



## Prion Protein Genotypes in Pakistani Goats

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**ABSTRACT :** The PCR-amplified prion protein (PrP) gene was sequenced to determine the frequency of scrapie-associated as well as novel PrP genotypes in 72 healthy goats representing five breeds. A total of six genotypes were detected, resulting from the three reported 143 (H/R), 154 (R/H) and 240 (S/P) and the two novel 39 (S/R) and 185 (I/F) amino acid polymorphisms. Of the four silent mutations 42 (a→g), 138 (c→t), 231 (c→a) and 237 (g→c) detected in this study, 237 (g→c) is novel. A genotype (SIP/RFP) harboring three amino acid polymorphisms 39 (S/R), 185 (I/F) and 240 (S/P) was found in few goats. Although both scrapie-associated genotypes with 143 (H/R) and 154 (R/H) polymorphisms and others with 39 (S/R), 185 (I/F) and 240 (S/P) polymorphisms were present in the studied Pakistani goats, their frequency was lower than that of the wild-type genotype SHRIS/SHRIS (34.7%). These results emphasize the need for further sequencing of the PrP gene in a large number of goats representing the five studied breeds, so that overall PrP variability can be assessed in these breeds in research addressing future concerns about scrapie. (**Key Words :** Prion Protein Genotypes, Scrapie, Goats, Pakistan)

### INTRODUCTION

Prions are lethal mammalian pathogens composed of aggregated conformational isomers (PrP<sup>Sc</sup>) of a host-encoded normal 33-35 kDa glycoprotein (PrP<sup>C</sup>) and are believed to be the sole causative agents of Transmissible Spongiform Encephalopathies (TSEs) (Prusiner, 2004; Collinge and Clark, 2007). TSEs are a group of fatal neurodegenerative diseases, which include scrapie in sheep and goats, chronic wasting disease in elk and deer, transmissible mink encephalopathy (TME) and Creutzfeldt-Jakob Disease (CJD) in humans. Among TSEs, scrapie in sheep and goats is clinically diagnosed by behavioral changes, hypersensitivity to touch, ataxia of hind limbs, head tremor, teeth grinding, chronic wasting (loss of weight), pruritus, wool loss, lack of co-ordination and, at a later stage, paralysis and death (Hurtado et al., 2002; [http://www.neurocenter-bern.ch/scrapie\\_e.shtml](http://www.neurocenter-bern.ch/scrapie_e.shtml)). However, the confirmed diagnosis of scrapie can only be made after performing immunohistochemistry and histopathology of CNS and lymphoid tissue of infected animals, which identifies in these tissues the PrP<sup>Sc</sup> accumulation and

spongiform lesions generated by a wide-spread vacuolisation of the neuropil (Prusiner, 1998; Vaccari et al., 2006).

The natural incidence of scrapie varies among animals indicating that the genotype of the host plays a role in modulating susceptibility to the disease (8). In sheep, genotypes based on the amino acid polymorphisms in codons 136 (A/V), 154 (R/H) and 171 (Q/R or H) of the prion protein (PrP) gene are associated with scrapie susceptibility. ARR/ARR is the most scrapie-resistant genotype; while VRQ/VRQ is the most scrapie-susceptible genotype (Baylis and Goldmann, 2004). In goats, the PrP genotypes based on the amino acid polymorphisms in codons 142 (I/M), 143 (H/R), 154 (R/H) and 222 (Q/K) are associated with scrapie susceptibility (Goldmann et al., 1996; Billinis et al., 2002; Vaccari et al., 2006). In addition to scrapie-associated polymorphisms 142 (I/M), 143 (H/R), 154 (R/H) and 222 (Q/K), the following PrP polymorphisms have been reported for goats: 21 (V/A), 23 (L/P), 37 (G/V), 49 (G/S), 102 (W/G), 110 (T/N) or 110 (T/P), 127 (G/S), 133 (L/Q), 137 (M/I), 146 (N/S) or 146 (N/D), 151 (R/H), 168 (P/Q), 211 (R/Q), 218 (I/L), 220 (Q/H), 222 (Q/K) and 240 (S/P) (Goldmann et al., 1996; Goldmann et al., 1998; Wopfner et al., 1999; Agrimi et al., 2003; Billinis et al., 2002; Goldmann et al., 2004; Zhang et al., 2004; Kurosaki et al., 2005; Acutis et al., 2006; Pappasavva-Stylianou et al.,

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2007). Some silent mutations of the PrP gene in goats have also been reported: 42 (a→g), 107 (g→a), 138 (c→t), 179 (g→t), 181 (c→t), 202 (c→t), 207 (g→a), 219 (c→t) and 231 (a→c) (Goldmann et al., 1996; Billinis et al., 2002; Zhang et al., 2004; Acutis et al., 2006; Vaccari et al., 2006; Papasavva-Stylianou et al., 2007).

Pakistan has more than 50 million each of sheep and goats. These sheep and goats serve the Pakistani nation in terms of meat, milk and wool and add to the annual income of the poor community of farmers (GOP, 2006). Despite having a huge population, studies regarding genetic resistance to scrapie remain to be undertaken in Pakistani goats and sheep. So, the present study was conducted to determine scrapie-resistance in five goat breeds of Punjab, Pakistan.

## MATERIALS AND METHODS

### Animal source and sample collection

A total of 207 healthy goats representing five breeds: Pak-Angora (PA), Dera Din Panah (DDP), Teddy (T), Naachi (N) and Beetal (B) were sampled for blood collection. Forty nine blood samples were collected from PA, 46 from DDP, 56 from T, 36 from N and 20 from B. Most of the sampling was done on Government Livestock Farms set up in the Punjab province of Pakistan i.e. Government Livestock Experiment Station (LES) Kherawala, Layyah; Livestock Production Research Institute (LPRI) Bahadarnagar, Okara; Barani Livestock Production Research Institute (BLPRI) Kherimurat, Attock; Livestock Experiment Station (LES) Rakh Ghulaman, Bakkar and Government Livestock Farm Chak Katora, Hasilpur. Earlier these farms purchased purebred goats (except Pak-Angora) from a large number of villages to expand the genetic base of their existing breeds. Information regarding morphological and production parameters of the five selected breeds is given in Table 1. A few animals from these breeds were also sampled from village flocks. A 10 ml blood sample was taken from the jugular vein of each goat into a vacutainer, containing 100 µl of 0.5 M (pH 8.0) EDTA, and immediately transferred to an icebox and stored at -20°C until further processing.

### Number of animals selected per breed for sequencing

The PrP sequencing of 72 animals was done after analyzing genotyping results for the PrP codons 136 and 154 in four of the five PA, DDP, T, N and B breeds, which resulted in only one goat of the T breed being polymorphic for PrP codon 154 (R/H). Fourteen goats were selected from PA for sequencing of the PrP gene, 14 from DDP, 16 (with inclusion of the PrP polymorphic goat) from T, 14 from N and 14 from B breed that was not PrP genotyped. The purpose of sequencing was to determine PrP variability,

additional to the amino acid polymorphism 154 (R/H).

### PCR and sequencing

Extraction of genomic DNA was performed using frozen peripheral whole blood, according to the protocol of Grimberg et al. (1989). The PCR amplification of 876 bp containing the entire coding region in exon 3 of the PrP gene (GeneBank accession no. DQ346682) was carried out using forward (5'-1CTTTAAGTGATTTTACGTGG21-3') and reverse (5'-854TGGCAAAGATTAAGAA GATA ATG876-3') primers. The PCR amplification reactions contained 50 ng genomic DNA, 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 10 pM each primer and 1.5 units Ampli Taq Gold (Applied Biosystems, Foster City, CA) in a reaction volume of 25 µl. Thermal conditions of the PCR program were set as initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 45 s and extension at 72°C for 1 min. Then a 10 min final extension program was set at 72°C. The PCR products were purified by Exo SAP-IT (Amersham) according to the manufacturer's instructions and were used in the sequencing reactions. In addition to the above-mentioned primer pair, PrP7-U (5'-369AGCAGTGGTAGGGGGCC TTGGT391-3') and PrP-L2 (5'-430TTTGGCTTACTG GGCTTG TTCC451-3') primers were used in sequencing PCR reactions for bidirectional sequencing of the entire coding region of the PrP gene. The amplified products of sequencing PCR were washed with 70% ethanol and resolved on an ABI 3130 capillary DNA analyzer (Applied Biosystems, Inc., Foster City, CA). The results were analyzed using the Chromas Lite (2.01) software.

## RESULTS

Pakistani goats revealed a single amino acid polymorphism (R/H) for the PrP codon 154 in only one T goat amongst 187 goats belonging to 4 different breeds (PA, DDP, T and N). The remaining 186 goats were monomorphic for the both RFLP (Restriction fragment length polymorphism)-analyzed PrP codons 136 and 154. After finding this low frequency of polymorphism, sequence analysis of the PrP gene was carried out to discover more polymorphism in this gene. The PrP sequence analysis in 72 goats, including 14 goats from each of the PA, DDP, N and B (a new breed) and 16 from the T breed, identified 4 more amino acid polymorphisms 39 (S/R), 143 (H/R), 185 (I/F) and 240 (S/P) in addition to 154 (R/H). The sequence analysis also identified 4 silent mutations 42 (a→g; P/P), 138 (c→t; S/S), 231 (c→a; R/R) and 237 (g→c; L/L) and an intronic nucleotide polymorphism on 38 nucleotide upstream (-38 (c→t)) of the first PrP codon. Among these, two 39 (S/R) and 185 (I/F)

**Table 1.** General description and morphological parameters of studied goat breeds

Breed	Type	Color	Size	Average body weight (kg)	Average wool yield/annum	General description
Dera Din Panah	Milk/meat	Black	Large	45-50	1.5 kg/head	Large head, Roman nose, hair on the chin; long broad ears; cartilaginous appendages on sides of neck, long and thick spiraled horns; hairy body, tail medium covered with rough hair; udder and teats well-developed, milk yield 160 liters in a 150 days lactation period; twin births common
Teddy	Meat	Cremy-white, brown black or patched with these colors	Small	25-30	Generally	Compact body, small droopy ears, slightly prominent nose, horns may have spirals, both horned and polled specimens found; bucks often have beards; udder moderately developed, short conical teats, 65 liters milk in 130 days; twin and triplet occur at about 50 and 15%; early maturity and high prolificacy
Nachi	Milk/meat	Black but black and white spotted too	Medium-large	40-50	0.6 kg/head	Medium head, Roman nose, small and thin horns, medium ears; udder well-developed, 150 liters milk in 100 days twin births common, dancing gait is a specific feature of this breed b/c of which it is so named
Beetal	Milk/meat	Golden	Large	45-55	Smooth	Massive head, Roman nose, long broad and pendulous ears; spiraled horns, longer in males; long stout legs; short tail; udder well-developed and long teats, milk yield 190 liters in a 150 days lactation; more than 50% twin or triplet births
Pak-angora	Wool/meat	White	-	-	3 kg/head	Pak Angora breed is kept at the Government Livestock Experiment Station Kherawala (Layyah). It is a composite of Angora and hair goat breeds and it produces soft Mohair. Adult males weigh on average 47 kg and females 27 kg with an average wither height of 75 cm and 65 cm respectively. The breed is reported to be heat tolerant and to have an unspecified disease resistance. This breed is raised exclusively on a few government farms and has not been propagated. Of females, 100% are bred to males of the same breed.

amino acid polymorphisms and a 237 (g→c; L/L) silent mutation and -38 (c→t) nucleotide polymorphism are novel to the best of our knowledge.

The five detected amino acid polymorphisms comprised 6 genotypes amongst which the wild-type genotype SHRIS/SHRIS was the most common (34.7%), followed by the genotype SHRIS/SHRIP (26.4%). Only 240 (S/P) polymorphism-harboring genotypes were present in all

studied breeds with the exception of the wild-type genotype. The breeds T and B were the most PrP variable in terms of genotypes. N and B had a genotype SHRIP/RHRFP containing 3 polymorphisms (Table 2).

The silent mutations 42 (a→g) and 138 (c→t) were linked with 240 (S/P) dimorphism: 42a and 138c were linked with S240, while 42g and 138t were linked with P240. Although 138t was found to be linked with P240 in

**Table 2.** Percentage frequencies of genotypes in five goat breeds

Genotype	Number of genotypes/goat breed (% age frequency of genotype)					Total number of genotypes/goats	Overall % age frequency
	PA	DDP	T	N	B		
<sup>1</sup> S <sup>29</sup> H <sup>143</sup> R <sup>154</sup> T <sup>185</sup> S <sup>240</sup> /SHRIS	2(14.3)	7(50)	7(43.8)	2(14.2)	7(50)	25	34.7
SHRIS/SHHIS	-	-	1(6.2)	-	-	1	1.4
SHRIS/SRRIS	6(42.8)	-	2(12.5)	-	1(7.1)	9	12.5
SHRIS/SHRIP	4(28.6)	4(28.6)	5(31.3)	4(28.6)	2(14.3)	19	26.4
SHRIP/SHRIP	2(14.3)	3(21.4)	1(6.2)	4(28.6)	1(7.1)	11	15.3
SHRIP/RHRFP	-	-	-	4(28.6)	3(21.5)	7	9.7
Total	14	14	16	14	14	72	100

<sup>1</sup> Superscripts over single-letter representing amino acids refer to amino acid polymorphism/codon position.

most of the PrP sequences, it was also detected in genotypes with S240 monomorphism. Other silent mutations 231 (c→a) and 237 (g→c) and an intronic nucleotide polymorphism -38 (c→t) were present only in homozygous pattern i.e. 231 (a/a).

## DISCUSSION

Although scrapie is currently an incurable disease, genetic resistance of sheep and goats to this disease can be increased through selection of resistant PrP genotypes to avoid scrapie incidence. We performed sequence analysis of the PrP gene to assess the presence of scrapie-associated and other genotypes in 72 goats from five breeds. A total of 6 genotypes were detected amongst which the wild-type genotype SHRIS/SHRIS was the most common (34.7%) (Table 2), indicating that the studied goat breeds harbor low genetic variability at the PrP locus.

Two genotypes (SHRIS/SHH<sup>154</sup>IS and SHRIS/SR<sup>143</sup>RIS) carrying amino acid polymorphisms, which are reported to be associated with scrapie resistance (Billinis et al., 2002), were present in some goats. The SHRIS/SR<sup>143</sup>RIS genotype was present in 3 (PA, T and B) out of the five breeds; while the SHRIS/SHH<sup>154</sup>IS genotype was detected in only one T goat, confirming the genotyping results which represented the same single goat as polymorphic for the PrP codon 154 (R/H). The other genotypes carrying 142 (I/M), 146 (N/S), 146 (N/D) and 222 (Q/K) polymorphisms, which have also been shown to be associated with scrapie resistance (Goldmann et al., 1996; Acutis et al., 2006; Vaccari et al., 2006), were not detected in the present study. These results indicate that the studied goats are susceptible to contracting scrapie under conditions of inadvertent exposure to prions.

After the wild-type SHRIS/SHRIS, the more prevalent genotypes were SHRIS/SHRIP and SHRIS/SHRIP harboring 240 (S/P) polymorphism. Two contradictory statements about 240 (S/P) association with scrapie susceptibility have been documented: Goldmann et al. (1996) and Billinis et al. (2002) proposed that 240 (S/P) has no association with the disease due to its possible

elimination during post-translational processing of the PrP protein; whereas Acutis et al. (18) favored the role of 240 (S/P) in scrapie susceptibility modulation through its interference at the mRNA level.

The genotype SHRIP/RHRFP harbors two novel 39 (S/R) and 185 (I/F) polymorphisms which may be specific for the Pakistani goats. These polymorphisms add to the existing pool of PrP variability, and the experimental inoculation of prions in goats with SHRIP/RHRFP genotype may help determine their role, if any, in scrapie susceptibility.

In accordance with previous studies (Goldmann et al., 1996; Kurosaki et al., 2005; Acutis et al., 2006) 42 (a→g) and 138 (c→t) silent mutations were detected linked with 240 (S/P) dimorphism in most of the goats carrying SHRIS/SHRIP and SHRIS/SHRIP genotypes: 42a and 138c were linked with S240, whereas 42g and 138t were linked with P240. In contrast, genotypes carrying 138 (c→t) mutations with 240 (S/S) monomorphism were also detected in some goats in this study, indicating that 138 (c→t) occurred independent of 240 (S/P).

In brief, the present study provides information about scrapie resistance in the five goat breeds of Pakistan and suggests that further studies regarding the PrP variability in Pakistani goats should be undertaken to obtain true estimates of the frequency of scrapie-associated and novel genotypes in these breeds.

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