Prion protein gene polymorphisms in natural goat scrapie

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A total of 51 goats, including seven clinical cases, from the first herd in Greece reported to have scrapie was examined to discern an association between scrapie susceptibility and polymorphisms of the gene encoding the prion protein (PrP). Each animal was evaluated for clinical signs of the disease, histopathological lesions associated with scrapie, the presence of detectable protease-resistant PrP in the brain and PrP genotype. Eleven different PrP genotypes encoding at least five unique predicted mature PrP amino acid sequences were found. These genotypes included the amino acid polymorphisms at codons 143 (H → R) and 240 (S → P) and ‘silent’ nucleotide alterations at codons 42 (a → g) and 138 (c → t). Additionally, novel caprine amino acid polymorphisms were detected at codons 21 (V → A), 23 (L → P), 49 (G → S), 154 (R → H), 168 (P → Q) and 220 (Q → H) and new silent mutations were found at codons 107 (g → a) and 207 (g → a). The following variants were found in scrapie-affected goats: VV21, LL23, GG49, SS49, HH143, RR154, PP240, SP240 and SS240. All scrapie-affected animals carried the HH143RR154 genotype, with the exception of two goats (HR143), both of which had detectable protease-resistant PrP but showed no clinical signs or histopathological lesions characteristic of scrapie.

Introduction

Scrapie is a fatal and infectious neurodegenerative disease affecting sheep and goats. It belongs to the group of transmissible sub-acute spongiform encephalopathies (TSEs) along with bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jakob disease in humans. A feature common to all TSE diseases is the accumulation of an aberrant isoform of the normal prion protein (PrP). This abnormal configuration of PrP, PrPSc, is considered to be infectious and resistant to proteolysis (Prusiner, 1991, 1998). Normal PrP, PrPc, is expressed in most tissues of the body, with the nervous tissues showing the highest levels of PrPc expression (Bendheim et al., 1992; Horiuchi et al., 1995).

The occurrence of natural scrapie is strongly influenced by alterations in the host gene that encodes PrP (Hunter, 1997). Such polymorphisms might influence the conversion of PrPc into the pathogenic isoform (Bossers et al., 1997). The mechanism by which the individual allelic variants lead to altered susceptibility or incubation periods has not been defined, but it has been proposed that, in humans, PrP polymorphisms may be present at critical sites involved in the conformational transition from PrPc to PrPSc (Glockshuber et al., 1999). The study of scrapie susceptibility is complicated due to the different PrP genotypes found in different breeds of animals. It is also difficult to predict the relationship between conformational changes and the existence of several infectious scrapie strains, each of which has a distinct affinity for host genotypes (Smits et al., 1997).

In sheep, several polymorphisms in the PrP open reading frame (ORF) are associated with differences in phenotypic expression of prion diseases, such as incubation period, pathology and clinical signs. Amino acid polymorphisms at positions 112 (M → T), 136 (A → V), 137 (M → T), 138 (S → N), 141 (L → F), 151 (R → C), 154 (R → H), 171 (Q → H or Q → R) and 211 (R → Q) have been described previously (Thorgeirsdottir et al., 1999; Bossers et al., 1996; Tranulis et al., 1999; Hunter et al., 1989, 1994; Laplanche et al., 1993; Westaway et al., 1994; Belt et al., 1995). Genotype
AA130RR at position 141 is associated with resistance to natural and experimental infections with scrapie and BSE (Bossers et al., 2000). Each of the above polymorphisms may represent an alternate conformation of PrP that influences the pathogenesis process.

In goats, PrP amino acid dimorphisms at codons 142 (I → M), 143 (H → R) and 240 (S → P) have been described. Only the dimorphism at codon 142 (I → M) was associated with an altered disease incubation period (Goldmann et al., 1996). However, another PrP variant containing only three instead of the usual five octapeptide repeats may also be associated with an increased scrapie incubation period in goats (Goldmann et al., 1998). It is conceivable that the different profiles of PrP polymorphisms reported for sheep and goats could lead to a differential phenotypic expression of individual scrapie strains in the two species. In mixed flocks of sheep and goats, this could lead to the phenomenon we have observed (unpublished observation), where the incidence of scrapie in goats is notably less frequent than that in sheep (approximate ratio of 1 : 3).

Naturally occurring scrapie in goats has been reported in France (Chelle, 1942), UK (Brotherston et al., 1968; Harcourt, 1974; Andrews et al., 1992; Goldmann et al., 1998), Switzerland (Fankhauser et al., 1982), USA (Hourrigan et al., 1969; Hadlow et al., 1980), Canada (Stemshorn, 1975), Cyprus (Toumazos & Alley, 1989; Toumazos, 1991) and Italy (Capucchio et al., 1998).

The first case of scrapie in Greece was diagnosed in sheep in 1986 (Leontides et al., 2000). Since then, the disease has been diagnosed in 19 Greek flocks, of which 18 were sheep flocks or mixed flocks of sheep and goats and one was a goat herd (Leontides et al., 1999). This first case of natural scrapie in a goat herd provided the material for the present study. The usual scrapie eradication scheme applied in Greece mandates that all animals of the flock in which scrapie cases have been diagnosed are slaughtered and appropriate decontamination measures taken.

The aim of this study was to determine PrP polymorphisms in goats in Greece, especially with regard to the incidence of scrapie. For analysis of genotypes relating to risk, goats affected with natural scrapie were compared to healthy goats from the same herd.

**Methods**

**Animals.** The goat population in Greece numbers around 4.5 million animals, the great majority of which belong to the indigenous Hellenic breed (Capra hircus) or crosses with other milking breeds, such as Saanen, Alpine or Maltese. They are reared mainly under free-range conditions (Zygoyiannis & Katsaounis, 1986).

A total of 51 goats from a scrapie-affected herd of 176 in number was studied. The herd was established 20 years ago as a mixed flock of sheep and goats and was maintained as such for 12 years, at which time the sheep in the flock were eliminated. In a sense, this herd could be considered a closed one, as goats have been neither imported nor exported from it during the past 8 years. However, there was contact of these animals with sheep and goats from other flocks grazing in the same pastures. Furthermore, scrapie was diagnosed in animals from these other flocks prior to the diagnosis of scrapie in the goat herd studied in the present work.

Seven clinically ‘suspect’ goats in the advanced stages of the disease were submitted to be euthanized within a 6 month period of observation. An additional 43 goats were selected randomly for study from ‘clinically healthy’ animals of the 3–6 year age range during the ‘stamping out’ procedure. This age group coincided with the age of the clinically affected animals. One 7-year-old goat was also included in the group of healthy herdmates examined in this study. EDTA-treated blood was collected for genotyping from each animal. The brain was removed and, except for aliquots that were stored at −70 °C for Western blotting and ELISA, was fixed immediately in 10% neutral-buffered formalin for histopathology.

**Scrapie diagnosis.** The most common clinical signs observed in the seven goats suspected to have scrapie were hyperexcitability and restlessness, followed by muscle tremor and ataxia, sometimes pruritus, and often progressive emaciation (Leontides et al., 1999). Clinical diagnosis was confirmed by histological examination and/or by immunohistochemical detection of PrPSc in the brain. For histological examination, eight coronal slices, 3–4 mm thick, of the brain (cerebrum, brainstem and cerebellum) were selected as follows: medulla at the obex and caudal cerebellar peduncles, including the trapezoidal body; middle of thepons; mesencephalon through the rostral colliculi, just posterior to thepineal body; middle transverse section of the cerebellum; diencephalon at the mammillary body and hypophyseal infundibulum–optic tract levels; and frontal cortex rostral to the corpus callosum. The slices were processed, embedded in paraffin and 4–6 µm thick sections were stained with hematoxylin and eosin. Sections were then scored for vacuolation of neuronal perikarya and status spongiosus on a scale of 0–5, as described previously (Fraser & Dickinson, 1967, 1968).

Two methods were utilized for the detection of PrPSc. Initially, all samples were tested by Western blot. A short protocol was applied (S. Verghese-Nikolakaki, M. Polymenidou, M. Groschup, M. J. Chaplin, M. J. Stack and T. Sklaviadis, unpublished data) for all but three of the goat samples (eartag numbers 365, 384 and 392) for which standard scrapie-associated fibril (SAF) preparations were utilized (Manousis et al., 2000). To summarize the short protocol, 10% brain homogenates were prepared by homogenizing brain tissue in ice-cold homogenization buffer (PBS containing 0.5% NP-40 and 0.5% sodium deoxycholate). The brain homogenates were treated with proteinase K (25 µg/ml) at 37 °C and the proteolized PrPSc fragments were pelleted by centrifugation. Samples were resuspended in O’Farrell’s buffer and 3 mg brain equivalent aliquots were resolved by SDS–PAGE on 13% gels. Proteins were electrophoresed onto PVDF membranes and, after blocking, the immobilized PrP fragments were detected (Sklaviadis et al., 1986) with a polyclonal antibody, SALL, which specifically recognizes PrPSc (S. Verghese-Nikolakaki & T. Sklaviadis, unpublished data). When a weak PrP signal was detected, up to 12 mg brain equivalents were loaded onto the polyacrylamide gels and another monoclonal antibody that recognizes PrPSc (P4 (Harmeyer et al., 1998), was used to probe the corresponding immunoblots. Using the short protocol, we calculate that over 95% of the proteinase K-resistant PrPSc present in the brain tissue samples is recovered in the final pellet.

Brain samples from goats that were assessed to be positive for scrapie by either histopathology or Western blot were also examined for PrPSc using the Platelex BSE Detection kit (Bio-Rad). Samples were tested following the manufacturer’s instructions, except that cerebellar tissue or pooled brain tissue was used instead of oex.

Immunoblots and micrographs were scanned using a model 6300C Hewlett Packard ScanJet and the HP Precision Scan Pro software package.
Genetic analysis. Genomic DNA was isolated from EDTA-treated blood using a DNA isolation kit for mammalian blood (Promega). PCR amplifications of the PrP gene were performed in a 100 µl reaction volume containing 0.5–1 µg genomic DNA, 200 µM dNTPs, 2 mM MgCl₂, 2.5 units Taq DNA polymerase and 30 pmol each of primer G1 (+), 5’ ATGGTGAAAAGCCACATAGGCAGT 3’, and G2 (−), 5’ CTATCCTACTAGAAGAAATGAG 3’. The G1 (+) and G2 (−) primers anneal at the extreme 5′ and 3′ regions of the PrP-coding sequence, respectively. Amplification reactions were performed in an MJR Cycler for 40 cycles of 2 min at 96 °C, 2 min at 60 °C and 3 min at 72 °C. Products were visualized by staining with ethidium bromide after the electrophoresis of a 10 µl reaction mixture on 2% agarose gels. PrP polymorphisms were detected by DNA sequencing on both strands of the PCR products (MWG Biotech). For rare genotypes, the amplification and sequencing of the PrP-coding region was repeated, starting with a new DNA isolation. In the present study, genotypes are described by the single letter amino acid code, whereas nucleotides are indicated with lowercase letters. The PrP genotype observed most frequently in the herd, genotype I, is designated as wild-type (wt) for the indigenous Hellenic goat breed, Capra priska.

Statistical analysis. Results were analysed using Fisher’s exact test to compare the frequencies of codons between groups.

Results

Neuropathological analysis and PrPSc detection

Histological examination revealed lesions in the brain of 15 goats (all seven clinically suspect goats plus eight clinically healthy goats), while characteristic lesions of the disease were not found in the remaining 36 goats. High levels of vacuolation (score 4 and 3) were detected in the brains of five affected goats carrying PrP genotype I (Table 1, Fig. 1a, b). In contrast, the remaining six affected goats with genotype I showed only a mild level of vacuolation (score 1), as did the affected goats with genotypes III (SP240) and V (SS240SS240) (Table 1, Fig. 1c). Additionally, a mild level of vacuolation was detected in the brains of two goats [PrP genotypes I and IV (SS240)] that were negative in both clinical and biochemical evaluations for scrapie.

In Western blot analyses (Table 1, Fig. 2), 11 of 51 goat brain samples tested were scored as positive for the presence of proteinase K-resistant PrPSc fragments (five of the clinically suspect goats plus six clinically healthy goats). For the remaining 40 samples, no PrPSc proteolytic fragments were detected by Western blot in proteinase K-treated brain homogenates, even when up to four times the standard amount of material was tested or another antibody recognizing PrP was used (data not shown). Three of these samples (corresponding to eartag numbers 393, 7006 and 1643), which showed limited vacuolation in the histological examination but were negative for PrP by Western blot, gave a positive signal for PrPSc by ELISA. Additionally, there was one goat (eartag number 391) that had clinical signs of scrapie, a high score for scrapie-associated brain lesions and a positive signal by ELISA, but gave a negative result in the Western blot test for PrPSc. These latter four cases were all assessed as scrapie-affected. This discrepancy might reflect a low PrP content of the sample that was used for Western blotting, which was possibly taken from an area of the affected tissue with poor levels of PrP.

The single scrapie-affected animals with PrP genotypes II (HR143SP210) and VI (HR143) were classified as positive for scrapie based on the detection of proteinase K-resistant PrPSc by Western blot (Table 1, Fig. 2) and ELISA. Both these animals were assessed as healthy by clinical evaluation and histopathological examination.

The scores from each of the four evaluations (clinical, histopathological, Western blot and ELISA) were analysed (Table 1) and a scrapie status determination was made for each animal. Out of 51 goats tested, 15 were assessed as scrapie-affected because they received, at minimum, positive scores in at least two of the four tests applied, including a positive score in at least one of the two tests for brain-associated PrPSc. Of the scrapie-affected animals, 47% (7 of 15) could be classified as sub-clinical or pre-clinical cases, since, in the absence of any clinical symptoms of scrapie, these cases were positive for PrPSc (with sub-clinical) or without (pre-clinical) accompanying histopathological changes in the brain. Another two goats (eartag numbers 8648 and 7008), hereafter referred to as suspect, gave no immunobiochemical or clinical manifestations of scrapie but did show a mild level of vacuolation in their brains. This discrepancy between pathology and PrP immunobiochemistry might be attributed to sampling of different parts of the brain (Hope, 2000). The remaining 34 goats were assessed as healthy, as they received negative scores in histology, Western blot, ELISA and clinical evaluations.

PrP genotypes detected in this study

The codon 21 polymorphism (Table 1), detected in six healthy goats, consisted of a t → c nucleotide substitution in the second codon position leading to an amino acid change of V → A. All these animals were heterozygous VA21. The codon 23 polymorphism, detected in one healthy goat, consisted of a t → c nucleotide substitution in the second codon position leading to an amino acid change of L → P. The codon 49 polymorphism, detected in one affected goat, consisted of a g → a nucleotide substitution in the first codon position leading to an amino acid change of G → S. The codon 143 dimorphism, found in 20 individuals, consisted of an a → g substitution in the second position of the codon leading to a change H → R. The codon 154 polymorphism, detected in eight healthy goats, consisted of a g → a nucleotide substitution in the second codon position leading to an amino acid change of R → H. All of these animals were heterozygous RH154. The codon 168 polymorphism, detected in one healthy goat, consisted of a c → a nucleotide substitution in the third codon position leading to an amino acid change of P → Q. The codon 220 polymorphism, detected in a single healthy goat, consisted of a g → t nucleotide substitution in the third codon position leading to an amino acid change of Q → H. The dimorphism at codon 240 stems from the identity of the initial nucleotide of
Table 1. Frequency of PrP genotypes in scrapie-affected goats compared to healthy control goats in a scrapie-affected herd

Genotype I comprises three unique PrP genotypes that differ only in silent mutations present at codons 42 or 138.

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the codon. The nucleotide c in the first position leads to the amino acid proline, while an initial t leads to the amino acid serine at codon 240. In addition to the silent mutations at codons 42 and 138 described previously (Goldmann et al., 1996), new silent mutations were found in codons 107 (K, g → a) (one goat, eartag number 6836) and 207 (K, g → a) (one goat, eartag number 1643).

The dimorphisms reported previously at codons 143 and 240 in goats were also present in our herd (Goldmann et al., 1996). The genotypic frequencies for these polymorphisms, however, were much higher in the herd we examined. In contrast to the combined frequency of about 3 % for the HR_{143} and RR_{143} genotypes in the goat population studied by Goldmann and colleagues, we found a combined genotypic frequency of 39 % (20 of 51) for HR_{143} and RR_{143} genotypes in the herd we examined. Similarly, the genotypic frequency of PP_{240} was 44 % in the goat population studied by Goldmann et al. (1996) compared to 90 % in the herd of goats we examined.

No polymorphisms were found at codons 136, 141 or 171, codons for which polymorphisms have been associated with differences in phenotypic expression of scrapie in sheep. Similarly, none of the goats we tested carried the codon 142
polymorphism (I → M) that has been associated with an altered disease incubation period in goats (Goldmann et al., 1996).

The PrP ORFs of all goats reported in this paper have five octapeptide repeats, as have sheep and most other species that have been studied.

Comparison of PrP genotypes in natural scrapie cases and healthy controls

Altogether, we detected 11 different PrP genotypes, predicted to encode either five or six unique mature PrP variants. If the Q^{154} and H^{220} alterations in the PrP of the single goat comprising group X are linked, then the number of predicted unique mature PrP sequences would be five. If, however, they are unlinked, six unique sequences would be predicted (codons 49, 143, 154, 168 and 220): GHHPQ, GHRPHQ, GHRPQ, GHRQP and GHRPQ. Group I (wt), the genotype observed most frequently in this herd (18 of 51 goats) comprises three unique PrP genotypes that differ only in the specific silent mutations present in codons 42 and 138. Seven of the PrP genotypes were associated with scrapie-affected goats and five with healthy control goats (clinically, histopathologically and biochemically negative). Two genotypes (I and IV) were common to both healthy control goats and the two suspect animals (eartag numbers 8648 and 7008).

Natural goat scrapie was strongly associated (11 of 15 goats, 73%) with PrP genotype I (wt) (Table 1). All scrapie cases were homozygous at codons 21, 23, 49, 136, 141, 142, 154, 168, 171 and 220. Of the 15 scrapie cases, 13 (86.6%) were homozygous (HH) at codon 143. The other two were heterogeneous (HR_{143}) and, interestingly, were positive for brain-associated PrP but negative in histopathology and clinical evaluations. On the other hand, less than half (16 of 34) of the healthy control goats were homozygous for histidine (HH_{143}) at this codon. Of the remaining 18 healthy goats, four were homozygous for arginine (RR_{143}) and 14 were heterozygous (HR) at this site. Based on a statistical analysis of these data using Fisher’s exact test, we conclude that the proportion of scrapie-affected individuals is significantly higher in goats carrying the HH_{143} genotype as compared to those carrying the HR_{143} or RR_{143} genotype ($P = 0.0121$). Similarly, our statistical analyses point to a protective effect for H^{154} against scrapie in goats. We observed a disproportionately high frequency of healthy goats carrying the RH_{154} genotype as compared to their scrapie-affected flockmates ($P = 0.0868$). Thus, individuals carrying the RH_{154} polymorphism were seen in 23.5% (8 of 34) of healthy goats from the flock but were not found at all among the scrapie-affected goats. Overall, 86.7% (13 of 15) of scrapie-affected goats were homozygous HH_{143}RH_{154}. While only 23.5% (8 of 34) of the healthy goats carried this genotype. These findings suggest, therefore, that the RH_{143} and H_{154} alleles may offer some protection against scrapie infection in Greek goats.

At codon 240, 12 of the scrapie cases (80%) were homozygous for proline (PP), two (13%) were heterozygous for glycine (GP) and one was homozygous for serine (SS). In healthy goats, 97% (33 of 34) were homozygous for proline (PP) at codon 240 and 3% (1 of 34) were homozygous for serine (SS). The VA_{21} alteration was not found in scrapie-affected goats, whereas 17.5% of healthy goats (6 of 34) carried this polymorphism. Interestingly, all of the goats with VA_{21} also carried the HR_{143} polymorphism. LP_{23'} PQ_{168} and QH_{220} polymorphisms were seen at low frequency (3%, 1 of 34) in healthy goats.

Discussion

The prion hypothesis, supported by PrP structural analysis, predicts that certain mutations in PrP may result in unstable PrP molecules with intermediate conformations that are more likely to convert from the normal cellular PrP^{wt} to the abnormal PrP^{Sc} thus initiating the disease process (Cohen et al., 1994; Huang et al., 1994; Prusiner, 1997). Comparison of promoter regions and partial exon sequences of caprine and ovine PrP has led to the suggestion that the two gene structures are homologous (Goldmann et al., 1996). Further comparison of the PrP-coding sequences of sheep and goats (Goldmann et al., 1996) revealed an extremely high level of genetic conservation between the two species. Thus, both PrP proteins have a deduced primary structure of 256 amino acids with a predicted 24 amino acid signal peptide and potential N-glycosylation sites at codons 189 and 200 (Goldmann et al., 1996). Despite this high degree of similarity, amino acid alterations associated with changes in scrapie susceptibility do not appear to be identical for the two species. Whereas polymorphisms at codons 136, 154 and 171 have been correlated with alterations in scrapie susceptibility for sheep, only polymorphisms at codon 154 (this study) were found to be associated with modulation of scrapie susceptibility in goats as well. For goats, it appears that polymorphisms at codon 143 (this study) also influence the animals’ risk for contracting scrapie.

The diagnosis of scrapie in goats and the application of the scrapie eradication policy in Greece afforded us the opportunity to compare the PrP genotypes of scrapie-affected and healthy control goats in the same herd. The results of our genotypic analysis (Table 1) revealed comparatively high genotypic frequencies for the dimorphisms identified previously at codons 143 and 240 and the existence of known silent mutations at codons 42 and 138. No polymorphisms were found at codon 142, which has been associated with altered disease incubation periods in goats (Goldmann et al., 1996). We have, however, found new polymorphisms for goat PrP at codons 21, 23, 49, 154, 168 and 220. Additionally, new silent mutations were detected at codons 107 and 207. It is possible that the different profiles of PrP polymorphisms observed for the goats of Greece and those of Northern European countries (Goldmann et al., 1996) may be reflected in
the different profiles of susceptibility to individual scrapie strains in these goat populations.

As Goldmann et al. (1996) observed previously in goats, we have found many individuals in our herd carrying at least one P240 allele, which has not been found in other ruminants but is present in mink, ferret, domestic dog and dingo PrP (Bartz et al., 1994; Wopfner et al., 1999). As in the earlier study (Goldmann et al., 1996), no significant association of this codon dimorphism was observed between scrapie-affected and healthy control goats. It is likely that, as has been demonstrated for rodent PrP (Stahl et al., 1990), the C-terminal region of goat PrP, including amino acid 240, is removed during the post-translational attachment of a glycosylphosphatidylinositol phospholipid tail. This may explain why neither we nor Goldmann and colleagues (Goldmann et al., 1996) observed any apparent association of codon 240 with disease. Similarly, the V → A alteration at codon 21 (allele VIII) is unlikely to affect susceptibility to scrapie as it is part of the N-terminal signal sequence that is removed during processing of PrP. The possibility does exist, however, that the polymorphism at codon 21 could lead to alternate or no splicing of the signal peptides.

The genotypes LP23, PQ168 and QH240 were absent in scrapie-affected goats but small numbers in each case (one goat) were too low to be significant for any consideration. Our data show that except for two goats that carried the HR143 dimorphism, all scrapie cases were homozygous, HH143 RR154. Moreover, of the animals that carried a PrP genotype other than HH143 RR154, only 7% were affected by scrapie. These findings support the notion that alterations at codons 143 and 154 are moderating protective against scrapie. Furthermore, it is possible that animals carrying the VA21 HR143 RR154, VV21 HH143 RH154, and VV21 RR143 RR154 PrP genotypes may be at an even lower risk, as no scrapie cases were found in these groups of goats.

In the present study, we observed clinical signs of scrapie in 7 of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but seven of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but seven of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but seven of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but seven of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but seven of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but seven of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but seven of 15 scrapie-affected goats.

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In the present study, we observed clinical signs of scrapie in 7 of 15 scrapie-affected goats. For the remaining eight scrapie-affected animals, no clinical signs of scrapie were observed but all tested positive for brain PrP^Sc. Of the eight asymptomatic scrapie cases, brain lesions characteristic of scrapie were found in all but the two HR143 goats. These latter two cases highlight the problem of diagnosing scrapie in asymptomatic animals, especially those with more resistant PrP genotypes. In such cases, where the animal may have been in either a pre-clinical or sub-clinical phase of the disease, the characteristic neuropathological changes usually observed with clinical scrapie cases may be absent or extremely limited. Thus, the determination that such animals were scrapie-affected relies mainly on immunobiochemical demonstration of brain PrP^Sc, with little or no corroborating evidence from clinical and histopathology examinations. It should be noted that even immunocytochemistry, which has been an invaluable technique for the diagnosis of TSE disease in many animals, has given false negative results for confirmed cases of scrapie in goats (Foster et al., 2001). In fact, in their study of goats experimentally infected with scrapie, Foster et al. (2001) found a partial dissociation of PrP^Sc deposition and vacuolation in the brain. While it is certainly possible that, in the present study, vague clinical signs of the disease may have gone unnoticed in some animals, it is most likely that at least a few of the eight asymptomatic scrapie-affected animals were either pre-clinical or sub-clinical scrapie cases.

It is the possibility of chronic sub-clinically infected animals that is the most worrisome aspect of finding so many apparently asymptomatic scrapie-affected goats in the herd we examined. Unlike pre-clinically affected animals that would eventually show clinical signs of the disease, chronic sub-clinically affected individuals could, potentially, remain seemingly healthy and go undetected for years. During this time, the scrapie strain they carry might be transmitted to susceptible herdmates. It is also possible that the scrapie agent might replicate and adapt in such carriers so that these individuals could harbour an altered, perhaps more virulent, scrapie strain(s), as has been reported recently in asymptomatic mice following inoculation with hamster scrapie strain 263K (Race et al., 2001). The scrapie eradication policy implemented in Greece requires that all herdmates be slaughtered following the diagnosis of a single scrapie case. This policy made it impossible for us to learn which of the healthy herdmates in our scrapie-affected population would have eventually developed clinical scrapie. It should, however, effectively solve the potential problem of chronic sub-clinically infected goats surviving in scrapie-affected herds and continuing to transmit scrapie undetected to their herdmates.

Due to the known variation in susceptibility to scrapie among the different breeds of sheep with specific PrP alleles, we consider the possibility that not only the PrP gene but other unidentified gene(s) as well may influence the susceptibility of goats and sheep to scrapie. The analysis of additional scrapie-affected herds for genotypic comparison with healthy goat populations that is currently under investigation in our facility may reveal a robust goat scrapie-resistance genotype. Additionally, cloning and overexpression of the described goat genotypes may also reveal structural alterations of PrP folding associated with more resistant genotypes.

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in vitro

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