REPORT

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Pilot project:

"Polymorphisms of caprine PrP gene and their association with resistance or susceptibility to natural scrapie"

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Abstract

The above project was funded by the European Commission following a request of the Veterinary Services of Cyprus in 2005. The project had a duration of two years, it started on 1st January 2006 and finished on 31st December of 2007. The main objectives of the project were to further investigate the PrP gene of the Cyprus goats in order to confirm the results of previous studies (Papasavva-Stylianou et al., 2007) where polymorphisms at codon 146 were found to have an association with resistance or susceptibility to natural scrapie and to evaluate the data in order to be able to determine the baseline prevalence of scrapie resistant PrP genes in goats. To confirm the original findings a case-control study was performed on 717 goats, including 218 scrapie-positive, 280 scrapie-negative and 219 healthy controls. The allelic variant 146D in the scrapie-negative and healthy control goats was found at the frequencies of 5.9% and 5.7%, respectively and the allelic variant 146S in the goats of the same groups at the frequencies of 6.1% and 7.3%, respectively. In contrast, in the goats of scrapie-positive group these allelic variants were found at the frequencies of 0.7% (146D) and 0.5% (146S). In order to find the frequency of the "resistant" alleles in the various goat breeds across Cyprus a breed survey was carried out in five main goat breeds. The Damascus breed was found to carry the allelic variants 146D and 146S at the frequencies 19.5% and 8.5%, respectively. In comparison, none of the goats of Saanen, Akamas Local and French Alpine breeds were found to carry these alleles.

Introduction

Scrapie is a fatal neurodegenerative disease of sheep and goats. Together with bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease in humans, scrapie belongs to the group of transmissible spongiform encephalopathies (TSEs). One common feature of all TSE diseases is the accumulation of an aberrant isoform (PrP^{Sc}) of the normal prion protein (PrP^{C}). It is widely accepted that PrP^{Sc} is identical to or a part of the infectious agent of TSEs, prion PrP^{Sc} can be discriminated from PrP^{C} by it partial resistance to proteases. 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 PrP^{C} into the pathogenic isoform (Bossers et al. 1997).

In sheep, several polymorphisms of the PrP gene are associated with differences in the phenotypic expression of prion diseases, such as incubation period, pathology and clinical signs. While in total more than 30 polymorphisms have been described previously, only a few of them are closely

associated with resistance or susceptibility to classical scrapie: in particular the codons for amino acids 136 [alanine (A) – valine (V)], 154 [arginine (R) – histidine (H)] and 171 [glutamine (Q) – R – H] (Belt et al., 1995; Bossers et al., 1996; Hunter et al., 1994, 1989; Laplanche et al., 1993). Although, the study of scrapie susceptibility is complicated due to the different PrP genotypes found in different breeds of sheep, almost all relevant studies suggest that the most resistant to classical scrapie is the ARR/ARR genotype. The recent finding of atypical scrapie (originally termed Nor98) in sheep and goats however has shown that the genetic susceptibility can be remarkably different in other strains of scrapie (Moum et al., 2005; Saunders et al., 2006).

In goats, the association of genetic variability of PrP with resistance or susceptibility to classical scrapie remains unsettled. Studies in the UK revealed high variability of the PrP gene in goats (Goldmann et al., 2004; Goldmann et al., 2008). Studies in Italy suggested that the variant 222K had an association with scrapie resistance in Ionica breed goats (Vaccari et al., 2006). Moreover, studies in Greek goats revealed that the variants 143R and 154H may give some protection against natural scrapie in Greek goats (Billinis et al., 2002). Similarly, a recent study in Cyprus goats, suggested that the variants 146D and 146S may provide protection against scrapie (Papasavva-Stylianou et al., 2007). In the same study, the presence of the variant 146N (wild type) in homozygous form was found to be strongly associated with susceptibility to natural scrapie. The variant 142M was found to be associated with varying disease incubation periods in British goats (Goldmann et al., 1996). Also a PrP variant having only three rather than the usual five copies of a short peptide repeat, reported by Goldmann et al., (1998), was found to be associated with increased scrapie incubation period in goats.

PrP amino acids polymorphisms described so far in the PrP gene of goats are at codons 21 (V→A), 23 (L→P), 37 (G→V), 39 (S→R), 49 (G→S), 102 (W→G), 110 (T→P or N), 127 (G→S), 133 (L→Q), 137 (M→I), 142 (I→M or T), 143 (H→R), 146 (N→D or S), 151 (R→H), 154(R→H), 168 (P→Q), 185(I→F),194 (T→P), 211 (R→Q), 218 (I→L), 220 (Q→H), 222 (Q→K), (240 (S→P) and "silent" mutations at codons 42(a→g), 107 (g→a), 138 (c→t), 179 (g→t), 181 (c→t), 202 (c→t), 207 (g→a) 219 (c→t), 231(a→c), 232(g→a) and 237 (g→ c)(Goldmann et al., 1996; 1998; 2004; Wopfner et al., 1999; Billinis et al., 2002; Agrimi et al., 2003; Zhang et al., 2004; Kurosaki et al., 2005; Acutis et al., 2006; Vaccari et al., 2006; Papasavva-Stylianou et al., 2007; Acutis et al., 2008, Babar et al., 2008).

Scrapie in the goat population of Cyprus was first recorded in 1986, in dairy goats run in the same mixed flocks as affected sheep (Toumazos and Alley, 1989). Since then scrapie has caused major losses in the goats of Cyprus and represents the major problem of the animal husbandry in the island.

The studies in this project are a continuation of a previous study in the goats of Cyprus (Papasavva-Stylianou et al., 2007) and aimed firstly to further investigate the PrP gene of the Cyprus goats in order to confirm the results of the above mentioned study, where polymorphisms at codon 146 were found to have an association with resistance or susceptibility to natural scrapie. Secondly, to find the frequency of the "resistant" alleles in the various goat breeds across Cyprus (breed survey) and, thirdly, to genotype the entire population of several scrapie-free herds in order to be able to operate these herds as "nuclei" units and be the basis of a future breeding programme. According to the animal registration database of the Department of Agriculture of Cyprus the caprine population comprises 252.000 goats, the majority of which belong to the Damascus breed or crosses with the two local breeds or Saanen breed. The breeds of goats found in Cyprus and their percentage of the

national herd are: Damascus: 15%, Machaeras Local: 1%, Damascus crossbred with other local breeds: 81.7%, Saanen: 0.3%, Akamas Local: 1% and French Alpine: 1%.

Materials and Methods

First year of the project (1.1.2006-31.12.2006)

Case-control study

This study was carried out on 717 goats, including 218 scrapie-positive, 280 scrapie-negative and 219 healthy controls from 75 different scrapie-affected herds (Table 1). The majority of the animals came from the Damascus breed and/or crossbred Damascus with local breeds.

The scrapie-positive goats were animals with clinical symptoms of scrapie and were confirmed positive after histological and biochemical examination of the brain (rapid or discriminatory testing). The scrapie-negative goats were animals with clinical symptoms similar to scrapie but confirmed negative after histological examination and/or rapid testing of the brain. The healthy control goats had no clinical symptoms and similar to the scrapie-negative animals were herd matched and, whenever this was possible, age, breed and sex matched with scrapie-positive animals. With regard to matching the following prioritization was used: First priority: herd matched; second priority: age matched; third priority: sex matched. The tested goats were between 2 and 8 years of age.

Second year of the project (1.1.2007-31.12.2007)

Scrapie-positive cases

In addition, to the 218 scrapie-positive goats of the above case-control study, a further 439 scrapie-positive goats derived from 120 different scrapie-affected herds were studied (Table 1). Thirty six of these herds were already part of the case-control study.
All these 439 goats had clinical symptoms of scrapie and were confirmed positive after histological examination and rapid testing of the brain.

The histological examination and the rapid testing of the brains were performed using the protocol of the OIE Manual and the TeSeE Biorad test, respectively.

Breed survey

For the breed survey, blood samples were collected from 504 goats, derived from six different herds and belonging to the five main goat breeds in Cyprus (Damascus n=100, Local Machaeras n=104, Saanen n=100, Local Akamas n=100 and French Alpine n=100) (Table 1). The herd with Damascus goats is scrapie-affected. The goats of Machaeras local breed were derived from two herds, one of which is governmental and scrapie-affected.

Goats from scrapie-free herds

In total 9615 samples from 25 different herds around Cyprus where scrapie has never been reported were tested during this task (Table 1). One of the herds is governmental.

Molecular analyses

All the 717 samples of the case-control study (first year of the project) were sent to Agrobiogen Laboratory in Germany in order to be analyzed by DNA sequencing. The genomic DNA of the samples was isolated from EDTA-treated blood with the nexttec^{TM} kit. PCR amplification of the entire coding region of the PrP gene was performed using the primers, ovPrP ex3 for: 5´actgctatacagtcattcattatgct 3´and ovPrP ex3 rev: 5´cgccaagggtattattatacagg 3´which refer respectively to base pair (bp) 22183-22208 and 23182-23160 (Gen bank no.U67922). DNA sequencing was then undertaken on both strands of the PCR products by using dye terminator cycle sequencing and the capillary sequencer ABI3100. The amplification reactions for the DNA sequencing were performed using the primers, PYellow for: 5´gatttttacgtgggcatttga 3´, PGreen for: 5´cccagtaagccaaaaaccaa 3´, PRed rev: 5´acagggctgcaggtagacac 3´and PBlue rev: 5´tggttggggtaacggtacat 3´which refer respectively to base pair (bp) 22222-22242, 22590-22609, 23123-23104 and 22765-22746.

The blood samples of the second year of the project, (439 scrapie - positive goats, 504 goats from breed survey and 9615 goats from scrapie-free herds), were examined by single nucleotide polymorphism (SNP) analysis (Medigenomix Laboratory, Germany) in order to determine only the polymorphisms of codon 146 of the PrP gene. Following DNA extraction and amplification of thr PrP gene, the analysis was performed using the Sequenom method, which is a short primer extension followed by mass analysis of the extended products. On the samples with the genotype SD, additional mass spectrometry was performed to determine the allele composition. For the PCR amplification the following primers were used: PCR_Primer 1: ACGTTGGATGGGGTAACGGTA CATGTTTTC, PCR_Primer 2: ACGTTGGATGTGGCTACATGCTGGGAAGTG. The primers for the extension reaction were: EXT_SNP1(forward): GCCTCTTATACATTTTGGC and EXT_SNP2(reverse): ACCGGTCCTCATAGTCA. The primers for the allele specific reaction had the following sequences: GOATALSPEXTF1: ACGTAACGGTCCTCATAGTCAC , GOATALSPEXTR1: GCCTCTTATACATTTTGGCG, GOATALSPEXTF2: AACGGTCCTCAT AGTCAT, GOATALSPEXTR2: ACGTGCCTCTTATACATTTTGGCA.

Statistical analysis

The results were analyzed statistically using χ^2 test by comparing genotype frequencies between the scrapie-positive, the scrapie-negative and healthy control goats.

Results

I. Case-control study

This study was undertaken to explore and possibly confirm the original findings in a small pilot study undertaken on scrapie infected goats in Cyprus (Papasavva-Stylianou et al., 2007). 218 confirmed scrapie cases were examined in parallel to scrapie negative and healthy controls (Table 1). The entire PrP gene coding sequence of all animals was sequenced.

Twelve amino acid polymorphisms and seven "silent" mutations of the caprine PrP gene were identified. (Throughout this report, genotypes are described by the single letter amino acid code and nucleotides are indicated with small letters.) Ten amino acid polymorphisms were previously been described: codon 102, W→G; codon 110, T→P; codon 142, I→M; codon 143, H→R; codon 146, N→D or S; codon 151, R→H; codon 154, R→H; codon 168, P→Q and codon 240, P→ S. Two amino acid polymorphisms were novel: at codon 163, a caa→taa substitution leading to an amino change of Q→Stop codon and at codon 208, an ata→aca substitution leading to an amino acid change of I→T. In addition, a novel four-octapeptide repeat (4OR) variant was found, which had a deletion of 24bp in comparison to the normal five-octapeptide repeat (5OR) allele. The seven "silent" mutations were at codons: 42(a→g), 138(c→t), 179(a→g), 181(c→t), 219 (c→t), 231(a→c) and 237(c→g).

Taking all the coding changes into consideration, many genotypes are possible, however only 39 genotypes were observed in this study. Table 2 shows all the genotypes in scrapie positive, scrapie negative and healthy control animals. The genotype 1 (Table 2) was the most frequent in all three groups and is identical to the wild type sequence of other studies of the PrP gene and similar to other studies the polymorphism at codon 240 (P or S) is the most common one. The polymorphisms at codon 146 (N→D or S) were also frequently observed after those at codon 240.

The genotype distribution for the three amino acids at codon 146 (see Table 3) were as follows: Of the 280 scrapie-negative goats, 26 (9.3%) were heterozygous (ND) at codon 146, 3 (1.1%) were homozygous (DD), 29 (10.4%) were heterozygous (NS), 2 other (0.7%) were homozygous (SS), a single goat (0.4%) was heterozygous (SD) and 219 (78.2%) were homozygous (NN) at the same codon (see also Table 2). Similarly, of the 219 healthy control goats, 21 (9.6%) were heterozygous (ND), one single goat (0.5%) was homozygous (DD), 26 (11.9%) were heterozygous (NS), 2 (0.9%) were homozygous (SS), two other (0.9%) were heterozygous (SD) and 167 (76.3%) were homozygous (NN). In comparison, of the 218 scrapie-positive goats, only 3 (1.4%) were heterozygous (ND) at codon 146 and 2 other (0.9%) were heterozygous (NS) at the same codon. None of the scrapie-positive goats was found to be homozygous (DD) or (SS) at codon 146. Also, none of the scrapie-positive goats was found to be heterozygous for the two rarer amino acids (SD). Based on statistical analysis of these data using the χ^2 test, we concluded that there was a significant difference in the proportions of the genotypes at codon 146 in scrapie-positive and scrapie-negative goats (χ^2 =40.5075, p<0.001). Similarly, there was a significant difference in the proportions of the genotypes at the same codon in scrapie-positive and healthy control goats (χ^2 =44.3208, p< 0.001). These findings show that the genotypes with the presence of the amino acids aspartic acid (D) and/ or serine (S) at codon 146 are significantly associated with reduced susceptibility to natural scrapie in goat.

Of the 218 scrapie-positive goats only 4 (1.8%) were heterozygous (RH) at codon 154 (Table 2). In comparison, of the 280 scrapie-negative goats, 23 (8.2%) were heterozygous (RH) and 3 other (1%) were homozygous (HH) at the same codon. Similarly, of the 219 healthy control goats, 24 (10.9%) were heterozygous (RH) and a single goat (0.5%) was homozygous (HH) at the same codon. The statistical analysis showed that there was a significant difference in the proportions of the genotypes at codon 154 in scrapie-positive and scrapie-negative goats (χ^2 =12.019561, p< 0.001). Similarly, there was a significant difference on the proportions of genotypes at the same codon in scrapie-positive and healthy control goats (χ^2 =16.185085, p<0.001).

Four (1.8%) scrapie-positive goats were heterozygous (WG) at codon 102 (Table 2). In the scrapie-negative and in the healthy control groups, 10 (3.6%) and 13 (5.9%) goats respectively, were heterozygous (WG) at the same codon. The statistical analysis showed that there was a significant

difference on the proportions of genotypes at codon 102 in scrapie-positive and healthy control goats (χ^2 =4.9148, p= 0.027). In comparison, there was no significant difference on the proportions of genotypes at the same codon in scrapie-positive and scrapie-negative goats (χ^2 =1.3528, p=0.245).

Four (1.8%) scrapie-positive goats, 11 (3.9%) scrapie-negative goats and 3 (1.4%) healthy control goats were heterozygous (RH) at codon 151(Table 2). Our statistical analysis, showed that there was no significant difference in the proportions of the genotypes at codon 151 in scrapie-positive and scrapie-negative goats (χ^2 =1.83924, p> 0.05). Similarly, there was no significant difference in the proportions of the genotypes at the same codon in scrapie-positive and healthy control goats (χ^2 =0.14987, p> 0.05).

From the 39 genotypes in Table 2, 15 allelic variations were derived (Table 4, see legend for details). Predominant alleles in all three groups were the wt and the 240S allele, which were distinguished by alteration at codon 240 (either P or S). The alleles 146D and 146S were two other main alleles found at the frequencies of 5.9% and 6.1%, respectively in the goats of the scrapie-negative group, 5.7% and 7.3%, respectively in the goats of the healthy control group and 0.7% and 0.5%, respectively in the goats of the scrapie-positive group.

The 154H allele in the scrapie-positive, the scrapie-negative and healthy control groups was found at the frequencies of 1%, 5.1% and 5.9%, respectively (Table 4).

The 102G allele in the scrapie-positive, the scrapie-negative and healthy control groups was found at the frequencies of 0.9%, 1.8% and 3%, respectively (Table 4).

The 151H allele was found at the frequencies 0.9%, 1.9% and 0.7% in scrapie-positive, scrapie-negative and healthy control goats, respectively (Table 4).

The alleles 110P and 168Q found in all three groups were too rare to consider any association with scrapie (Table 4). Similarly, the frequencies 0.2%, 0.9% and 0.5% of the four-octapeptide repeat variant in scrapie-positive, scrapie-negative and healthy control goats, respectively, were very low to be considered for any relation with scrapie (Table 4).

The 142M allele was found only in one single (0.4%) scrapie-negative goat and in two (0.9%) healthy control goats and the 143R allele was found only in a single (0.4%) scrapie-negative goat (Table 4). Again, they were too rare to be significant for any consideration.

One scrapie negative animal carries the novel 163Stop allele and the 208T allele. With regard to the 163Stop allele, it is of interest to note the same nonsense mutation (Q to Stop) on the homologous position in the human PrP gene (codon 160) has been described in two cases of familial prion disease (Finckh et al., 2000).

II. Scrapie-positive cases

In the second year of the project a further 439 scrapie-positive goats were analysed for the polymorphic position 146 only (Table 1). Of the 439 goats, 437 (99.5%) were homozygous (NN) at codon 146 and only one single goat (0.2%) was heterozygous (NS) at the same codon. The remaining one goat (0.2%) of this task was found to have three alleles (146N, 146D and 146S). Similar genotypes termed “complex”, have been reported in a low percentage of British sheep by

Dawson et al., (2003). None of the scrapie-positive goats of this task was found to carry the allele 146D. Similarly to the data obtained during case-control study, the data of SNP analysis of codon 146 of the scrapie-positive cases revealed that the allele 146N had an association with susceptibility to natural scrapie. In contrast, the alleles 146D and 146S showed high association with resistance to the disease.

III. Breed survey

In order to find the frequency of the different 146 alleles in various goat breeds, the codon 146 was examined using SNP analysis in 504 animals of 5 different breeds (Table 1). A total of six genotypes (Table 5) were detected. In the goats of Damascus breed all six genotypes (NN, ND, DD, NS, SS and SD) were found. In the goats of Machaeras Local breed were found two genotypes (NN and NS) and only one (NN) in the goats of Saanen, French Alpine and Akamas Local breeds. Only one genotype (NN) was common in the five goat breeds, one other (NS) was common only in the goats of Damascus and Machaeras Local breeds and four other (ND, DD, SS and SD) were found only in the goats of Damascus breed. The predominant genotype in the goats of all breeds was the NN genotype. None of the goats of Saanen, French Alpine and Akamas Local breeds was found to have the alleles 146D and 146S. All the animals of these breeds were homozygous NN at this codon. In Machaeras Local breed only 7 goats (6.7%) were heterozygous (NS) at codon 146 and the rest 97 goats (93.3%) were homozygous (NN) at the same codon. In comparison, in the goats of Damascus breed, 27 goats (27%) were heterozygous (ND) at codon 146, 4 goats (4%) were homozygous (DD), 9 other goats (9%) were heterozygous (NS), 2 goats (2%) were homozygous (SS), 4 goats (4%) were heterozygous (SD) and the rest 54 goats (54%) were homozygous (NN) at the same codon. The frequencies of the alleles 146N, 146D and 146S in the goats of Damascus breed were 72%, 19.5% and 8.5%, respectively (Table 6).

IV. Goats from scrapie-free herds

The frequency of the different 146 alleles was in animals from herds where scrapie has never been reported. The results of SNP analysis of codon 146 of the 9615 blood samples of this task are shown in Table 7. The predominant allele in this task was the allele 146N found at the frequency of 86 %. The alleles 146D and 146S were present at the frequencies of 6.9% and 7.1 %, respectively. Specifically, of the 9615 tested goats, 7127 (74.1%) were homozygous (NN) at codon 146, 1126 (11.7%) were heterozygous (ND), 62 (0.7%) were homozygous (DD), 1153 (12%) were heterozygous (NS), 59 (0.6%) were homozygous (SS) and 88 (0.9%) were heterozygous (SD) at the same codon.

Discussion

In the first year of this study the PrP genotypes were examined in a case-control study. The polymorphisms reported previously at codons 102 (W→G), 110 (T→P), 142(I→M), 143(H→R), 146 (N→D or S), 151(R→H), 154(R→H), 168(P→Q) and 240(P→S) (Goldmann et al., 1996; Billinis et al., 2002; Agrimi et al., 2003; Papasavva-Stylianou et al., 2007) were also found in this case-control study. Statistical analysis of the data revealed that there are at least two main PrP polymorphisms that can influence scrapie susceptibility/resistance, the polymorphisms at codons 146 and 154. The alleles 146D and 146S showed a strong association with resistance to natural scrapie. Further examination of scrapie-positive cases in the second year of the study confirmed the association of these alleles with resistance to the disease. Similarly, in the Cypriot goats studied

previously by Papasavva-Stylianou et al. (2007) the amino acids aspartic acid (D) and serine (S) have a protective role against scrapie. In the same study the amino acid asparagine at codon 146 (146N) was found to have an association with susceptibility to natural scrapie (Papasavva-Stylianou et al., 2007). This was confirmed during this case-control study and the examination of the scrapie-positive animals.

The allele 154H showed also an association with resistance to natural scrapie during our case-control study. A similar effect has been previously reported in Greek and Cypriot goats, where this allele is held to be moderately protective against scrapie (Billinis et al., 2002; Papasavva-Stylianou et al., 2007). In the Italian goats studied by Vaccari et al. (2006) the same allele plays a role in the increase of scrapie incubation time. In comparison, in the goats studied by Acutis et al. (2006) the same allele appears at similar frequencies in both scrapie-affected and healthy goats. In addition, this allele has been detected in Nor98-affected goats in France and Switzerland (LeDur et al., 2005; Seuberlich et al., 2007) suggesting that it could give high susceptibility to Nor98 (or atypical scrapie) also in goats. The same allele has been found to have an association with susceptibility to atypical scrapie strain in sheep (Buschmann et al., 2004; Moum et al., 2005; Saunders et al., 2006).

The data obtained from our case-control study and the examination of scrapie-positive goats confirmed the results of Papasavva-Stylianou et al. (2007) where the presence of the amino acids aspartic acid (D) and serine (S) at codon 146 provide protection against scrapie in the goats of Cyprus. Additionally, the current data clarified the association of codon 154 with scrapie. The results of the breed survey revealed that the main source of the alleles 146D and 146S in Cypriot goats was the Damascus breed. The results of the examination of goats from several scrapie-free herds indicated that the alleles 146D and 146S were found in high numbers in the goat population of Cyprus.

Overall these data strongly suggest that the polymorphisms at codon 146 can offer a tool for controlling scrapie in the goats of Cyprus based on the selection of genitors carrying the amino acids aspartic acid (D) and serine (S) at codon 146 and as the Damascus breed was found to carry the "resistant" alleles 146D and 146S at high frequencies the selected breeding of genitors from this common Cypriot breed may solve the scrapie problem in Cyprus. Also, the scrapie-free herds we examined may operate as "nuclei" herds and be the basis of a future breeding programme.

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Table 1: The project plan

Year	Task	Animal Group	Description	No of animals	Total
First year (1.1.2006-31.12.2006)	Case-control study	Scrapie-positive	Animals with suspect clinical symptoms to scrapie and confirmed positive after histological and biochemical examination of the brain	218	717
		Scrapie-negative	Animals with clinical symptoms similar to scrapie but confirmed negative after histological examination and/or rapid testing of the brain. Herd matched and whenever this was possible, age, breed and sex matched with positive animals	280	
		Healthy control	Animals without clinical symptoms to scrapie herd matched and whenever this was possible, age, breed and sex matched with positive animals	219	
Second year (1.1.2007-31.12.2007)	Study of scrapie-positive cases	Scrapie-positive	Animals with suspect clinical symptoms to scrapie and confirmed positive after histological examination and rapid testing of the brain	439	10558
	Breed survey	Damascus breed	Animals from scrapie-affected herd	100	
		Local Machaeras breed	Animals were either from one scrapie-affected herd or from one herd where scrapie has never been reported	104	
		Saanen breed	Animals from herd where scrapie has never been reported	100	
		Local Akamas breed	Animals from herd where scrapie has never been reported	100	
		French Alpine breed	Animals from herd where scrapie has never been reported	100	
	Study of animals from scrapie-free herds	Animals without clinical symptoms of scrapie	Animals from herd where scrapie has never been reported	9615	

Table 2: Distribution of PrP genotypes in scrapie-positive, scrapie-negative and healthy control goats

PrP genotype	PrP codons												No of goats			
	54-95	102	110	142	143	146	151	154	163	168	208	240	Scrapie-positive	Scrapie-negative	Healthy control	Total
1	Five Octapeptide repeat (5OR):5OR	WW	TT	II	HH	NN	RR	RR	QQ	PP	II	PP	119	104	82	305
2	5OR:5OR	WW	TT	II	HH	NN	RR	RR	QQ	PP	II	SS*	15	25	4	44
3	5OR: 5OR	WW	TT	II	HH	NN	RR	RR	QQ	PP	II	PS	64	40	34	138
4	Four Octapeptide repeat (4OR):5OR	WW	TT	II	HH	NN	RR	RR	QQ	PP	II	PP	1	3	1	5
5	4OR:5OR	WW	TT	II	HH	NN	RR	RR	QQ	PP	II	PS	0	1	1	2
6	4OR:5OR	WW	TT	II	HH	NN	RR	RH	QQ	PP	II	PS	0	1	0	1
7	5OR:5OR	WG	TT	II	HH	NN	RR	RR	QQ	PP	II	PS	3	4	10	17
8	5OR:5OR	WG	TT	II	HH	NN	RR	RR	QQ	PP	II	SS	1	2	1	4
9	5OR:5OR	WG	TT	II	HH	ND	RR	RR	QQ	PP	II	PS	0	1	2	3
10	5OR:5OR	WG	TT	II	HH	NN	RH	RR	QQ	PP	II	SS	0	1	0	1
11	5OR:5OR	WG	TT	II	HH	NN	RR	RH	QQ	PP	II	SS	0	2	0	2
12	5OR:5OR	WW	TP	II	HH	NN	RR	RR	QQ	PP	II	PS	0	1	3	4
13	5OR:5OR	WW	TP	II	HH	NN	RR	RR	QQ	PP	II	SS	1	0	0	1
14	5OR:5OR	WW	TT	IM	HH	NN	RR	RR	QQ	PP	II	PP	0	1	1	2
15	5OR:5OR	WW	TT	MM	HH	NN	RR	RR	QQ	PP	II	PP	0	0	1	1
16	5OR:5OR	WW	TT	II	HR	NN	RR	RR	QQ	PP	II	PP	0	1	0	1
17	5OR:5OR	WW	TT	II	HH	ND	RR	RR	QQ	PP	II	PP	3	20	16	39
18	5OR:5OR	WW	TT	II	HH	ND	RR	RR	QQ	PP	II	PS	0	4	2	6
19	5OR:5OR	WW	TT	II	HH	DD	RR	RR	QQ	PP	II	PP	0	3	1	4
20	5OR:5OR	WW	TT	II	HH	SD	RR	RR	QQ	PP	II	PP	0	1	2	3
21	5OR:5OR	WW	TT	II	HH	ND	RR	RH	QQ	PP	II	PS	0	1	0	1
22	5OR:5OR	WW	TT	II	HH	ND	RR	RH	QQ	PP	II	PP	0	0	1	1
23	5OR:5OR	WW	TT	II	HH	NS	RR	RR	QQ	PP	II	PP	2	25	20	47
24	5OR:5OR	WW	TT	II	HH	SS	RR	RR	QQ	PP	II	PP	0	2	2	4
25	5OR:5OR	WW	TT	II	HH	NS	RR	RR	QQ	PP	II	PS	0	1	3	4
26	5OR:5OR	WW	TT	II	HH	NS	RH	RR	QQ	PP	II	PS	0	1	1	2
27	5OR:5OR	WW	TT	II	HH	NS	RR	RH	QQ	PP	II	PS	0	2	1	3
28	5OR:5OR	WW	TT	II	HH	NS	RR	RR	QQ	PQ	II	PP	0	0	1	1
29	5OR:5OR	WW	TT	II	HH	NN	RH	RR	QQ	PP	II	PS	2	7	2	11
30	5OR:5OR	WW	TT	II	HH	NN	RH	RR	QQ	PP	II	SS	2	1	0	3
31	5OR:5OR	WW	TT	II	HH	NN	RH	RH	QQ	PP	II	SS	0	1	0	1
32	5OR:5OR	WW	TT	II	HH	NN	RR	RH	QQ	PP	II	PS	2	12	17	31
33	5OR:5OR	WW	TT	II	HH	NN	RR	RH	QQ	PP	II	PP	0	4	3	7
34	5OR:5OR	WW	TT	II	HH	NN	RR	RH	QQ	PP	II	SS	2	0	2	4
35	5OR:5OR	WW	TT	II	HH	NN	RR	HH	QQ	PP	II	PP	0	3	0	3
36	5OR:5OR	WW	TT	II	HH	NN	RR	HH	QQ	PP	II	PS	0	0	1	1
37	5OR:5OR	WW	TT	II	HH	NN	RR	RR	QQ	PQ	II	PP	1	3	4	8
38	5OR:5OR	WW	TT	II	HH	NN	RR	RR	QQ	PQ	II	PS	0	1	0	1
39	5OR:5OR	WW	TT	II	HH	NN	RR	RR	QStop codon	PP	IT	PS	0	1	0	1
Total													218	280	219	717

*In blue are emphasized the positions which are heterozygous or homozygous with amino acids which differ from the wild type

Table 3: Frequencies of the PrP genotypes of codon 146 in scrapie-positive, scrapie-negative and healthy control goats during case-control study

PrP Genotype at codon 146	Scrapie-positive		Scrapie-negative		Healthy control	
	No	%	No	%	No	%
ND	3	1.4	26	9.3	21	9.6
DD	0	0	3	1	1	0.5
NS	2	0.9	29	10.4	26	11.9
SS	0	0	2	0.7	2	0.9
SD	0	0	1	0.4	2	0.9
NN	213	97.7	219	78.2	167	76.2

Table 4: Allelic variations of caprine PrP gene

Allele	PrP codon												Allelic frequencies **					
	54-95	102	110	142	143	146	151	154	163	168	208	240	Scrapie-positive*		Scrapie-negative *		Healthy control*	
													No	%	No	%	No	%
Wt	Five Octapeptide repeat (5OR)	W	T	I	H	N	R	R	Q	P	I	P	314	72	317	56.6	258	58.9
4OR	Four Octapeptide repeat (4OR)	-	-	-	-	-	-	-	-	-	-	P	1	0.2	5	0.9	2	0.5
102G	-	G	-	-	-	-	-	-	-	-	-	S	4	0.9	10	1.8	13	3
110P	-	-	P	-	-	-	-	-	-	-	-	S	1	0.2	1	0.2	3	0.7
142M	-	-	-	M	-	-	-	-	-	-	-	P	0	0	1	0.2	3	0.7
143R	-	-	-	-	R	-	-	-	-	-	-	P	0	0	1	0.2	0	0
146D	-	-	-	-	-	D	-	-	-	-	-	P	3	0.7	33	5.9	25	5.7
146S	-	-	-	-	-	S	-	-	-	-	-	P	2	0.5	34	6.1	32	7.3
151H	-	-	-	-	-	-	H	-	-	-	-	S	4	0.9	11	1.9	3	0.7
154H-240S	-	-	-	-	-	-	-	H	-	-	-	S	2	0.5	7	1.3	4	0.9
154H-240P	-	-	-	-	-	-	-	H	-	-	-	P	0	0	10	1.7	5	1.1
154H-240P or 240S***	-	-	-	-	-	-	-	H	-	-	-	P or S	2	0.5	12	2.1	17	3.9
Wt or 240S***	-	-	-	-	-	-	-	R	-	-	-	P or S	2	0.5	12	2.1	17	3.9
163Stop	-	-	-	-	-	-	-	-	Stop codon	-	-	P or S	0	0	1	0.2	0	0
168Q	-	-	-	-	-	-	-	-	-	Q	-	P	1	0.2	4	0.7	5	1.1
208T	-	-	-	-	-	-	-	-	-	-	T	P or S	0	0	1	0.2	0	0
240S	-	-	-	-	-	-	-	-	-	-	-	S	100	22.9	100	17.9	51	11.6

* Scrapie-positive goats, n=218 (436 alleles); scrapie-negative goats, n=280 (560 alleles); healthy control goats, n=219 (438 alleles)

** The alleles were assigned from Table 1. In most cases this was unequivocal. For any genotypes, where more than one position was polymorphic, the following assumptions were made to avoid that an allele is counted more than once:

(i) The 4OR, the 142M, the 143R, the 146D, the 146S and the 168Q allele were coupled with 240P as there are animals homozygous for 240P that carry the respective polymorphism and all animals heterozygous for these polymorphisms always carry at least one 240P polymorphism.

(ii) The 102G, the 110P and the 151H alleles were coupled with 240S as there are animals homozygous for 240S that carry the respective polymorphism and all animals heterozygous for these polymorphisms always carry at least one 240S.

(iii) The 154 wild type codon (R) and the polymorphic codon (H) can both occur on two alleles, one with P at position 240 and one with S at position 240. This is shown by animals with 154RH – 240PP, 154RH – 240SS and 154HH – 240PS genotypes, resulting in four alleles 154H -240P, 154H – 240S, 154R – 240P and 154R – 240S. This makes animals heterozygous at both position 154 and position 240 much more difficult to assign to exact alleles. However, in animals carrying a third polymorphic codon such as at 146, the 146S and 146D polymorphisms are both associated with 154R and the P polymorphism at codon 240 therefore the allele with the 154H polymorphism will by default be associated with the 240S polymorphism and therefore the 240S allele. This is based on the assumption that the 154 mutation does not exist on the same allele as another (excluding the 240) polymorphism, this is supported by the observation of the goats heterozygous or homozygous at 154, 46 are in animals without a further mutation and of those associated with a further mutation

these include a variety of mutations including 4OR, 102G, 146D, 146S and 151H. Goats heterozygous at positions 154 and 240 without additional polymorphisms cannot however be accurately assigned to the 4 possible alleles as possible genotypes for this group are 154R – 240P / 154H – 240S or 154H – 240P / 154R – 240S, and from the data there is no way of knowing which is correct, this group is therefore listed separately***.

(iv) The 163Stop and the 208T occurred in one animal and it was not possible to couple either of them with 240S or 240P.

(v) In case of three polymorphic positions, the 240 codon was always polymorphic and it was assumed that the non – wt codons of the two other polymorphisms are on the two different 240 alleles and not on the same one.

Table 5: PrP genotyping results at codon 146 during breed survey

	PrP genotype at codon 146						
Breed	ND	DD	NS	SS	SD	NN	TOTAL
Damascus	27	4	9	2	4	54	100
Saanen	0	0	0	0	0	100	100
Machaeras Local	0	0	7	0	0	97	104
Akamas Local	0	0	0	0	0	100	100
French Alpine	0	0	0	0	0	100	100

Table 6: Frequencies of the PrP allelic variants of the goats of Damascus breed

PrP allelic variant	Goats from Damascus breed*	
	No	%
N	144	72%
D	39	19.5%
S	17	8.5%

*** n=100 (200 alleles)**

Table 7: Frequencies of PrP genotypes and allelic variants in the goats of scrapie – free herds

PrP Genotype	Goats of scrapie – free herds*	
	No	%
NN	7127	74.1
ND	1126	11.7
DD	62	0.7
NS	1153	12
SS	59	0.6
SD	88	0.9
PrP allelic variant		
N	16533	86
D	1338	6.9
S	1359	7.1

*** Goats from scrapie – free herds, n= 9615(19230 alleles)**