Evaluation of PRNP polymorphisms in Brazilian local adapted breeds.

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Introduction

PRNP polimorphisms have been associated with resistance/susceptibility to Transmissible Spongiformes Encephalopaties (TSEs) or prion diseases, including the ovine and caprine scrapie. Over 40 polymorphisms have been reported so far for the ovine PRNP gene (L'Homme *et al.* 2008). However, polymorphisms at codons 136 (A/V – substitution of alanine by valine), 154 (R/H – arginine by hystidine) and 171 (Q/R/H – glutamine by arginine or hystidine) have been frequently correlated to host susceptibility to natural and experimentally induced classic scrapie (Goldmann *et al.* 1990; Hunter 1997; Benkel *et al.* 2007). Many countries have implemented routine genotyping of the PRNP gene to identify and select ARR rams as breeding stock to increase this alleles frequency in commercial flocks. In Brazil, PRNP genotyping has not been incorporated by sheep evaluation and breeding programs. Furthermore, only a few studies have been performed to evaluate PRNP variant frequencies in commercial flocks (Sotomaior *et al.,* 2008; Pacheco *et al.,* 2007; Lima *et al.,* 2007). The present study was conducted to genotype and estimate haplotypes and haplotypic and genotypic frequencies on three previously reported PRNP polymorphisms in Brazilian local adapted/naturalized breeds, and to evaluate the flock's genetic potential in relation to scrapie susceptibility/resistance.

Material and methods

Sample. A total of 1,275 sheep DNA samples from Embrapa Genetic Resources and Biotechnology Animal Genetics Laboratory's repository were used in the study. Animals sampled are from local adapted breeds (Brazilian Bergamasca, Brazilian Creole, Morada Nova, Rabo Largo, Creole of Pantanal, Santa Inês and Brazilian Somali) and cover all the main breeds used in Brazilian production systems.

SNPs interrogation: A fragment of the PRNP coding region from nucleotide 287 to 613 (GenBank accession number AJ223072) was PCR-amplified from genomic DNA using primers 5`-GGTAGCCACAGTCAGTGG-3` and 5`-CAGTTTCGGTGAAGTTCTCC-3'. Post PCR clean-up was performed using ExoSAP-IT (GE Healthcare) and SNP interrogation

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at positions 136, 154 and 171 was carried out using a primer extension method (SNaPShot – Applied Biosystems). Labeled products were analyzed by capillary electrophoresis with an ABI3100 automated sequencer (Applied Biosystems) and electropherograms were analyzed with GeneMapper v4.0 software (Applied Biosystems).

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

Results and discussion

Four of the five previously reported haplotypes were found in the studied samples (Table 1). All four haplotypes were found in Morada Nova, Creole of Pantanal and Santa Inês. Codon 171 was polymorphic in all breeds however, the H variant was not found in any of the tested samples, as previously described by Sotomaior *et al.* (2008) – this variant is usually found in small frequencies only in some breeds such as Texel and Suffolk (Silk *et al.,* 2006; Drogemuller *et al.,* 2008). Codon 154 was polymorphic in all breeds, except in Brazilian Somali. Brazilian Bergamasca, Brazilian Creole, Brazilian Fat Tail and Brazilian Somali did not show the V variant in codon 154. The ARQ haplotype was the most frequent in all breeds, followed by ARR - the sum of these two variants total more than 78% in all breeds. ARQ is considered to be the archetypal allele, which explains its high frequencies in all breeds (Goldmann, 2008).

Table 1: Haplotypic frequencies (%) of PRNP gene in seven Brazilian local adapted sheep breeds (Total N = 1275)

Breed	Haplotype				N	N of sampled flocks
	ARR	ARQ	AHQ	VRQ		
OB	20.65	71.74	7.61	-	46	2
OCL	38.83	59.50	1.67	-	300	2
OMN	20.06	77.47	1.85	0.62	162	4
OPT	33.85	54.17	10.68	1.30	192	2
ORL	12.07	85.78	2.16	-	116	3
OSI	24.88	54.00	17.35	3.76	412	5
OSO	8.51	91.49	-	-	47	1

OB: Brazilian Bergamasca; **OCL:** Brazilian Creole; **OMN:** Morada Nova; **OPT:** Creole of Pantanal; **ORL:** Brazilian Fat Tail; **OSI:** Santa Inês; **OSO:** Brazilian Somalis.

Nine out the 10 possible genotypes for the four observed PRNP haplotypes were identified in the analyzed samples (Table 2). All of the nine genotypes were found in Creole of Pantanal and Santa Inês breeds, evidencing a higher variability for this locus compared with the other breeds. The susceptible genotypes (ARQ/VRQ and AHQ/VRQ) were not present in Brazilian

Bergamasca, Brazilian Creole, Brazilian Fat Tail and Brazilian Somalis. AHQ/VRQ was not found in Morada Nova. The ancestral genotype (ARQ/ARQ) was the most frequent genotype in Brazilian Bergamasca, Morada Nova, Brazilian Fat Tail and Brazilian Somalis. VRQ/VRQ animals, which are considered to be the most susceptible were not observed. The ARR/ARQ and ARQ/ARQ genotypes were the most common in all studied breeds and are the most frequent in sheep breeds around the world (Luhken *et al.* 2008; L'Homme *et al.* 2008; Babar *et al.* 2009).

Table 2: PRNP genotypic frequencies (%) in seven Brazilian local adapted sheep breeds (n = 1275)

NSP type		Genotypic Frequencies								
		I	II			III		IV	V	
Breed	N	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	-	-	-
OCL	300	15.4	44.7	2.3	-	36.3	1.3	-	-	-
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	-	-	-	-

NSP: National Scrapie Plan. **OB:** Brazilian Bergamasca; **OCL:** Brazilian Creole; **OMN:** Morada Nova; **OPT:** Creole of Pantanal; **ORL:** Brazilian Fat Tail; **OSI:** Santa Inês; **OSO:** Brazilian Sommalis.

The National Scrapie Plan of Gran Britain (DEFRA, 2009) classified the 15 possible genotypes according to five levels of resistance/susceptibility to scrapie. NSP type 1, the most resistant (ARR/ARR), is found in the highest frequency in Brazilian Creole (15.4%). The ARR/ARQ genotype, classified as NSP type 2, was found in all breeds as described by Sotomaior *et al.* (2008). The ARQ/ARQ and ARQ/AHQ genotypes (NSP type 3) were the most frequent genotypes in all breeds, with exception of Brazilian Creole. VRQ carrying animals (NSP type 4 and 5), the most susceptible allele to classic scrapie, were found in small frequencies in all breeds, reaching a maximum of 7.2% in Santa Inês. These findings can be explained by the lack of natural or artificial selective pressures in local adapted breeds in Brazil, which also has not contributed to fix the ARR allele. The prevalence of the ARQ allele was also observed in other studies carried out in flocks from countries where the scrapie is not endemic and where this gene does not seem to be under selection (Babar *et al.*, 2009, Bossers, *et al.,* 1999, Hunter *et al.*, 1998, Cooper, 1973).

Conclusion

This work represents the first study to estimate PRNP haplotypes in Brazilian sheep and the first solid study of the PRNP haplotypic and genotypic frequencies in Brazilian local adapted breeds. The wild type ARQ allele and the ARR allele were found to be the most frequent in Brazilian local adapted breeds. Genotypes that confer intermediate risk to classic scrapie infection (ARR/ARQ and ARQ/ARQ) were found to be the most frequent. Our findings reveal that a national breeding program to improve the genetic potential for scrapie resistance in the studied breeds could easily increase ARR frequencies. This study represents the first comprehensive initiative to generate data and establish genetic/molecular tools to support a National program for scrapie prevention in Brazil.

References

Barret, J. C. (2005). *Bioinformatics,* 21:263-265.

Basbar, M. E. *et al.* (2009). *Molecular Biology Reports*, 36:561-565.

Benkel, B. F. *et al.* (2007). *Molecular Evolution Probes,*21:363-367.

Bossers, A. *et al.* (1999). *Arch Virol*, 144:829-834.

Cooper, J. E. (1973). *Br Vet J*, 129:2.

Drogemuller, C, *et al.* (2001). *Veterinary Research,* 149:349-352.

Goldmann, W. *et al.* (1990). *PNAS*, 80:2527-2534.

Goldmann, W. *et al.* (2008). *Veterinary Research*, 39:30.

Goudet, J. (2002). *<http//www2.unil.ch/popgen/softwares/fstat.htm>*.

Hunter, N. *et* al. (1997) *Vet Rec, 141:137-140.*

Hunter, N. and Cairns, D. (1999). *J Gen Virol.* 79:2079-2082.

L'Homme , Y., Leboeuf, A. and Cameron, J. (2008) *The Canadian Journl of Veterinary Research.* 71:320-324.

Lima, A. C. B. *et al.* (2007) *Veterinary Records,* 160:336-337.

Luhken, G. *et al.* (2008). *Small Ruminant Research.* 75:43-47.

Pacheco, A. C. L. *et al.* (2007). *Ciência Animal*, 17:27-36.

Sild, *et al.* (2006). *Veterinary Records,* 159:247-250.

Sotomaior, C. S. *et al.* (2008). *Animal Genetics*, 39:659-661.