



STREP

Thematic priority: Food quality and safety

FOOD-CT-2006-36353

goatBSE

**Proposal for improvement of goat TSE discriminative diagnosis  
and susceptibility based assessment  
of BSE infectivity in goat milk and meat.**

**Deliverable 1.2**

**PrP allele and genotype frequencies for common and rare goat breeds.**

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Dissemination Level		
<b>PU</b>	Public	X
<b>PP</b>	Restricted to other programme participants (including the Commission Services)	
<b>RE</b>	Restricted to a group specified by the consortium (including the Commission Services)	
<b>CO</b>	Confidential, only for members of the consortium (including the Commission Services)	

## Deliverable 1.2

### PrP allele and genotype frequencies for common and rare goat breeds.



As indicated in WP 1.2 and Deliverable 1.1 a PrP coding region variation study has been carried out in order to evaluate the presence of alleles which influence the susceptibility of goats to TSE. Several caprine PrP polymorphisms have been published, the most common of which are in codons 142, 154, 211, 222 and 240 (see figures below). It is likely that analysing a larger number of samples from EU breeds would reveal further polymorphisms. We therefore collected samples for analysing the PrP coding region of roughly 6000 samples. These samples originated from several countries, collected from the most common breeds (eg. Saanen, Alpine) as well as some rare goat breeds. Genomic DNA was produced from blood/tissue samples. The PrP coding region was sequenced in full by direct sequencing of PCR-generated DNA as specified in the year 1 report.

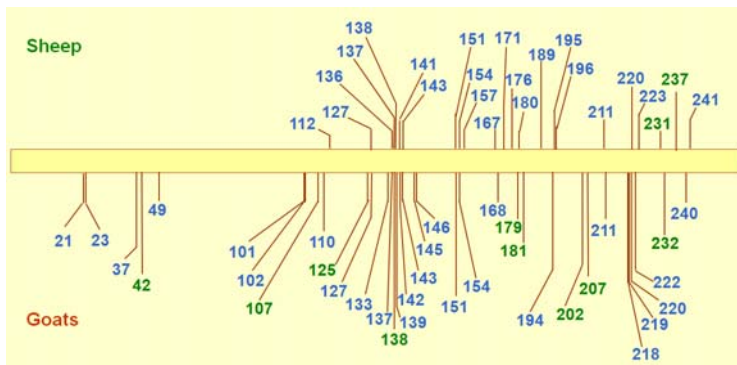


Figure a: Schematic representation of the sheep and caprine PrP polymorphisms based on literature and data from the current survey (special thanks to Cindy Panagiotidis P9 for the figures).

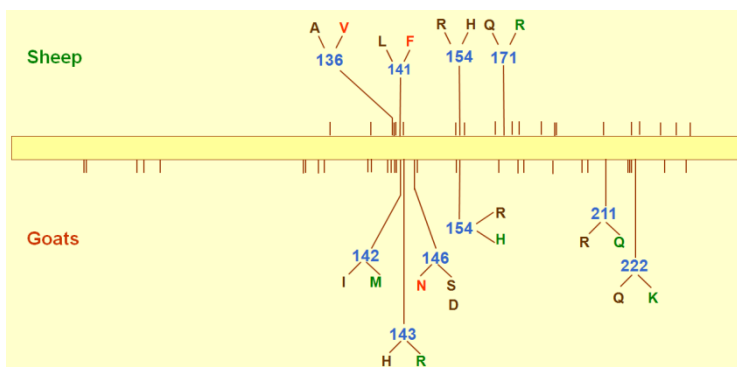


Figure b: Schematic representation of the sheep and caprine PrP polymorphisms that have a strong or indicative association with scrapie susceptibility..

Most of the sample collection was done in time as indicated in D 1.1 and only a minority of samples were finished after a delay in full-ORF sequencing by late arrival of sequencing equipment; these delays do not have an impact on other parts of the project. Some details on breeds could not be collected since these were undefined crosses or they concerned samples from other sampling regimes where no specific breeds track was possible.

Sample planning and sample analysis: the planned numbers and the achieved sequencing of samples are in the next table.

Table: Collected samples for full-ORF sequencing of various goat breeds from various countries.

	country							total
	NL	FR	UK	SP	IT	GR	CY	
<b>planned</b>	1000	1000	1000	1500	600	500	500	<b>6100</b>
<b>achieved</b>	1534	810	1173	1321	638	471	452	<b>6399</b>

These data here presented complete those acquired until May 2009, month 30 of the project. At the end of year 4, the analyzes of goat PrP allele frequencies were completed and are delivered here as a final result from this work package (WP) 1.2 survey. On the next pages these results are presented in the following way:

1. overviews per country, where possible for a number of specific breeds. In countries like The Netherlands, United Kingdom and Greece no specific breed analysis was possible; in those countries the data are derived from production breeds as indicated in the tables per country.
2. a consolidated overview from all countries in tabular format.
3. a consolidated graphical presentation per country for the most interesting susceptibility/resistance related alleles.

We further refer to a goatBSE collaborative review of Vaccari et al., published in 2009 in *Veterinary Research* as a reference for the worldwide state of the art on Prnp genetics and TSE epidemiology, and to two EFSA opinions made with the help of the project members in the light of potential breeding for resistance from EFSA:

- EFSA-BIOHAZ-panel (2009). Scientific Opinion on genetic TSE resistance in goats in all European Union Member States. *EFSA Journal* 7, 42.
- EFSA-BIOHAZpanel (2009). Genetic TSE resistance in goats. *The EFSA Journal* 995, 1-25.
- Vaccari, G., Panagiotidis, C. H., Acin, C., Peletto, S., Barillet, F., Acutis, P., Bossers, A., Langeveld, J., van Keulen, L., Sklaviadis, T., Badiola, J. J., Andreoletti, O., Groschup, M. H., Agrimi, U., Foster, J. & Goldmann, W. (2009). State-of-the-art review of goat TSE in the European Union, with special emphasis on PRNP genetics and epidemiology. *Veterinary research* 40, 48.

## 1 OVERVIEW PER COUNTRY

Survey data of each partner for the period year 1-4 is listed in the tables below (with special emphasis on the more common polymorphisms/alleles):

### The Netherlands (P1).

Allele frequencies over all animals (2005+2008). Polymorphisms at other positions than 127, 142, 154, 211, 222 and 240 are indicated in the lower section. The data are obtained mostly from the Saanen and Alpine dairy breeds, and are a representative cross-section of the Dutch farmed goats.

Allele (codons 127, 142, 154, 211, 222, and 240)*	frequency	
	N	%
GIRRQS	895	29,2%
GIRRQP	1334	43,5%
SIRRQP	181	5,9%
GMRQP	463	15,1%
GIHRQS	11	0,4%
GIRQQS	97	3,2%
GIRRKS	87	2,8%
<b>Total</b>	<b>3068</b>	<b>100,0%</b>

72,7%

\* Additional polymorphisms are being analyzed (in progress). Variation of 240S and 240P are grouped as wildtype. All other variants can be derived by single point mutation compared to this "wildtype".

### France (P2c).

Haplotype frequencies for the goat PrP gene at indicated codons in 404 AI bucks and 264 goats from the scrapie affected herd A

Haplotype	Amino acid position						Haplotype frequency		
	127	142	154	211	222	240	Alpine bucks	Saanen bucks	Herd A
1	G	I	R	R	Q	S	0.350	0.212	0.282
2	-	-	-	-	-	P	0.314	0.451	0.341
3	S	-	-	-	-	P	0.098	0.011	0.110
4	-	M	-	-	-	P	0.039	0.087	0.053
5	-	-	H	-	-	-	0.054	0.005	0.032
6	-	-	-	Q	-	-	0.071	0.185	0.104
7	-	-	-	-	K	-	0.074	0.049	0.078

### United Kingdom (P3).

Codon 127 142 211 222 240	Frequency	Frequency
	N	%
G I R Q P	682	50.9
G I R Q S	165	12.3
G I Q Q S	20	1.5
S I R Q P	128	9.6
G M R Q P	338	25.2
G I R K S	7	0.5

In United Kingdom, P3 detected other, but less common amino acid polymorphisms in the genotyped animals. A change at codon 143 (histidine (H) to arginine (R)), which has been published before, was present in two herds. The frequencies were 14% and 5%, respectively.

A polymorphism at codon 146 (asparagines (N) to serine (S)) was seen for the first time in UK goats. It was found in two herds (54% in D7; 1.5% in L4). This polymorphism is frequent in Cypriot goats and appears to be associated with scrapie resistance in their studies.

A novel polymorphism was found in a few goats within one herd. The polymorphism at codon 101 is glutamine (Q) to arginine (R) change, which has been described for Spanish sheep before.

Approximately 1200 goats from dairy breeds (D), meat breeds (M) and fiber breeds (F) were genotyped and the allele frequencies are shown in Table 1.7. The three most common genotypes making up almost 60% of all animals were wt-P/wt-P (21%), wt-P/M142 (20.5%) and wt-P/wt-S (16%). When the common polymorphism at codon 240 is disregarded the number of genotypes was 22, but half of those had frequencies of less than 1%. When all wt/wt homozygotes are removed from the frequency calculations, M142 carriers are 61% followed by S127 (15%), S146 (11%) and Q211 (8%). Rare genotypes containing R143 and K222 had frequencies of only 3.5% and 2.9%, respectively. The D breed category was not very different from the all-breed average with the highest frequencies for wt/wt of 38% and wt/M142 of 32.7%. Seven genotypes were less frequent than 1%. Q211 carrying genotypes and K222 carrying genotypes were slightly more infrequent with 4.4% and 1.4%, respectively, than in the overall assessment. The M breed group showed a significant increase of S146 carriers (44%) which is not linked to a reduction in the M142 carriers (21%) when compared to the results from all breeds.

United Kingdom: allele frequencies in UK goat herds, with totals in bold print and frequency percentages in regular print. D = dairy, M = Meat, F = fiber (production breeds).

Country	UK	UK	UK	UK	France <sup>1</sup>	Italy <sup>2</sup>
Breed Type	D	M	F	ALL	D	D
<b>Number</b>	<b>932</b>	<b>157</b>	<b>84</b>	<b>1195</b>	<b>404</b>	<b>478</b>
Wt-S, wt-P <sup>3</sup>	61.8	56.1	89.2	62.7	66.4	67.8
R101	0.1	-	-	0.1	-	-
S127	8.2	0.3	-	6.4	5.8	1.8
M142	26.6	13.2	1.2	22.6	6.1	8.4
R143	0.1	4.5	5.4	1.0	-	1.3
S146	0.2	24.5	-	3.6	-	-
H154	-	0.3	0.6	0.1	3.2	5.3
Q168	-	-	-	-	-	0.4
Q211	2.3	0.3	-	2.3	12.2	7.4
L218	-	0.7	3.6	0.4	-	-
K222	0.7	-	-	0.9	6.3	7.6

Footnotes

<sup>1</sup> Barillet *et al* (2009) J Gen Virol 90:769-776.

<sup>2</sup> Acutis *et al* (2008) J Appl Microbiol 104:1782-1789.

<sup>3</sup> summary of wild type coding polymorphisms found

codon	101	127	142	143	146	154	168	211	218	222	240
wt-S, wt-P	Q	G	I	H	N	R	P	R	I	Q	S, P

### Spain (P5).

Remarkable is the low frequency of 222K in the goat populations sampled in Spain and also that several heterozygotes were found in scrapie positives (in total 1321 samples)\*.

Population	Alleles									
	Codon 127		Codon 154		Codon 211		Codon 222		Codon 240	
	G	S	R	H	R	Q	Q	K	P	S
Moncaina negative	1	0	0.949	0.051	0.816	0.184	1	0	0.653	0.347
Moncaina negative 2	1	0	0.845	0.155	0.877	0.123	1	0	0.621	0.379
Pirenaica negative	1	0	0.854	0.146	0.938	0.063	1	0	0.938	0.063
Retinta negative	1	0	0.967	0.033	0.733	0.267	1	0	0.7	0.3
Retinta negative 2	1	0	0.986	0.014	0.681	0.319	1	0	0.625	0.375
Alpine negative	1	0	0.974	0.026	0.872	0.128	0.974	0.026	0.577	0.423
Saanen negative	0.976	0.024	0.988	0.012	0.94	0.06	1	0	0.655	0.345
Alpine positive herd	0.948	0.052	0.958	0.042	0.813	0.188	0.948	0.052	0.458	0.542
Saanen positive herd	1	0	1	0	0.745	0.255	1	0	0.559	0.441
Crossbreed positive herd	1	0	0.85	0.15	1	0	1	0	0.55	0.45
Crossbreed positive herd 2	1	0	0.927	0.073	0.804	0.196	1	0	0.631	0.369
Positive animals	1	0	1	0	0.889	0.111	1	0	0.778	0.222

\*Rare polymorphisms found: 18R<sup>240S</sup>; 37V<sup>240S</sup>; 74D<sup>240S</sup>; 112T<sup>240S</sup>; 127S<sup>240p</sup>; 137I<sup>240p</sup>; 139S<sup>240S</sup>; 141F<sup>240S</sup>; 143R<sup>240S</sup>; 151H<sup>240p</sup>; 215R<sup>240p</sup>; 232W<sup>240S</sup>

### Italy (P7).

The other Italian data (458 samples) have been published together with P1 and P3/P12 in Acutis et al., 2008. Genetic variability of the PRNP gene in goat breeds from Northern and Southern Italy. J. Appl. Microbiol. **104**:1782. The data can be found in the consolidated table at the end of this report.

Lateron, about 180 samples more were collected from a few breeds that had considerable 222K allele frequencies (table below).

allele (127, 142, 154, 211, 222, 240)	Garganica	Maltese	Red Mediterranean
G I R R Q S	0.105	0.073	0.114
G I R R Q P	0.492	0.516	0.675
G M R R Q P	0.024	0	0
G I H R Q P	0.113	0.097	0.105
G I R R K S	0.177	0.0725	0.044

### Greece (P9).

Allele frequencies were obtained from 471 samples from 55 separate herds incl. ±20 rare Skopelos, and all initially bred for dairy purposes. The local Greek goat breed consist of crosses of both local, Damascus (not Cypriot version) and Alpine breed.

Codon	Allele	No	%															
37c	G (ggg)	941	99,9	142c	I (ata)	927	98,4	173c	S (agt)	940	99,8	179 c	S (agt)	941	99,9			
	V (gtg)	1	0,1		M (atg)	14	1,5		N (aat)	2	0,2		gtg	941	99,9	gtt	1	0,1
					T (aca)	1	0,1									acc	941	99,9
42c	ccg	596	63,3	143c	H (cat)	938	99,6	202 c	act	1	0,1	211c	R (cga)	890	94,5			
	cca	346	36,7		R (cgt)	4	0,4		S (aga)	27	2,9		Q (caa)	52	5,5	I (atc)	941	99,9
102c	W (tgg)	922	97,9	146c	D (gat)	4	0,4	218c	I (ctc)	1	0,1	222c	Q (cag)	888	94,3			
	G (ggg)	20	2,1		R (cgt)	940	99,8		Q (aag)	54	5,7		K (aag)	54	5,7			
110c	T (acc)	905	96,1	151c	H (cat)	2	0,2	240c	S (ccc)	338	35,9	240c	P (tcc)	604	64,1			
	P (ccc)	37	3,9		R (cgt)	926	98,3		Ch No	942			Animal No	471				
127c	G (ggc)	923	98,0	154c	H (cat)	16	1,7											
	S (agc)	19	2,0		P (cca)	921	97,8											
138 c	agt	594	63,1	168c	Q (caa)	21	2,2											
	agc	348	36,9															

### Cyprus (P9)

Partner 9 also successfully analyzed 452 goats from three Cypriot (CY), and these results are detailed below and also presented in the consolidated table. The three herds consisted of a Damascus breed, a cross-breed, and a Machaeras breed.

PrP allele frequencies in Cypriot selection									
polymorphism	wt	wt	M142	S146	D146	H154	H154	Q211	K222
240 codon	S	P	P	P	P	S	P	S	S
frequency (%)	21.1	62.9	0.2	4.3	2.4	3.1	0.6	0.0	0.0
polymorphism	G102	P110	I125	H151	Q168				
240 codon	S	S	S	S	P				
frequency (%)	2.0	0.9	1.1	0.3	1.0				

## 2 CONSOLIDATED OVERVIEW

EU goat PrP allele frequencies, with specific attention to the TSE susceptibility related codons per country and where possible per breed detected within the “GoatBSE” consortium (2007-2010).

country	breed/production type	sample numbers sequenced	allele frequency (%)								other alleles (if >4% percentage is mentioned)
			wt <sup>240P, 240S</sup>	M <sup>240P</sup>	S <sup>240P</sup>	D <sup>240P</sup>	H <sup>240S</sup>	Q <sup>240S</sup>	K <sup>240S</sup>	P/(S+P)*100%	
NL	dairy (Saanen/Alpine)	1534	72.7	15.1	0.06	0	0.4	3.2	2.8	60%	101R <sup>5.7%</sup> ; 106S; 127S <sup>5.0%</sup> ; 153S; 220L
FR	total	810									one 154H <sup>240P</sup> ; other alleles not investigated
	Alpine bucks	200	76.2	3.9	0	0	5.4	7.1	7.4	54%	
	Saanen bucks	194	67.4	8.7	0	0	0.5	18.5	4.9	68%	
	Corsica bucks	94	88.6	0.5	0	0	1	0.5	9.4	64%	
	Poitevine bucks	104	45.6	19.7	0	0	0	31.3	3.4	42%	
UK	D(airy)	932	61.8	26.6	0.2	0	0	2.3	0.7	76%	101R, 127S, 143R
	M(eat)	157	56.1	13.2	24.5	0	0.3	0.3	0	30%	127S, 143R, 218L
	F(fibre, wool)	84	89.2	1.2	0	0	0.6	0	0	63%	143R, 218L
	all	1173	63.0	23	3.5	0	0.1	1.8	0.6	69%	
SP	total	1321									18R; 37V; 74D; 112T; 127S; 137I; 139S; 141F; 151H; 215R; 232W
	Moncaina	386	62.6	12.6	0	0	14	12.5	0.3	65%	
	Pireneica	44	44.1	38.1	0	0	13.6	6.8	0	94%	
	Retinta	71	50.8	21.8	0	0	3.1	28.2	0	70%	
	Alpine	39	76.9	3.8	0	0	2.6	10.3	6.4	58%	
	Saanen	42	69	20.2	0	0	1.2	6	1.2	65%	
	Murciano Granadina	50	23	60	0	0	0	18	0	83%	
	Alpine (scrapie herd)	51	62.7	5.9	0	0	4.9	17.6	5.2	56%	
	Saanen (scrapie herd)	52	67.3	6.7	0	0	0	25	0	66%	
	cross breed (scrapie herd)	10	70	15	0	0	15	0	0	55%	
	cross breed (scrapie herd)	563	58	16.1	0	0	7.7	17.9	0	63%	
	Scrapie affected	13	70.8	15.4	0	0	0	11.5	0	84%	
IT	total	638									new alleles?
	Garganica	60	59.7	2.4	0	0	11.3	0	17.7	82.4%	37V, 110P, 142T, 143R
	Maltese	60	58.9	0	0	0	9.7	0	7.3	87.6%	37V <sup>21.8%</sup> ; 143R, 168Q
	Red Mediterranean	60	78.9	0	0	0	10.5	0	4.4	85.5%	37V, 110P, 143R, 168Q
IT (North)	2008 (Acutis et al)										
	Camosciata delle Alpi	84	62.5	8.9	0	0	11.3	13.7	2.4	52.3	127S
	Saanen	69	77.5	7.2	0	0	0	10.2	3	75.7	110P, 127S
	Roccamerano	70	70	5.7	0	0	3.6	13.6	4.3	63.3	127S, 168Q
	Valdostana	77	54.5	28.2	0	0	0	9.6	1.3	37.6	127S
IT (South)	Garganica	58	61.3	2.6	0	0	11.2	0	17.2	83.2	37V, 110P, 143R
	Maltese	25	62	0	0	0	6	2	12	83.7	37V <sup>14%</sup> ; 143R <sup>4%</sup>
	Ionica	27	72.2	0	0	0	7.4	1.9	7.3	82	37V <sup>5.6%</sup> ; 137I, 143R
	Mediterranean	28	81.4	0	0	0	5.3	1.8	5.4	73.9	143R <sup>5%</sup>
	South cross	20	65	0	0	0	5	0	22.5	80.8	143R <sup>5%</sup>
GR	dairy; cross local with Alpine breed (+20 Skopelos)	471	70.8	1.5	2.9	0.4	1.7	5.5	5.7	76.4	37V, 102S, 110P, 127S, 142T, 143R, 151H, 168Q, 173N, 218L
CY	Damascus + Dam. cross + Machaeras	452	84	0.2	4.3	2.4	3.7	0	0	74.9	102G, 110P, 125I, 151H, 168Q
			wt	142M <sup>240P</sup>	146S <sup>240P</sup>	146D <sup>240P</sup>	154H <sup>240S/P</sup>	211Q <sup>240S</sup>	222K <sup>240S</sup>	wt <sup>240P, 240S</sup>	



### 3 CONSOLIDATED GRAPHICAL PRESENTATION PER COUNTRY

#### Graphical display of the allele frequencies for codons wt, 142M, 146S, 146D, 154H, 211Q and 222K: overall averages per country.

The codon 240 polymorphism in goats is different from that in sheep: in sheep the codon is a serine (S) while in goats this can be a proline (P) or a serine (S) with a majority for P in most breeds. Therefore, for each specific allele at other codons than 240, the 240 polymorphism is specified. The bars above “rest alleles” do reflect the remainder of polymorphisms together, which can be associated with either 240P or 240S.

