ANALYSIS OF PRION PROTEIN GENE (*PRNP*) POLYMORPHISMS IN HEALTHY MORADA NOVA SHEEP REVEALS THE PRESENCE OF GENOTYPES SUSCEPTIBLE TO SCRAPIE

(Análise de polimorfimos do gene da proteína prion (PRNP) em ovinos Morada Nova revela presença de genótipo susceptível ao *Scrapie*)

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ABSTRACT

Prion diseases, such as sheep scrapie, are usually associated with certain genotypes of prion protein gene (*PRNP*). Polymorphisms at codons 136, 154 and 171 of ovine *PRNP* open reading frame (ORF) are believed to confer either resistance or susceptibility to scrapie. In this study 72 healthy sheep from two different flocks, representing two meat type color variants from hair breed Morada Nova, from the State of Ceará, Brazil, were analyzed. *PRNP* ORF sequences were investigated for single nucleotide polymorphisms (SNPs) detection, followed by genotype analysis of codons 136, 154 and 171. Well known polymorphisms at codons 136 (coding for A/V) and 171 (coding for Q/R) were identified among the subjects, while at codon 154 only codon R has been observed. *PRNP* genotypes observed among Morada Nova subjects were ARQ/ ARQ (34.75%), ARQ/ARR (30.49%), ARR/ARR (31.92%) and the rare VRR/VRR (2.78%). We suggest here that the observed high homozygote frequency among Morada Nova *PRNP* genotypes AA at codon 136 and RR at codon 171 could be a genetic element for a putative natural resistance to scrapie. This is the first report of *PRNP* genotyping in Morada Nova breed and the first time the scrapie susceptible allele VRR has been identified in Brazil.

KEY WORDS: Prion protein gene (*PRNP*); scrapie; single nucleotide polymorphisms (SNPs); genotyping; hair breed sheep.

RESUMO

Doenças priônicas, como o *scrapie* (Paraplexia Enzoótica dos Ovinos) estão usualmente associadas com determinados genótipos do gene da proteína priônica (PRNP). Polimorfismos na matriz aberta de leitura (ORF) dos códons 136, 154 e 171 de PRNP de ovinos estão associados a resistência ou suscetibilidade a *scrapie.* Neste estudo, um total de 72 ovinos saudáveis, de 2 rebanhos e diferente variação de cor de pelagem, da raça deslanada Morada Nova no Estado do Ceará, foram analisadas. Seqüências *PRNP,* compreendendo as ORF, do gene PRNP foram investigadas para a identificação

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dos Polimorfismos de único nucleotídeo (SNPs), seguida de detalhado seqüenciamento do DNA e análise bioinformática para os códons 136, 154 e 171. As freqüências genotípicas dos polimorfismos do PRNP são relatadas. Os polimorfismos já identificados no códon 136 (codificando A/V), 171 (codificando Q/R), entretanto no códon 154 não houve variação (somente R). As variantes alélicas e genotípicas para o PRNP observadas entre os ovinos Morada Nova foram: ARQ/ARQ (34,75%), ARQ/ARR (30,49%), ARR/ARR (31,92%) além do genótipo raro VRR/VRR (2,78%). Além das variantes alélicas e genótipicas, nós discutimos a freqüência da homozigozidade dos alelos AA no códon 136 e RR no códon 171 do PRNP e correspondentes haplótipos nos ovinos Morada Nova como elementos genéticos como um suposto elemento de resistência natural a *scrapie*. Este é o primeiro relato de genotipagem para PRNP em ovinos da raça Morada Nova e primeira observação do genótipo VRR/VRR em rebanho brasileiro.
PALAVRAS-CHAVE: Gene da Proteína Prion (PRNP); Polimorfismo de único nucleotídeo (SNPs); genotipagem; ovinos deslanados; *scrapie*.

INTRODUCTION

In several animal species, including humans, polymorphisms within the Open Reading Frame (ORF) of the Prion Protein (PrP) gene (*PRNP*) are associated with the occurrence and the pathological lesions of the Transmissible Spongiform Encephalopathies (TSEs), (POCCHIARI, 1994). TSEs form a group of diseases which are characterized by fatal and progressive degenerative disorders of the central nervous system. TSEs are associated with the conversion of normal PrP (PrPC) into a protease resistant pathological isoform (PrPSc) in which â-strand content increases and á- helix decreases (PRUSINER, 1996; HORIUCHI et al., 2002). PrPSc is formed from PrPC by a post-translational process and such conversion seems to be the key event in the pathogenesis of scrapie in sheep and in several mammalian species, including man (GORODINSKY & HARRIS, 1995; TRABOULOS et al., 1995; VEY et al., 1996). According to the prion theory PrPSc would be the sole component of the infectious particle (PRUSINER, 1998). Therefore, molecular differences in PrPSc must build the basis of the different scrapie phenotypes (BIRKETT et al. 2001). Recent evidences suggest that PrPSc cannot kill neurons on their own, but rather require normal cellular prions (PrPC) to do so (SOLFOROSI et al., 2004). Occurrence of natural scrapie in sheep, as a result of genetic susceptibility, is strongly influenced by polymorphisms in the host gene that encodes the PrP (GOLDMANN et al.,1990; HUNTER et al., 1997).

The PrP gene is 31,4 kb long, organized in three exons, respectively 52, 98 and 4028 nucleotides long, which are separated by two introns, respectively 2421 and 14031 nucleotides long (WESTAWAY et al., 1994; LEE et al., 1998). The coding sequence is located at exon 3. A number of single nucleotide polymorphisms (SNPs) in the *PRNP* ORF is said to be associated with differences in phenotypic expression of prion diseases, such as incubation period, pathology and clinical signs (BOSSERS et al., 2000). In fact, the exact mechanisms by which the different allelic variants of the *PRNP* contribute to susceptibility to scrapie are not completely understood. However, genetic studies suggest that naturally occurring variants of PrP, including those associated with a high risk for scrapie, cannot induce spontaneous development of the disease in sheep (HUNTER et al., 1997, 1998; BOSSERS, 1999). Biological aspects of TSE diseases, such as species barriers, polymorphism barriers, and the nongenetic propagation of prion strain phenotypes are reflected in the specificities of PrP conversion reactions (BESSEN, 1995; KOCISKO et al., 1995; BOSSERS et al., 1997; RAYMOND, 1997).

For sheep *PRNP*, 17 different and mutually exclusive amino acid polymorphisms

have been described at the time of writing this manuscript (GOLDMANN et al., 1990, 1991; LAPLANCHE et al., 1993; BELT et al., 1995; BOSSERS et al., 1996; HUNTER et al., 1996; TRANULIS et al., 1999; DeSILVA et al., 2003; GOMBOJAV et al., 2003; GUO et al., 2003; HEATON et al., 2003; ACIN et al., 2004; McINTYRE et al., 2006). However, among these, only three codons (136, 154 and 171) have an actual reported influence in the susceptibility to the disease. The polymorphisms at codons 136 (Ala/Val), 171 (Gln/Arg/His/Lys) and, to a lesser extent, 154 (Arg/His) have been reported as determining factors in scrapie susceptibility (BELT et al., 1995, HUNTER et al., 1996, HEATON et al., 2003). There is no described mechanism to explain why these particular codons should be the most relevant on scrapie genetic susceptibility. In this paper, we attempt to analyze *PRNP* genotype frequencies in healthy sheep, from the scrapie-free Brazilian Northeast. Scrapie in Brazil is not a real problem as occurring disease, where 4 cases of scrapie in sheep had been notified since 2003, with 2 in 2006 (MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO, 2007), but *PRNP* genotyping in relation to resistance/ susceptibility to the ovine TSE (scrapie) is expected to contribute for genetic selection programs and avoid the breakout of the disease in scrapie-free areas.

In order to contribute both to genetic improvement and health surveillance of typical Brazilian hair breeds of sheep, we have previously genotyped *PRNP* for Santa Ines breed (LIMA et al, 2007). In the present work we focused on *PRNP* analyses of Morada Nova breed, seeking to identify genotypes resistant or susceptible to scrapie witch, in general, it is accepted that mutations coding for A136 and R171 confer higher resistance, and those for V136 and Q171 render animals more susceptible to scrapie, while polymorphisms in codon 154 are considered of minor importance (GAMA et al., 2006). As a result, animals of the ARR/ARR homozygous genotype are considered highly resistant and those of the VRQ/VRQ genotype highly susceptible to clinical scrapie (HUNTER et al., 1997; ELSEN et al., 1999).

Morada Nova is a meat type breed which has a significant importance for agriculture and social inclusion for countryside human populations living in semi-desert regions of Brazil, where raising hair sheep is a part of subsistence agriculture. Along the years Morada Nova sheep has proven to be a hair breed perfectly adapted to the dry Northeastern regions of Brazil. The reported *PRNP* genotypes provide the first survey of Morada Nova breed for TSE susceptibility in Brazil.

MATERIAL AND METHODS

Sampling

In this study 72 purebred healthy subjects from two different flocks and two color variants (29 red and 43 white) of the meat type, hair breed Morada Nova have been analyzed. The studied flocks belonged to the 'Centro Nacional de Pesquisa em Caprinos' (CNPC-EMBRAPA) and to the experimental farm of 'Universidade Federal do Ceará', both located in the State of Ceará (NE Brazil), a completely scrapie-free environment. The estimate minimum allele frequency required for detection, assuming 10% sharing of haploid genomes, and a sample size of 72 individuals, is less than 0.02 (HEATON et al., 2001, 2003). Animals were genotyped for scrapie-linked polymorphisms in the *PRNP* gene and partial nucleotide sequences were analyzed and translated into prion haplotypes.

DNA extraction and amplification

Genomic DNA was isolated from mononuclear cells obtained from EDTA treated blood, using a CLB (Sucrose, Tris HCL 2 M, MgCl2 and Triton X-100), SLB (Tris HCL 2 M, EDTA 0.5M, NaCl 5 M, SDS10%) and Proteinase K protocol, modified from the salting out method (Miller et al. 1988). To obtain nucleotide sequences for *PRNP*, 794-bp amplifications of exon 3 ORF were obtained through Polymerase Chain Reaction (PCR). PCR assays were performed in a 10 µl reaction volume containing 1 µL (50 ng) genomic DNA, 1 µM of each primer (P8: 5'-

CAGGTTAACGATGGTGAAAAGCCACATAGG-3'; P143: 5'-CTGGGAT TCTCTCTGGTACTG-3') and 5µL of ReadyMix™ Taq PCR Reaction Mix (Sigma-Aldrich Corp., USA) containing 20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3 mM MgCl2, 0.002% gelatin, 0.4 mM dNTP mix, stabilizers, 0.06 units Taq DNA Polymerase per µL). Amplification was performed in a Primus 96 Plus thermal cycler (MWG-Biotech AG, Germany) starting with one cycle of 1 min at 95°C, 1.5 min at 58°C and 1.5 min at 72°C followed by 39 cycles of 1 min at 94°C, 1.5 min at 58°C and 1.5 min at 72°C, followed by a final extension step of 10 min at 72°C. PCR Products were checked through electrophoresis on 1% agarose gels containing 0.1 µg / ml ethidium bromide.

DNA Sequencing

PCR products were treated with shrimp alkaline phosphatase (1 unit/µL; Amersham Biosciences) and Exonuclease III (10 units/µl; Amersham Biosciences) using a volume equaling 5% that of the PCR, incubated at 37°C for 30 min followed by 80°C for 10 min. PCR products were then submitted to ethanol precipitation and directly cycle-sequenced. Cycle sequence reactions were performed in 10 ìL reactions, using 0.5 ìM primer P143 and BigDye® Terminator 3.0 Kit (Applied Biosystems), following manufacturer instructions. Sequence reactions were read on an ABI Prism 3100® Genetic Analyzer (Applied Biosystems).

Sequence Analysis for Genotype Frequency Determination

Individual sequences were initially edited to remove noise and ambiguities both at the 5' and 3' ends. Only high-quality sequences were analyzed and about 15% of the samples were re-sequenced or analyzed more than once. All sequences were analyzed using the Phred/Phrap/Consed suit of programs (EWING & GREEN, 1998, GORDON et al., 1998). PolyPhred version 4 (NICKERSON et al., 1997) was used to identify single nucleotide polymorphisms (SNPs). SNPs were also detected by electropherogram data manually checked for accuracy at codons where polymorphisms were noted. Any ambiguous codon identifications were discarded from the final analysis. Amino acid polymorphisms at codons 136 (Ala/Val), 154 (Arg/His) and 171 (Gln/Arg/His/Lys) were used for distinction of the ovine PrP genotypes. The three combined codons were used to build the *PRNP* haplotypes. The online Basic Local Alignment Search Tool (BLAST, ALTSCHUL et al., 1997) has been used to perform homology searches and to deduce the respective amino acid sequences.

Quantitative and Statistical Analysis of Genotype Data

In order to partially automate the manual interpretation of sequences, the relative quantity of nucleotides (1 or 2) in each position sample was determined using an Excel template. For this purpose, the relative peak areas for each position were calculated as fractions of the total sum of peak areas in a certain position sample. Subsequently, the fraction of each peak was divided by the average peak fractions of the corresponding positions in each sample. Finally, the values were normalized using the values obtained for the peak, as a reference for the reliability. Data is presented in terms of both allelic variant and genotype frequency of the *PRNP* gene highly polymorphic sites. Allele and genotype distributions were used to infer haplotypes for combinations of polymorphisms, using the program HAPLOTYPER 1.0 (NIU et al., 2002). Polymorphisms at codons 136, 154, and 171 were compared using Chi-square contingency tables and comparisons served as references for indicating the genotypes.

RESULTS

PRNP allele and genotype variation

Among the 72 examined sheep we have found the well known dimorphism at codon 136 (Ala/Val). However, the reported dimorphism at codon 154 (Arg/Hist) and tetramorphism at codon 171 (Gln/Arg/His/Lys) have not been characterized. At codon 171 only Gln and Arg have been detected, and at codon 154 only Arg. Among four possible combinations, only three

different allelic variants were found (ARQ, ARR e VRR). Further than that, among nine possible genotypes only four have been detected (Table 1). Only two animals showed the rare genotype VRR/VRR (2.78%), which is believed to be associated with high scrapie susceptibility (BELT et al., 1995). The low frequency at which this genotype has been observed allows no major concern for potential risk among the Brazilian hair breed Morada Nova. The genotype VRR/VRR must be considered extremely rare when compared to other ovine breeds studied to date (Kutzer et al. 2002). The analysis revealed four different PrP genotypes, including two of high risk of scrapie susceptibility, ARQ/ARQ and VRR/VRR.

PRNP genotype and allele differences between color variants

A contingency chi-square test has shown that the genotype differences observed between color variants is highly significant (P<0.01). It was not possible to study the interaction between genotype and sex because there were only four males among the sampled animals. ARQ/ARQ is the most frequent genotype in the red variant (65.52%), while on the white variant its frequency reaches only 14.0%. In the white variant the most frequent genotype is ARR/ARR (44.19%). Following that genotype distribution it is clear that allelic frequencies should be also significantly different between variants. Allele VRR has been observed only in the white variant, and has not been observed among the four sampled males.

DISCUSSION

Around the world there are more than 300 recognized meat and wool breeds of sheep. Among them hair breeds are selected for a non-wool producing characteristic. Morada Nova is an example of a hair-shedding breed perfectly adapted to the drought Northeastern region of Brazil where features such as hardness and prolificacy that, associated with good meat characteristics, constitute specially interesting advantages for the human population that rely on it for small farming enterprises as well as subsistence. *PRNP* genotyping in relation to resistance/susceptibility to the ovine TSE is

Table 1. Prion Protein gene (*PNRP*) genotype and allele frequencies for two color variants of the hair sheep Morada Nova, from NE Brazil. Allele types represent the observed variation at codons 136 (A/V), 154 (R) and 171 (Q/R) among 72 subjects (observed numbers in parenthesis). The differences on genotype and allele frequencies among color variants have proven to be highly significant after chi-square contingency tests (both at P<0.01).

	Color Variant		
PRNP Genotype	Red	White	Total
ARQ/ARQ	0.655 (19)	0.140 (6)	0.347 (25)
ARQ/ARR	0.207 (6)	0.372 (16)	0.306 (22)
ARR/ARR	0.138 (4)	0.442 (19)	0.319 (23)
VRR/VRR	0.000 (0)	0.047 (2)	0.028 (2)
Allele			
ARQ	0.759	0.326	0.500
ARR	0.241	0.628	0.472
VRR	0.000	0.047	0.028

expected to contribute both for health surveillance and genetic selection programs. Although scrapie in Brazil is not a real problem as occurring disease, where 4 cases of scrapie in sheep had been notified since 2003, with 2 in 2006 (Agriculture Ministery- Brazil, 2007), it is a worldwide concern that has impelled many countries to develop policies aimed at eliminating TSE-affected animals from their food chains. Key components of TSE eradication programs include restocking with animals from TSE-free regions (HEATON et al., 2003), such as tropical countries, where *PRNP* genotypes of sheep have not yet been properly studied. Sheep with genetic resistance to scrapie are desirable for restocking because disease recurrence from exposure to environmental prion contamination may occur in endemic areas (THORGEIRSDOTTIR et al., 1999). Thus, identifying genetic variation related to scrapie resistance is an important step towards a strategy to eliminate TSE from the food chain. Various PrP isoforms may influence TSE susceptibility in sheep and at least 17 SNPs in the *PRNP* coding region have been published and/or reported in GenBank. Nucleotide variants affecting the translation of codons 136, 154, and 171 are the most often studied polymorphisms associated with variation in susceptibility to scrapie. *PRNP* allele encoding respectively for alanine, arginine, and arginine (ARR) at the cited positions is linked to increased scrapie resistance, whereas valine, arginine, and glutamine allele (VRQ) is related to increased scrapie susceptibility (BELT et al., 1995; HUNTER et al., 1996; BAYLIS et al., 2002; HEATON et al., 2003). It is believed that the least susceptible genotype is ARR/ARR, while the most susceptible one should be VRQ/VRQ. Scrapie susceptibility for other known *PRNP* alleles (ARQ, AHQ, ARH, ARR) is less clear, because susceptibility appears to vary somewhat among breeds and populations (GOLDMANN et al., 2005). Nevertheless, individuals with two alleles encoding glutamine at codon 171 (*i.e.* 171QQ) have been reported as susceptible to natural scrapie (GOLDMANN et al., 1994; WESTAWAY et al., 1994; O'ROURKE et al., 1997). *PRNP* alleles encoding arginine at position 171 are dominant for increased scrapie resistance, and thus, heterozygous individuals (171QR) are usually considered resistant (HEATON et al., 2003).

This study aimed at identifying polymorphisms on Morada Nova sheep *PNRP*, in order to set the start for a nationwide scrapie evaluation program. *PRNP* genotypes reported here are part of the first surveys of *PRNP* ovine Morada Nova breed genotypes reported in Brazil. The results obtained have indicated a consistent homozygosis for *PRNP* alleles, as well as low allelic diversity, when compared to other sheep breeds where up to 10 allelic variants have been reported (DROGEMULLER et al., 2001; GOMBOJAV et al., 2003; HEATON et al, 2003; DeSILVA et al, 2003; Billinis et al., 2004). Results for another hair-shedding breed in Brazil, Santa Inês (LIMA et al., 2007), has shown a considerably greater variation, including four allelic variants and six different genotypes for a smaller scale survey of only 29 subjects. In all ovine breeds studied to date an amino acid change from arginine (R) to glutamine (Q) at codon 171 in *PRNP* seems to render the animal more susceptible to natural and experimental scrapie. In addition to that scrapie susceptibility seems to be significantly increased by the presence of valine (V) at codon 136 (CLOUSCARD et al., 1995). In breeds where the VRQ allele is rare or absent, such as the Morada Nova here studied, the wild type ARQ allele is associated with susceptibility to scrapie (KUTZER et al. 2002). According to this Morada Nova would be potentially susceptible, especially the red type, where allele ARQ is present at high frequency.

Regarding observed site polymorphisms alanine (A) at codon 136 seems to be far more common than valine (V) in Brazilian sheep breeds. The predominance of alanine at codon 136 has been reported several times for other breeds around the world (O'DOHERTY et al., 2000; GOMBOJAV et al,. 2003; HEATON et al., 2003; DeSILVA et al., 2003; BILLINIS et al., 2004). In Morada Nova breed, the susceptible V136 has been found only in combination with the resistant R171. However, the high frequency of allele Q171 among Morada Nova subjects

raises a potential risk in cross-breeding these *PRNP* genotypes. Allele VRQ is rare or absent in several breeds, such as Suffolk and Mongolia, and was not found in any of the 72 animals genotyped. On the other hand, the allele ARQ, associated to scrapie susceptibility have been observed in Morada Nova. Allele VRR seems to rarely occur at more than low frequencies among sheep (KUTZER et al., 2002). However, VRR has been found in homozygosis among white Morada Nova. Further investigation is necessary to explain this observation. The results presented here show previously undescribed genetic diversity of *PRNP* and provide allele frequency estimates for healthy Morada Nova sheep, from NE Brazil. Knowledge about less frequent alleles is critical in estimating genotyping error rates and in evaluating their association with TSE susceptibility (HEATON et al., 2003), Further and wider *PRNP* surveys in Brazilian herds will be necessary for establishing selection strategies that comply to national and international scrapie eradication programs. It may be possible to reduce the incidence of ovine scrapie by increasing the frequency of resistant genotypes (AA-136, RR-171 or QR-171). By identifying individuals genetically resistant to scrapie and selectively breeding them, we can increase the efficiency with which the disease is avoided from the food chain. In many countries, breeding programs for resistance to scrapie in sheep have already been established. The demand for a better knowledge of livestock genotype composition is steadily increasing in Brazil. Initiatives such as the one reported here, for scrapie susceptible genotypes, can contribute significantly for the improvement of genetic quality and health surveillance of the local sheep herds.

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REFERENCES

ACÍN, C.; MARTÍN-BURRIEL, I.; GOLDMANN, W.; LYAHYAI, J.; MONZON, M.; BOLEA, R.; SMITH, A.; RODELLAR, C.; BADIOLA, J. J.; ZARAGOZA, P. Prion protein gene polymorphisms in healthy and scrapie-affected Spanish sheep. *Journal General Virology*, v. 85, p. 2103-2110, 2004.

ALTSCHUL, S.F.; MADDEN, T.L.; SCHAFFER, A.A.; ZHANG, J.; ZHANG, Z.; MILLER, W.; LIPMAN, D.J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, v. 25, p. 3389-3402, 1997.

APPLIED BIOSYSTEMS, ABI Prism 3100® Genetic Analyzer, Sequencing Chemistry Guide, © Copyright 53p, 2001.

BAYLIS, M.; GOLDMANN, W.; HOUSTON, F.; CAIRNS, D.; CHONG, A.; ROSS, A.; SMITH, A.; HUNTER, N.; MCLEAN, A. R. Scrapie epidemic in a fully PrP-genotyped sheep flock. *Journal General Virology*, v. 83, p. 2907-2914, 2002.

BELT, P.B.G.; BROWN, H.R.; RUDELLI, R.D.; SCALA, L.F.; GOLLER, N.L.;WEN, G.Y.; KASCSAK, R.J.; CASHMMAN, N.R.; BOLTON, D.C. Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. *Journal General Virology*, v. 76, p. 509-517, 1995.

BESSEN, R. A.; KOCISKO, D. A.; RAYMOND, G. J.; NANDAN, S.; LANSBURY, P. T.; CAUGHEY, B. Non genetic propagation of strain-specific properties of scrapie prion protein. *Nature*, v. 375, p. 698-700, 1995.

BILLINIS, C.; PSYCHAS, V.; LEONTIDES, L.; SPYROU, V.; ARGYROUDIS, S.; VLEMMAS, I.; LEONTIDES, S.; SKLAVIADIS, T.; PAPADOPOULOS, O. Prion protein gene polymorphisms in healthy and scrapie affected sheep in Greece. *Journal General Virology*, v. 85, p. 547-54, 2004.

BIRKETT, C. R.; HENNION, R. M.; BEMBRIDGE, D. A.; CLARKE, M. C.; CHREE, A.; BRUCE, M. E.; BOSTOCK, C. J. Scrapie strains maintain biological phenotypes on propagation in a cell line in culture. *EMBO Journal*, v. 20, p. 3351-3358, 2001.

BOSSERS, A.; SCHREUDER, B.E.; MUILEMAN, I.H.; BELT, P.B.; SMITS, M.A.; PrP genotype contributes to determining survival times of sheep with natural scrapie. *Journal General Virology*, v. 77, p. 2669-2673, 1996.

BOSSERS, A.; BELT, P.B.G.M.; RAYMOND, G.J.; CAUGHEY, B.; DE VRIES, R.; SMITS, M.A. Scrapie susceptibility-linked polymorphisms modulate the *in vitro* conversion of sheep prion

protein to protease-resistant forms. *Proceedings National Academy Sciences*, v. 94, p. 4931-4936,1997.

BOSSERS, A.; HARDERS, F. L.; SMITS, M.A. PrP genotype frequencies of the most dominant sheep breed in a coutry free from scrapie. *Archives of Virology*, v. 144, p. 829-834, 1999.

BOSSERS, A.; DE VRIES, R.; SMITS, M. A. Susceptibility of Sheep for Scrapie as Assessed by *In Vitro* Conversion of Nine Naturally Occurring Variants of PrP. *Journal Virology*, v. 74, p.1407-1414, 2000.

CLOUSCARD, C.; BEAUDRY, P.; ELSEN, J.M.; MILAN, D.; DUSSAUCY, M.; BOUNNEAU, C.; SCHELCHER, F.; CHATELAIN, J.; LAUNAY, J.M.; LAPLANCHE, J.L. Different allelic effects of the codons 136 and 171 of the prion protein gene in sheep with natural scrapie. *Journal General Virology*, v. 76, p. 2097-2101, 1995.

DeSILVA, U.; GUO, X.; KUPFER, D.M.; FERNANDO, S.C.; PILLAI, A.T.; NAJAR, F.Z.; SO, S.; FITCH, G.Q.; ROE, B.A. Allelic variants of ovine prion protein gene (PRNP) in Oklahoma sheep. *Cytogenetics Genome Research*, v. 102, p. 89-94, 2003.

DROGEMULLER, C. ; LEEB, T.; DISTL, O. PrP genotype frequencies in German breeding sheep and the potential to breed for resistance to scrapie. *Veterinary Record* v. 149, p. 349-52, 2001.

ELSEN, J.M.; AMIGUES, Y.; SCHELCHER, F.; DUCROCQ, V.; ANDREOLETTI,O.; EYCHENNE, F.; TIEN KHANG, J.V.; POIVEY, J.P.; LANTIER, F.; LAPLANCHE, J.P. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. *Archives of Virology*, v. 144, p. 431–445, 1999.

EWING, B.; GREEN, P. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Research*, v. 8, p.186-94, 1998.

GAMA, L.T.; CAROLINO, M.I.; SANTOS-SILVA, M.F.; PIMENTA, J.A.; COSTA, M.S., Prion protein genetic polymorphisms and breeding strategies in Portuguese breeds of sheep. *Livestock Science*, v. 99, p.175– 184, 2006.

GOLDMANN, W.; HUNTER, N.; FOSTER, J.D.; SALBAUM, J.M.; BEYREUTHER, K.; HOPE, J. Two alleles of a neural protein gene linked to scrapie in sheep. *Proceedings National Academy Sciences*, v. 87, p. 2476-2480, 1990.

GOLDMANN, W.; HUNTER, N.; BENSON, G.; FOSTER, J.D.; HOPE, J. Different scrapie-associated fibril proteins (PrP) are encoded by lines of sheep selected for different alleles of the Sip gene. *Journal General Virology*, v. 72, p. 2411-2417, 1991.

GOLDMANN, W.; HUNTER, N.; SMITH, G.; FOSTER, J.; HOPE, J. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *Journal General Virology*, v. 75, p. 989-995, 1994.

GOLDMANN, W., BAYLIS, M., CHIHOTA, C., STEVENSON, E., HUNTER, N. Frequencies of PrP gene haplotypes in British sheep flocks and the implications for breeding programmes. *Journal of Applied Microbiology*, v. 6, P. 1294–1302, 2005.

GOMBOJAV, A.; ISHIGURO, N.; HORIUCHI, M.; SERJMYADAG, D.; BYAMBAA, B.; SHINAGAWA, M. Amino acid polymorphisms of PrP gene in Mongolian sheep. *Journal Veterinary Medicine Science*, v. 65, p. 75-81, 2003.

GORDON, I.; ABDULLA, E.M.; CAMPBELL, I.C.; WHATLEY, S.A. Phosmet induces up-regulation of surface levels of the cellular prion protein. *Neuroreport*, v. 9, p. 1391-1395,1998.

GORODINSKY, A.; HARRIS, D.A. Glycolipid-anchored proteins in neuroblastoma cells from detergent-resistant complexes without caveolin. *Journal Cell Biology* v.129, p. 711-723, 1995.

GUO, X.; KUPFER, D.M.; FITCH, G.Q.; ROE, B.A.; DESILVA, U. Identification of a novel lysine-171 allele in the ovine prion protein (PRNP) gene. *Animal Genetics* v. 34, p. 303-305, 2003.

HEATON, M. P.; LEYMASTER, K. A.; FREKING, B. A.; HAWK, D.A.; SMITH, T.P.; KEELE, J.W.; SNELLING, W.M.; FOX, J.M.; CHITKO-MCKOWN, C.G.; LAEGREID, W.W. Prion gene sequence variation within diverse groups of U.S. sheep, beef cattle, and deer. *Mammalian Genome*, v. 14, 765-777, 2003.

HEATON, M.P.; CHITKO-MCKNOWN, C.G.; GROSSE, W.M.; KEELE, J.W.; KEEN, J.E.; LAEGREID, W.W. Interleukin-8 haplotype structure from nucleotide sequence variation in commercial populations of U.S. beef cattle. *Mammalian Genome*, v. 12, p. 219-226, 2001.

HORIUCHI, M.; NEMOTO, T.; ISHIGURO, N.; FURUOKA, H.; MOHRI, S.; SHINAGAWA, M. Biological and biochemical characterization of sheep scrapie in Japan. *Journal Clinical Microbiology*, v. 40, p. 3421-3426, 2002.

HUNTER, N.; FOSTER, J.D.; GOLDMANN, W.; STEAR, M.J.; HOPE, J.; BOSTOCK, C. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. *Archives Virology*, v. 141, p. 809-824, 1996.

HUNTER, N.; GOLDMANN, W.; FOSTER, J.D.; CAIRNS, D.; SMITH, G. Natural scrapie and PrP genotype: case-control studies in British sheep. *Veterinary Record*, v. 141, p.137-140,1997.

HUNTER, N.; CAIRNS, D. Scrapie-free Merino and Poll Dorset sheep from Australia and New Zealand have normal frequencies of scrapie-susceptible PrP genotypes. *Journal General Virology*, v. 79, p. 2079-2082, 1998.

KOCISKO, D.A.; PRIOLA, S.A.; RAYMOND, G.J.; CHESEBRO, B.; LANSBURY, P.T.J.R.; CAUGHEY, B. Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. *Proceedings National Academy Sciences*, v. 92, p. 3923-3927, 1995.

KUTZER, T.; PFEIFER, I.; BRENING B. Identification of new allelic variant in the ovine prion protein (PrP) gene. *Journal Animal Breeding Genetics*, v. 119, p. 201-208, 2002.

LAPLANCHE, J. L.; CHATELAIN, J.; Recebido em:26.06.07Aceito em: 18.07.07WESTAWAY D.; THOMAS S.; DUSSAUCY, M.; BRUGERE-PICOUX, J.; LAUNAY, J.M. PrP polymorphisms associated with natural scrapie discovered by denaturing gradient gel eletrophoresis. *Genomics* v. 15, p.30-37, 1993.

LEE, I.Y.; WESTAWAY, D.; SMIT, A.F.A.; WANG, K.; SETO, J.; CHEN, L., ACHARYA, C.; ANKENER, M.; BASKIN, D.; COOPER C.; YAO H.; PRUSINER, S.B.; HOOD, L.E.C. Complete genomic sequence and analysis of the prion protein gene region from three mammalian species. *Genome Research*, v. 8, p. 1022-1037,1998.

LIMA, A. C.; BOSSERS, A.; SOUZA, C.; OLIVEIRA, S. M. P.; OLIVEIRA, D. M.. PrP genotypes in a pedigree flock of Santa Inês sheep. *Veterinary Record*, v. 160, p. 336-337, 2007

McINTYRE, K.M.; GUBBINS S.; GOLDMANN W.; STEVENSON E.; BAYLIS M. The time-course of a scrapie outbreak. *BMC Veterinary Research,* v. 2:20, p. 1-6, 2006.

MILLER, S.A.; DYKES, D.D.; POLESKY, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, v. 16, 1215-1221,1988.

MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO. Informação técnica: Situação atual do Brasil frente à *Scrapie*.http://www.agricultura.gov.br/portal/page?_pageid=33,3271785&_dad=portal&_schema=PORTAL 2007.

NICKERSON, D.A.; TOBE, V.O.; TAYLOR, S.L. Polyphred: Automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. *Nucleic Acids Research*, v. 25, p.2745–2751, 1997.

NIU, T.; QIN, Z.S.; XU, X.; LIU, J.S. Bayesian haplotype inference for multiple linked single nucleotide polymorphisms. *American Journal Human Genetics*, v. 70, 157-169, 2002.

O'DOHERTY, E.; AHERNE, M.; ENNIS, S.; WEAVERS, E.; HUNTER, N.; ROCHE, J.F.; SWEENEY, T. Detection of polymorphism in the prion protein gene in a population of Irish Suffolk sheep. *Veterinary Record*, v. 146, p.335-338, 2000.

O'ROURKE, K. I.; HOLYOAK, G. R.; CLARK, W. W.; MICKELSON, J. R.; WANG, S.; MELCO, R. P.; BESSER, T. E.; FOOTE, W. C. PrP genotypes and experimental scrapie in orally inoculated Suffolk sheep in the United States. *Journal General Virology*, v. 78, p. 975-978, 1997.

POCCHIARI, M. Prions and related neurological disorders. *Molecular Aspects Medicine*, v. 15, p.195-291,1994.

PRUSINER, S.B. Introduction: Prion diseases. *Seminars in Virology* v. 7, p. 157-161, 1996.

PRUSINER, S.B. Prions. *In* Proceedings National Academy Sciences Nobel Lecture v. 95, p.13363-13383, 1998.

RAYMOND, G. J.; HOPE, J.; KOCISKO, D. A.; PRIOLA, S. A.; RAYMOND, L. D.; BOSSERS, A.; IRONSIDE, J.; WILL, R. G.; CHEN, S. G.; PETERSEN, R. B.; GAMBETTI, P.; RUBENSTEIN, R.; SMITS, M. A.; LANSBURY, P. T. JR.; CAUGHEY, B. Molecular assessment of the potential transmissibilities of BSE and scrapie to humans. *Nature*, v. 388, p. 285.288, 1997.

SOLFOROSI, L.; CRIADO, J. R.; MCGAVERN, D. B.; WIRZ, S.; SÁNCHEZ-ALAVEZ M.; SUGAMA, S.; DEGIORGIO, L. A.; VOLPE, B. T.; WISEMAN, E.; ABALOS, G.; MASLIAH, E.; GILDEN, D.; OLDSTONE, M. B.; CONTI, B.; WILLIAMSON, A. Crosslinking cellular prion protein triggers neuronal apoptosis in vivo. *Science*, v. 303, p. 1514-1516, 2004.

THORGEIRSDOTTIR, S.; SIGURDAESON, S.; THORISSON, H. M.; GEORGSSON, G.; PALSDOTTIR, A. Prp gene polymorphism and natural scrapie in Iceland sheep. *Journal General Virology*, v. 80, p. 2527-2534,1999.

TRABOULOS, A.; SCOTT, M.; SEMENOV, A.; AVRAHAMI, D.; LASZLO, L.; PRUSINER, S.B. Cholesterol depletion and modification of COOH-

terminal targeting sequence of the prion protein inhibits formation of the scrapie isoform. *Journal Cell Biology*, v. 129, p. 121-132,1995.

TRANULIS, M. A.; OSLAND, A.; BRATBERG, B.; ULVUND, M. J. Prion protein gene polymorphisms in sheep with natural scrapie and healthy controls in Norway. *Journal General Virology*, v. 80, p.1073-1077, 1999.

VEY, M.; PILKUHN, S.; WILLE, H.; NIXON, R.; DEARMOND S.J.; SMART, E.J.; ANDERSON, R.G.; TARABOULOS, A.; PRUSINER, S.B. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. *Proceedings National Academy Sciences*, v. 93, p.14945-14949, 1996.

WESTAWAY, D.; ZULIANI, V.; COOPER, C.M.; DA COSTA, M.; NEUMAN, S., JENNY, A.L.; DETWILER, L.; PRUSINER, S.B. Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. *Genes & Development*, v. 8, p. 959-969, 1994.

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