PrP-associated resistance to scrapie in five highly infected goat herds

Fabien Corbière, Cécile Perrin-Chauvineau, Caroline Lacroux, Pierrette Costes, Myriam Thomas, Isabelle Brémaud, Samuel Martin, Séverine Lugan, Christophe Chartier, François Schelcher, Francis Barillet and Olivier Andreoletti

1INRA, UMR 1225, Interactions Hôtes Agents Pathogènes, Ecole Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, 31076 Toulouse Cedex, France
2ANSES, Laboratoire d’Etudes et de Recherches Caprines, BP 3081, 79012 Niort Cedex, France
3INRA, UR 631, Station d’Amélioration Génétique des Animaux, BP 52627, 31326 Castanet-Tolosan Cedex, France

Received 18 August 2012
Accepted 18 October 2012

The PrP gene polymorphisms at codons 142 (I/M), 154 (R/H), 211 (R/Q), 222 (Q/K) and 240 (S/P) and their association with susceptibility to classical scrapie infection were investigated in five French goat herds displaying a high disease prevalence (>10%). On the basis of PrPSc detection in the central nervous system and in various lymphoid tissues, 301 of 1343 goats were found to be scrapie infected. The statistical analyses indicated that while P 240 mutation had no direct impact on scrapie infection risk, the H 154 , Q 211 and K 222 mutations were associated with high resistance to scrapie. The M 142 mutated allele was associated with a limited protection level against the disease. These results further reinforce the view that, like in sheep, the control and eradication of classical scrapie through the selection of certain PrP alleles could be envisaged in commercial goat population.

In sheep, the susceptibility to transmissible spongiform encephalopathies (TSE) is strongly modulated by polymorphisms of the prion protein (PrP) gene and the nature of the prion disease agent (strain) (Baylis & Goldmann, 2004). The A136R154H171 allele is associated with a highly protective effect against natural or experimental infection with classical scrapie and bovine spongiform encephalopathy (BSE) agents, while the V136L154Q171 or A136R154Q171 alleles are associated with susceptibility (Elsen et al., 1999; Hunter, 1997). However, in sheep, the ARR allele does not provide any particular protection against atypical scrapie, whereas the R154H or L141F amino acid substitutions are associated with an increased risk of occurrence of this TSE (Fediaevsky et al., 2010; Goldmann, 2008; Moreno et al., 2007; Moum et al., 2005).

At the European level, the selection of the ARR allele carriers was successfully applied for controlling and eradicating classical scrapie in infected sheep flocks (Nodelijk et al., 2011). At the population level, large-scale selection programmes were also implemented. They aimed at increasing the frequency of the ARR allele in the general population making it less favourable for TSE agent circulation and spreading. This ‘breeding for resistance policy’ in combination with the other eradication measures, resulted in a significant reduction of the classical scrapie prevalence in populations where it was comprehensively applied (Dawson et al., 2008; Fediaevsky et al., 2008; Hagenaaars et al., 2010).

In goats, several field studies have identified coding mutations of the PrP gene that are associated with lower risk of developing classical scrapie; namely the I/M142,N/D146 and S146,R/Q211 and Q/K222 (Acutis et al., 2006; Barillet et al., 2009; Bouzalas et al., 2010; Goldmann et al., 1996, 2011; González et al., 2009; Papasavva-Stylianou et al., 2007, 2011; Vaccari et al., 2006).

The development of a PrP genotype selection programme is now considered by the EU authorities as a potential tool for the control and eradication of scrapie in the commercial goat population. However, the available data related to PrP polymorphisms associated with TSE resistance in goat are still considered insufficient to recommend a breeding for resistance policy (EFSA, 2009). Indeed, because (i) the low frequency of certain alleles in goat populations and (ii) the relatively limited number of scrapie cases usually involved in certain studies, it still remains difficult to estimate the real level of resistance associated with the different goat PrP genotypes (Acutis et al., 2006; Baylis & Goldmann, 2004; EFSA, 2009; Vaccari et al., 2006).
In this study, five naturally scrapie-infected herds (A–E) were selected on the basis of their high infection prevalence (Table 1). Index cases were identified through the EU active TSE surveillance system either at the rendering plant or slaughter house. According to the EU regulation (EU999/2001) those TSE-affected goat herds were destroyed. In more than 99.4% of the animals (1343 of 1350), the ileum, the mesenteric lymph node, the tonsil and the posterior brainstem were sampled at the rendering plant. For each sample, PrPSc detection was carried out by immunohistochemistry using the 8G8 antibody (epitope amino acid sequence 95–108 of the human PrP), as described previously (Lacroix et al., 2007). An animal was considered scrapie positive when at least one of its tissues was found to be positive. From the 1343 sampled goats a total of 301 scrapie cases were identified (Table 1).

The five herds comprised Alpine, Saanen and Alpine/Saanen cross-bred goats. The PrP haplotype diversity in French Saanen and Alpine breeds has already been described, showing that the PrP gene coding polymorphisms in this population are restricted to positions 142, 154, 211, 222 and 240. In particular, no polymorphism was reported at codon 146 of the PrP gene in the French goat population (Barillet et al., 2009). Therefore, in this study genotypes were established at codons 142, 154, 211, 222 and 240 by snapshot PCR (Labogena, Jouy en Josas, France) (Table 1). In scrapie cases, the PrP gene ORF was sequenced (Barillet et al., 2009). Sequencing confirmed that no additional coding mutations to those observed at codons 142, 154, 211, 222 and 240 were present. Genotyping and sequencing were established using blood samples that had been collected in the herds several weeks before stamping out.

In this study, we made the deliberate choice to restrict genetic/scrapie infection risk analysis only to those animals for which traceable and complete information (PrP genotype, scrapie infection status and age) was available. In four of the five herds (A–D), such information was established in more than 93% of the individuals. In the fifth herd (E), traceable information was only available in 60% of the animals. Finally, 259 scrapie-infected goats and 868 apparently healthy controls were considered. To check if a potential bias could have been introduced to the analysis by excluding animals with incomplete information, a sensitivity analysis was performed based on multiple imputations (Meng & Rubin, 1992). Assuming that these data were missing at random, unknown information were randomly imputed based on the within flock distributions of age and PrP haplotypes/genotypes derived from animals with complete data. This was done separately for scrapie-positive and -negative animals, to take into account the distribution disequilibrium between these two groups. Statistical analyses were performed on 100 imputed datasets as described below. Results were not different from those reported here (not shown). We therefore concluded that no strong bias in the estimation of PrP haplotypes/genotypes effect could be evidenced.

Six different haplotypes were identified: four (IH154RQS, IRQ211QS, IRR222S and IRR240) derived from the archetype I142R154R211Q222S240 (subsequently noted as IRQQS) allele by a single codon mutation, and one (M142RRQP240) by a double mutation. Strong differences in allele frequencies were observed between herds and apart from the IRQQS the PrP mutated alleles were represented at a low level (Table 1). These results are consistent with those previously reported in the French Saanen and Alpine breeds (Barillet et al., 2009; Vaccari et al., 2009) and support the view that the five studied herds did not display any particularities in their PrP genetic structure.

Associations between PrP haplotypes/genotypes and the scrapie infection status were assessed using logistic regression models with a random ‘herd’ effect to control potential clustering of data (Glimmix macro, SAS 9.2; SAS Institute). In order to consider the potential confounding effect of animals’ age, models were adjusted for three age groups (younger than 2 years, 2–4 years old and older than 4 years). In the absence of scrapie cases, some haplotypes/genotypes could not be included in the mixed logistic model analysis and the Fisher exact test was used.

<table>
<thead>
<tr>
<th>Herd</th>
<th>No. goats</th>
<th>No. positive/ sampled</th>
<th>Prevalence (%)</th>
<th>Genotyped goats</th>
<th>PrP allele frequencies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scrape infected</td>
<td>Healthy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IRRQS</td>
<td>M142RRQP240</td>
</tr>
<tr>
<td>A</td>
<td>290</td>
<td>38/290</td>
<td>13.1</td>
<td>38</td>
<td>247</td>
</tr>
<tr>
<td>B</td>
<td>247</td>
<td>42/245</td>
<td>17.1</td>
<td>39</td>
<td>193</td>
</tr>
<tr>
<td>C</td>
<td>162</td>
<td>37/162</td>
<td>22.8</td>
<td>32</td>
<td>120</td>
</tr>
<tr>
<td>D</td>
<td>208</td>
<td>64/208</td>
<td>30.8</td>
<td>60</td>
<td>134</td>
</tr>
<tr>
<td>E</td>
<td>443</td>
<td>120/438</td>
<td>27.4</td>
<td>90</td>
<td>174</td>
</tr>
<tr>
<td>Overall</td>
<td>1350*</td>
<td>301/1343</td>
<td>22.4</td>
<td>259</td>
<td>868</td>
</tr>
</tbody>
</table>

*Of these 1350 animals 223 were not fully traceable (PrP genotype, scrapie infection status, age) and therefore not used to assess the effect of the PrP haplotype (Table 2) and PrP genotype (Table 3) on the risk of infection with scrapie.
Scrapie cases were mainly observed in the IRRQS or the IRRQP_{240} haplotype carriers and using IRRQS as a baseline, the IRRQP_{240} and M_{142}RRQP_{240} alleles were associated with a low to moderate decrease in scrapie infection risk (odds ratio, OR=0.79, \( P=0.036 \) and OR=0.47, \( P=0.003 \), respectively) (Table 2). Conversely, the H_{154}, Q_{211} and K_{222} mutated alleles were associated with a strong protection against scrapie (OR <0.1, \( P<10^{-4} \)).

Twenty-one different PrP genotypes were observed in the studied goat population (Table 3). The IRRQP_{240}/IRRQS \((n=238, 21.1\%)\) and IRRQP_{240}/IRRQP_{240} \((n=182, 16.1\%)\) genotypes were the most frequent and no difference in scrapie susceptibility was observed between these genotypes and the wild-type IRRQS homozygote animals.

All the other genotypes were represented at substantially lower frequencies and for some genotypes, like homozygotes K_{222} and homozygotes H_{154}, the low number of individuals precluded any statistical comparison.

However, still using IRRQS homozygote animals as a baseline, all the K_{222}, H_{154}, Q_{211} heterozygote genotypes (for which sufficient data were available) and the Q_{211} homozygote animals displayed a strongly reduced risk of scrapie infection. In M_{142} allele carriers, the situation was more complex. While homozygotes M_{142} and heterozygotes IM_{142}RQP_{240}/IRRQP_{240} displayed a reduced risk of scrapie infection, such a risk reduction was not significant in the IM_{142}RQP_{240}/IRRQS genotype \((P=0.07)\).

The R/H_{154} PrP gene mutation was also associated with a strong protective effect against classical scrapie infection in heterozygotes animals (Table 3). However, the R/H_{154} mutation has been demonstrated in goats, like in sheep, to be associated with higher risk of atypical scrapie occurrence (Colussi et al., 2008). In our opinion, this precludes the use of this allele for genetic selection against TSE in goats.

In this study some scrapie cases, although only few, were identified in heterozygote Q_{211} and K_{222} goats. These findings could suggest a limited protective effect of Q_{211} and K_{222} alleles against scrapie. In sheep, whereas homozygote ARR sheep display a high resistance, some classical scrapie cases have also been reported in heterozygote ARR sheep (Baylis et al., 2004). Therefore, a clear assessment of the level of resistance to scrapie infection in homozygote Q_{211} and K_{222} animals appears to be crucial before ‘a breeding for resistance’ programme to control and eradicate scrapie in goats could be envisaged. However, despite the large number of scrapie infected/total goats involved in this study, the final number of goats with homozygous mutation at codons 211 \((n=23)\) and 222 \((n=2)\) remained too limited to address this point. Considering the low frequencies of such genotypes in goats, it is unlikely that any observational studies might provide sufficient information to answer this question.

In this context, the experimental challenge of Q_{211} and K_{222} homozygote animals with a panel of TSE isolates (different classical scrapie isolates and BSE) is probably the most suitable way to answer this question. Such experimental challenges have already been initiated and the first results obtained in K_{222} heterozygote goats inoculated with a classical scrapie isolate are consistent with a high level of resistance to infection in those animals (Acutis et al., 2012).

In the infected goats, 121 of the 259 animals displayed PrP\textsuperscript{Sc} accumulation in one or several investigated lymphoid tissues but were negative in the central nervous system (CNS; obex).

Conversely to what has been reported in an infected goat herd in the UK (González et al., 2009) none of the infected animals showed PrP\textsuperscript{Sc} in the CNS, while being negative in the lymphoreticular system (LRS). This discrepancy could be the consequence of the involvement of different scrapie agents (strains) in the studied populations. Alternatively, it might reflect the possibility of different dissemination pathways of the TSE agent in infected individuals.

In our infected goat population no significant difference was observed in the proportion of the PrP\textsuperscript{Sc} LRS-positive/
CNS-negative animals between the different age groups of IRRQS and IRRQP homozygotes and IRRQS/IRRQP heterozygote-infected animals (Fisher’s exact test, all P-values >0.3) (Table S1, available in JGV Online). This observation supports the view that the S/P240 mutation has no strong influence on the kinetics of the scrapie agent’s dissemination from peripheral tissues to the CNS.

In the M142,Q211 and K222 allele carriers, a lower proportion of CNS-positive goats (3 of 16) was observed in animals younger than 48 months than in IRRQS and IRRQP homozygote and IRRQS/IRRQP heterozygous goats (70 of 130) (Fisher’s exact test, P=0.015). This result suggests that the expression of the M142,Q211 and K222 PrP alleles might slow the scrapie agent dissemination in the body of infected animals. While these results are in line with those reported in M142 allele carriers goat experimentally infected with BSE (Goldmann et al., 1996), it is our opinion that the number of infected animals with appropriate genotype involved in our study remains too low to draw definitive conclusions on that question.

Therefore, experimental oral challenges with scrapie in animals carrying appropriate genotypes is certainly the most suitable approach to definitively clarify the impact of the M142, Q211 and K222 PrP alleles on scrapie agents’ dissemination in the tissues of goats.

As in sheep naturally infected with scrapie (Gomez et al., 2007; Reckzeh et al., 2007), the substantial proportion of LRS-positive/CNS-negative-infected adult goats that we observed in our study probably has some consequences for the sensitivity of the EU TSE active surveillance programme (PrPSc detection in the obex on a random sample of animals more than 18 months old). We are currently developing a simulation model that takes into account both the characteristics of the French commercial goat population (zootechnic management, demography, etc) and the sensitivity of the PrPSc detection in the CNS as a tool for identifying scrapie-infected animals to quantitatively estimate the impact of this phenomenon on the efficiency of the scrapie active surveillance programme.

Beyond these epidemiological considerations, it should also be noted that according to the EU regulation 746/20081 modifying the regulation 999/20012, EU member states can decide to put for human consumption carcasses prepared from animals born and/or raised in scrapie-infected flocks providing that they have been tested and are negative for PrPSc in the CNS. In that perspective, the relatively high proportion of scrapie-infected but CNS-negative animals that can be found in infected herds is also likely to have consequence in terms of the consumer’s dietary exposure to scrapie agents.

### Acknowledgements

This work was funded by GIS PRION grant no. 31B06134, by INRA grant no. AIP P00297, by the Poitou-Charentes region grants nos 04/RPC-A-103 and 05/RPC-A-13, by the EU FP7 ‘Goat BSE’ project CT-2006-36353 and EMIDA 2012 ‘Goat TSE free’ project.
References


Genetic resistance to scrapie in goats

http://vir.sgmjournals.org