Protective Effect Of The $I_{142}R_{154}R_{211}K_{222}S_{240}$ PrP Allele Against Classical Scrapie In French Alpine And Saanen Goat Breeds

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Introduction

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of transmissible neurodegenerative diseases including Creutzfeldt-Jacob disease (CJD), variant CJD of man, bovine spongiform encephalopathy (BSE) of cattle, and scrapie of sheep and goats. The *PrP* gene, encoding the prion protein (PrP), is a major gene in sheep controlling scrapie in this species. Thus to reduce the hazard to public health from the theoretical risk of acquisition of BSE by sheep, the European Union has adopted innovative strategies against classical scrapie and BSE strain, by promoting breeding programmes for resistance to TSEs in sheep (EFSA, 2006), based on the selection of the $A_{136}R_{154}R_{171}$ allele of the *PrP* gene (expressed in single-letter amino acid code at positions 136, 154 and 171).

Genetic selection as an approach to control scrapie in goat is also considered by the European food safety authorities (EFSA, 2009) and goat breeding sector as the ideal option for long term prevention against sanitary and economical risks that are associated to TSEs in goat. The caprine *PrP* coding region is highly polymorphic, with 21 polymorphic codons described to date (Goldmann (2008)). But until 2005 only a few polymorphisms have been associated with TSEs resistance in goats (Goldmann *et al.* (1996)). Over the last five years, new and significant results have spotlighted the IRRKS allele (simplified notation for $I_{142}R_{154}R_{211}K_{222}S_{240}$ also noted shortly K_{222} allele), which is presumed presently as providing a protective effect against classical scrapie as high as the ARR allele in sheep, according to the following results : (i) case control studies in natural contaminations (Acutis *et al*. (2006), Vaccari *et al.* (2006), Barillet *et al*. (2009)); (ii) *in vitro* conversion assay of the prion protein (PrP) into PrP^{Sc} by TSE agents depending on the *PrP* allele, developed by partners of the European GoatBSE project (Bossers (2006)); (iii) in progress experimental challenges with classical scrapie or BSE carried out by partners of the same GoatBSE project.

The 3 case control studies were based respectively on 25 cases and 152 control (Acutis *et al*. (2006)), 39 cases and 61 control (Vaccari *et al*. (2006), 90 cases and 174 control (Barillet *et al*. (2009)).

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The power of the 2 first analyses was partly impaired by the relatively low number of scrapie cases (25 and 39) compared to the third analysis (90 cases). A new French study to be published (Corbière *et al.* (2010)), based on 169 cases and 694 control goats, will correspond to the most powerful case control design presently available in goats.
This paper presents the results of a meta-analysis including all the data of the 2 French case control studies in natural contaminations (259 scrapie cases and 868 control goats).

Material and methods

Scrapie affected herds. Five herds were included in the meta-analysis, herd A of the first study (Barillet *et al.* (2009) and herds B to E of the second study to be published (Corbière *et al.* (2010)). These 5 herds were composed of French Alpine, Saanen, and Alpine x Saanen crosses breeds. The samples of blood and various tissues for PrP^{Sc} detection were described in Barillet *et al*. (2009). A total of 259 scrapie-positive cases were found over 1127 sampled goats (22.9 %): the prevalence ranged between 13.1 % (herd D) and 34.1 % (herd A).

***PrP* gene sequencing or genotyping.** The *PrP* gene open reading frame (ORF) of goats of the herd A was sequenced as described previously by Barillet *et al.* (2009). Based on results obtained in this first study including haplotype determination, the genotype at codons 142, 154, 211, 222 and 240 was determined for goats of the herds B to E (Corbière *et al.* (2010)) using a snapshot technique (GIE Labogena). The meta-analysis was performed on the genotypes at these 5 codons, *i.e*. based on 6 possible alleles $I_{142}R_{154}R_{211}Q_{222}S_{240}$ or IRRQS allele being the wild-type one, and 5 mutually exclusive mutated alleles $IRRQP_{240}$, $M_{142}RRQP_{240}$, $IH_{154}RQS$, $IRQ_{211}QS$, and $IRRK_{222}S$ (Barillet *et al*. (2009)).

Statistical analysis. Association analysis was carried out using a mixed logistic regression model adjusted on age (younger than 2 years, 2 to 4 years old and older than 4 years), with a random "herd" effect to control for clustering of data, *PrP* genotypes being the main covariate (Glimmix macro, SAS 9.1, SAS Institute). When there were no positive scrapie cases in some genotypes the Fisher's exact test was used instead. $P<0.05$ was considered to be statistically significant in all analyses.

Results and discussion

Association of *PrP* genotypes with scrapie susceptibility. Twenty one *PrP* genotypes were found in herds A to E. Table 1 shows the results from the mixed logistic model applied to *PrP* genotypes with IRRQS/IRRQS goats (homozygous wild-type genotype) as the baseline in the 5 highly infected herds. There were insufficient data for 5 of the 21 *PrP* genotypes.
No significant differences were found between IRRQS/IRRQS, $IRRQP_{240}$/IRRQS and $IRRQP_{240}$/$IRRQP_{240}$ genotypes ($P=0.962$ and $P=0.220$, respectively). Compared to IRRQS/IRRQS goats, **the M142 mutation** was associated with reduced odds, ratio being significant only in $M_{142}RRQP_{240}$/ $IRRQP_{240}$ goats, in agreement with only an increase of the incubation length (Goldmann *et al.* (1996)). The frequency of scrapie PrP^{Sc} positive cases in $IH_{154}RQS$/ $IRRQP_{240}$ and $IH_{154}RQS$/ IRRQS heterozygous goats was significantly lower than in the IRRQS/IRRQS baseline group (Fisher's exact tests, $P<10^{-4}$ and 0.015 respectively).
But **the H154 mutation** is now recognized to be a risk factor for atypical scrapie in goat (Colussi *et al.* (2008)) as in sheep (Moum *et al.* (2005).

The Q211 mutation was associated with a highly reduced odds for $IRQ_{211}QS$/ $IRRQP_{240}$ and $IRQ_{211}QS$/IRRQS heterozygous goats compared to IRRQS/IRRQS goats (OR=0.06 and $P<10^{-4}$), *i.e.* an OR 17 times lower than in the baseline homozygous wild-type IRRQS/IRRQS goats. The protective effect of the Q211 mutation was also illustrated by the absence of scrapie case in homozygous Q_{211}/Q_{211} goats (n=25, Fisher exact test, $P<10^{-3}$), or heterozygous Q_{211}/M_{142} and Q_{211}/H_{154} goats (n=13, P=0.009; and n=10, P=0.028, respectively).

Table 1 : **Association between PrP genotypes at codons 142, 154, 211, 222 and 240 and scrapie infectious status, with reference to IRRQS/IRRQS genotype, in 5 enzootic scrapie herds.**

PrP genotype	# Scrapie positive / # scrapie negative	% scrapie positive	OR (95 % CI) *	P-value
IRRQS/IRRQS	45 / 66	40.54	1	
$IRRQP_{240}$/ $IRRQP_{240}$	70 / 112	38.46	0.73 [0.44 - 1.21]	0.220
$IRRQP_{240}$/IRRQS	108 / 130	45.38	0.99 [0.61 - 1.59]	0.962
$M_{142}RRQP_{240}$/ $M_{142}RRQP_{240}$	0 / 9	0.00	ND†	0.029
$M_{142}RRQP_{240}$/ $IRRQP_{240}$	10 / 50	16.67	0.30 [0.13 – 0.66]	0.003
$M_{142}RRQP_{240}$/IRRQS	11 / 36	23.40	0.53 [0.24 - 1.17]	0.119
$IH_{154}RQS$/ $IH_{154}RQS$	0 / 5		Insufficient data	
$IH_{154}RQS$/ $M_{142}RRQP_{240}$	0 / 3		Insufficient data	
$IH_{154}RQS$/ $IRRQP_{240}$	0 / 38	0.00	ND†	$<10^{-4}$
$IH_{154}RQS$/IRRQS	0 / 18	0.00	ND†	0.015
$IRQ_{211}QS$/ $IRQ_{211}QS$	0 / 25	0.00	ND†	$<10^{-3}$
$IRQ_{211}QS$/ $M_{142}RRQP_{240}$	0 / 13	0.00	ND†	0.009
$IRQ_{211}QS$/ $IH_{154}RQS$	0 / 10	0.00	ND†	0.028
$IRQ_{211}QS$/ $IRRQP_{240}$	7 / 140	4.76	0.06 [0.02 - 0.13]	$<10^{-4}$
$IRQ_{211}QS$/IRRQS	5 / 95	5.00	0.06 [0.02 - 0.17]	$<10^{-4}$
$IRRK_{222}S$/ $IRRK_{222}S$	0 / 2		Insufficient data	
$IRRK_{222}S$/ $M_{142}RRQP_{240}$	0 / 1		Insufficient data	
$IRRK_{222}S$/ $IH_{154}RQS$	0 / 4		Insufficient data	
$IRRK_{222}S$/ $IRQ_{211}QS$	1 / 22	4.54	0.04 [0.00 – 0.33]	0.002
$IRRK_{222}S$/ $IRRQP_{240}$	1 / 52	1.89	0.01 [0.00 – 0.11]	$<10^{-4}$
$IRRK_{222}S$/IRRQS	1 / 37	2.63	0.02 [0.00 – 0.18]	$<10^{-3}$
Total	259 / 868	22.98		

* OR (95% CI) adjusted from the mixed logistic regression model : IRQS/IRRQS genotype used as the baseline.

† ND : not determinated. For genotypes with sufficient data but without any PrP^{Sc} positive case, OR could not be determined (ND) and the comparison was performed using the Fischer's exact test.

The K222 mutation was associated with the highest reduced OR for $IRRK_{222}S$/ $IRRQP_{240}$ and $IRRK_{222}S$/ IRRQS heterozygous goats compared to the baseline IRRQS/IRRQS goats (OR=0.01, $P<10^{-4}$; and OR=0.02, $P<10^{-3}$, respectively), *i.e*. a decrease by 100 or 50 times respectively.

Discussion The wild-type allele IRRQS was very susceptible to classical scrapie since in enzootic situation (flocks A to E) 40 % of homozygous IRRQS/IRRQS goats were scrapie positive animals. It was the same trend for the $IRRQP_{240}$ allele (prevalence of 38 % and 45 % respectively for P_{240}/P_{240} or P_{240}/S genotypes). If we pass over the H154 mutation due to its susceptibilty to atypical scrapie, the Q211 and K222 mutations were significantly associated with resistance to classical scrapie, and $IRQ_{211}QS$ or $IRRK_{222}S$ alleles were dominant over IRRQS or $IRRQP_{240}$ alleles, since susceptibility in heterozygous $IRQ_{211}QS$ or $IRRK_{222}S$ goats was dramatically reduced compared to homozygous wild-type goats (prevalence in the range of 1.9 % to 5 % in enzootic situation compared to 40 % for IRRQS/IRRQS goats).

Conclusion

In our meta-analysis, the K_{222} allele was clearly the most resistant allele: the OR of scrapie cases in K_{222}/Q_{222} heterozygous goats compared with susceptible Q_{222}/Q_{222} homozygous goats was in a similar range than the OR in ARR/ARQ heterozygous sheep compared to susceptible ARQ/ARQ sheep (Tongue *et al*. (2006), Corbière *et al*. (2007)) in similar enzootic situation (risk divided by 50 to 100 times). Moreover *in vitro* conversion assay of the prion protein (PrP) and in progress experimental challenges with classical scrapie and BSE using goats and transgenic mice (in the framework of the European GoatBSE project) spotlight also the K_{222} allele: the K222 mutation is therefore presently presumed as the best candidate mutation for a genetic approach to eradicate TSEs in goats. On-going European researchs aim at validating this option, which would have to face the fact that the frequency of the K_{222} allele seems so far to be low in numerous breeds.

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