PrP genotypes in a pedigree flock of Santa Inês sheep

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SCRAPIE is a prion disease of sheep and goats, and is characterised by the accumulation of pathologically folded prion protein (PrP) derived from host-encoded PrP. For sheep, over 40 polymorphisms of PrP have been described (Laplanche and others 1993, Goldmann and others 1994, Belt and others 1995, Bossers and others 1996, Tranulis and others 1999, O'Rourke and others 2000, Vaccari and others 2001, Gombojav and others 2003, 2004, Seabury and Derr 2003, Acin and others 2004, Hunter and Bossers 2006). The polymorphisms at codons 136, 154 and 171 have a significant association with scrapie susceptibility.

In Brazil, scrapie has been detected in Hampshire Down and Suffolk sheep since the late 1970s (Driemeier 1998). Recently, scrapie cases were reported in Hampshire Down sheep imported from the USA, in the city of Candóia/PR (Felício 2001) and in the city of Curitiba/PR (World Organisation for Animal Health [OIE] 2003). Despite the occurrence of clinical cases, there is still scant information on scrapie in Brazil. This small-scale study was conducted to evaluate the PrP genotypes in a pedigree flock of sheep in Brazil.

Blood samples were collected from 29 Santa Inês sheep from a commercial flock in the state of Ceará. Genomic DNA was isolated using Dynabeads (DNA Direct System II; Dynal, Norway) (A. Bossers, personal communication). Thereafter the PrP open-reading frame was screened for polymorphisms by TaqMan real-time PCR amplification (A. Bossers, F. L. Harders, unpublished observations). Screening for hitherto unknown mutations, and confirmation of TaqMan PrP genotypes, was performed using PCR amplification of the entire PrP open-reading frame and classical Sanger sequencing (Belt and others 1995) on an ABI automated sequencer. Samples were sequenced forwards and in reverse. PrP alleles were reconstructed on the assumption that all mutations are mutually exclusive. New polymorphisms were checked for haplotype linkage by cloning into a PCR-topo vector (Invitrogen Technologies) and subsequent sequencing.

In total, nine alleles had a polymorphism at a position other than 136, 154 or 171 (Table 1). These alleles resulted in 13 additional PrP genotypes. Two of the nine polymorphisms formed hitherto non-described PrP alleles, with polymorphisms at codons 142 (t→c; I to T) and 167 (g→c; R to T) (Table 1). All polymorphisms were confirmed as mutually exclusive by single allele sequencing. The most common alleles were ARR (31·0 per cent), followed by ARQ (20·7 per cent). Other more common alleles such as AHQ (8·6 per cent) and VRQ (1·7 per cent) were found at a lower frequency (Table 1). Of the less common alleles, the ARQ(D172) and (P116)ARQ alleles were found most frequently at 8·6 and 6·9 per cent, respectively.

For the more common PrP alleles (VRQ, ARQ, AHQ and ARR), a clear association with scrapie susceptibility has been described for many breeds in different countries (Hunter and others 1996, Hunter 1997, Laplanche and others 1993, Goldmann and others 1994, Bossers and others 1996, 2000, Tranulis and others 1999). The A(F141)RQ allele, which is frequently found among various breeds in Europe, was also found in this study, albeit with a low frequency (5·2 per cent). Some of the rare polymorphisms have been described before for native herds from Norway and Iceland, in Mongolian sheep, and in sheep from the USA (Thorgeirsdottir and others 1999, Tranulis and others 1999, O'Rourke and others 2000, Gombojav and others 2003).

TABLE 1: PrP allele frequencies (n=58), with polymorphisms at positions other than 136, 154 and 171

Allele*	Frequency (%)
ARQ	20·7
VRQ	1·7
AHQ	8·6
ARR	31·0
(P116)ARQ	6·9
(S127)ARQ	1·7
A(N138)RQ	5·2
A(F141)RQ	5·2
A(T142)RQ	1·7
A(R143)RQ	1·7
AR(T167)Q	1·7
ARQ(D172)	8·6
ARQ(L189)	5·2
Total	100

* A Alanine, R Arginine, Q Glutamine, H Histidine – these are at the most important codons 136, 154 and 171 of the sheep PrP open reading frame. Polymorphisms at other positions are indicated separately: P Proline, S Serine, N Asparagine, F Phenylalanine, T Threonine, D Aspartic acid, L Leucine

In this study, 19 different PrP genotypes formed by 13 different PrP alleles were found. The PrP genotype frequencies found in the samples were as follows: ARQ*/ARQ* (37·9 per cent) (ARQ* comprises all alleles with amino acid positions at 136A and 154R but not 171R, including alleles with additional polymorphisms at different codons); ARQ*/ARR (31·0 per cent); AHQ/ARQ* (10·3 per cent); VRQ/ARR (3·4 per cent); AHQ/ARR (6·9 per cent); and ARR/ARR (10·3 per cent). Among the population studied, 41·3 per cent of the animals (ARQ*/ARQ* and VRQ/ARR) were characterised as susceptible to scrapie (Dawson and others 1998). Interestingly, although the VRQ allele was present, no highly susceptible animals were found with the VRQ/VRQ or VRQ/ARQ* PrP genotypes.

Although Santa Inês hairy sheep have potentially susceptible genotypes for scrapie, the disease outbreaks in Brazil in 2001 and 2003 did not affect the breed. Both of the cases reported were Hampshire Down sheep. However, although no scrapie cases have been reported in hairy sheep, it is important to know the genotypic profile of these sheep, because crossbreeding of Santa Inês dams with Suffolk and Hampshire Down rams is becoming more frequent.

In conclusion, almost half of the animals could be classified as being potentially susceptible to scrapie. Fortunately the most resistant genotype ARR was also present, providing a future tool for scrapie control through selective breeding.

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