29–35.

Gama, L.T., Carolino, M.I., Santos-Silva, M.F., Pimenta, J.A., Costa, M.S., 2006. Prion protein genetics polymorphisms and breeding strategies in Portuguese breeds of sheep. Livestock Science 99, 175–184.

Goldmann, W., 2008. PrP genetics in ruminant transmissible spongiform encephalopatie. Veterinary Research 39, 30.

Goldmann, W., Hunter, N., Foster, J.D., Salbaum, J.M., Beyreuther, K., Hope, J., 1990. Two alleles of a neural protein gene linked to scrapie in sheep. PNAS 80, 2527–2534.

Goudet, J. 2002. FSTAT Version 2.9.3.2 for Windows: A Computer Program to Calculate *F*-Statistics. <http://www2.unil.ch/popgen/softwares/fstat.htm>.

Haybaeck, J., Heikenwalder, M., Klevenz, B., Schwarz, P., Margalith, I., Bridel, C., Mertz, K., Zirdum, E., Petsch, B., Fuchs, T.J., Stitz, L., Aguzzi, A., 2011. Aerosols transmit prions to immunocompetent and immunodeficient mice. PLOS Pathogens 7, e101257.

Hunter, N., Cairns, D., 1998. Scrapie-free merino and Poll Dorset sheep from Australia and New Zealand have normal frequencies of scrapie-susceptible genotypes. Journal of General Virology 79, 2079–2082.

Hunter, N., Foster, J.D., Goldmann, W., Stear, M.J., Hope, J., Bostock, C., 1996. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. Archives of Virology 141, 809–824.

Hunter, N., Goldmann, W., Foster, J.D., Cairns, D., Smith, G., 1997. Natural scrapie and PrP genotype: case control studies in British sheep. Veterinary Record 141, 137–140.

Ianella, P., McManus, C.M., Paiva, S.R., Caetano, A.R., 2011. Adaptation of a medium-throughput PRNP genotyping system for use in Brazilian sheep flocks. Genetics and Molecular Research.

L'Homme, Y., Leboeuf, A., Cameron, J., 2008. PrP genotype frequency of Quebec sheep breeds determined by real-time PCR and molecular beacons. Canadian Journal of Veterinary Research 72, 320–324.

Laplanche, J.L., Chatelain, J., Westaway, D., Thomas, S., Dussaucy, M., Brugere-Picoux, J., Launay, J.M., 1993. Prp polymorphisms associated with natural scrapie discovered by denaturing gradient gel electrophoresis. Genomics 15, 30–37.

Lima, A.C.B., Bossers, A., Souza, C.E., Oliveira, S.M.P., Oliveira, D.M., 2007. Prp genotypes in a pedigree flock of a Santa Inês sheep. Veterinary Records 160, 336–337.

Luhken, G., Lipsky, S., Peter, C., Erhardt, G., 2007. Prion protein polymorphisms in autochthonous European sheep breeds in respect to scrapie eradication in affected flocks. Small Ruminant Research 75, 43–47.

McManus, C., Paiva, S.R., Araujo, R.O., 2010. Genetics and breeding of sheep in Brazil. Revista Brasileira de Zootecnia (Special Suppl.).

Moum, T., Olsaker, I., Hopp, P., Moldal, T., Valheim, M., Moum, T., Benestad, S.L., 2005. Polymorphisms at codons 141 and 154 in the ovine prion gene protein gene are associated with scrapie Nor98 cases. Journal of General Virology 86, 231–235.

Pacheco, A.C.L., Oliveira, S.M.P., Gouveia, J.J.S., Dini, M.C., Vasconcelos, E.J.R., Viana, D.A., Rosinha, G.M.S., Costa, R.B., Maggioni, R., Oliveira, D.M., 2007. Analysis of prion protein gene (PRNP) polymorphisms in healthy Morada Nova sheep reveals the presence of genotypes susceptible to scrapie. Ciência Animal 17, 27–36.

Passos, D.T., Ribeiro, L.A.O., Rodrigues, N.C., Hepp, D., Weimer, T.A., 2008. PrP polymorphisms in Brazilian sheep. Small Ruminant Research 74, 130–133.

Pongolini, S., Bergamini, F., Iori, A., Migliore, S., Corradi, A., Bassi, S., 2009. Prion protein genotypes of Italian sheep breeds with lysine-171 and phenylalanine-141 detection. Veterinary Microbiology 137, 18–23.

Prusiner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. Science 21, 136–144.

Prusiner, S.B., 1998. Prions. PNAS 10, 13363–13383.

Saunders, G.C., Cawthraw, S., Mountjoy, S.J., Hope, J., Windl, O., 2006. PrP genotypes of atypical scrapie cases in Great Britain. Journal of General Virology 87 (2006), 3141–3149.

Sild, E., Volskiene, R., Viinalass, H., Miceikiene, I., Grislis, Z., Distl, O., Drogemuller, C., 2006. Detection of prion protein gene polymorphisms in Baltics breeds of sheep. Veterinary Records 159, 247–250.

Smith, M.C., Sherman, D.M., 2009. Goat Medicine, second ed. Wiley-Blackwell, p. 177.

Sotomaior, C.S., Sotomaior, V.S., Madeira, H.M.F., Thomaz-Soccol, V., 2008. Prion protein gene polymorphisms in sheep in the state of Parana, Brazil. Animal Genetics 39, 659–661.

Vaccari, G., Conte, M., Morelli, L., Guardo, G.D., Petraroli, R., Agrimini, U., 2004. Primer extension assay for prion protein genotype determination in sheep. Molecular and Cellular Probes 18, 33–37.

Wisniewska, E., Mroczkowski, S., 2009. Different breeding strategies for scrapie resistance depending on breed-specific PrP allele and genotype frequencies in the Polish sheep. Züchtungskunde 81, 180–189.