# BREEDING FOR SCRAPIE RESISTANCE USING *PrP* GENOTYPING IN THE FRENCH DAIRY SHEEP BREEDS

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## INTRODUCTION

Scrapie, the sheep prion disease, is the most common natural form of the transmissible spongiform encephalopathies (TSE), which also include Creutzfeld-Jakob disease in man and bovine spongiform encephalopathy (BSE) in cattle. Genetic susceptibility to scrapie is strongly modulated by allelic variations at three different codons (136, 154 and 171) in the sheep *PrP* gene encoding for protein PrP (Hunter, 1997). Therefore breeding for scrapie resistance has been recently considered as an attractive solution to control this disease (Dawson *et al.*, 1998; Smits *et al.*, 2000). However such a strategy requires first to accumulate knowledge on two critical points (i) do sheep exist, fully resistant to all scrapie and BSE strains ? (ii) may resistant sheep be healthy carriers of infectivity ? Both physiopathological and large epidemiological studies are needed to answer these questions.

The purpose of this paper is to present epidemiological results on the *PrP* susceptibility in French dairy sheep breeds. Then keeping in mind that sheep are first selected on production traits, preliminary analyses were carried out to check out any possible genetic relationship between the *PrP* locus and milk production traits.

## *PrP* POLYMORPHISM AND SCRAPIE SUSCEPTIBILITY

*PrP* genotyping at the 3 codons 136, 154 and 171 was performed using a PCR-RFLP method as described in Elsen *et al*. (1999), which did not distinguish alleles H and Q at the codon 171, so that only 4 alleles were described at the 3 codons: ARR, AHQ, ARQ and VRQ.

**Material and methods.** The association between scrapie and polymorphism in the sheep *PrP* gene was studied in the Lacaune and Manech blond faced breeds : 58 and 368 scrapie affected animals were recorded respectively, between 1992 and 1994 in 10 Lacaune flocks, and between 1992 and 2001 in 32 Manech flocks. The clinical diagnosis of scrapie was confirmed by brain histology. A case-control study was carried out at the population level, the control being either 937 AI Lacaune rams born between 1995 and 1997, or 3149 elite Manech blond faced ewes *PrP* genotyped in 152 healthy nucleus flocks in 1999-2000. The risk factors according to the *PrP* genotype were estimated using a logistic regression. The reference level was ARQ/ARQ animals when computing the odds ratios (table 1).

**Results and discussion.** Despite large differences in the *PrP* genotype structure between Lacaune and Manech blond faced breeds, the case-control study showed comparable general picture for the two breeds (table 1) : (i) when compared to ARQ/ARQ animals, the risk to be scrapie affected increased significantly by almost 4 times for ARQ/VRQ or VRQ/VRQ sheep,

(ii) the risk was extremely low for ARR/ARQ animals (odds ratio = 0.02 in the Manech breed), and (iii) ARR/ARR sheep were never affected. The results for the AHQ allele were not discussed due to its very low frequency in these 2 breeds. In agreement with the abundant literature reviewed in Hunter (1997) and Smits *et al.* (2000), susceptibility appears to be associated with the ARQ and especially the VRQ alleles, and resistance with the ARR allele. ARR is nearly dominant over ARQ and VRQ since in most cases ARR/ARQ and ARR/VRQ are resistant. ARR/ARR sheep appear to be resistant based on clinical criteria. In addition since it is always the rule for all the results of an European survey implemented in a number of countries x breeds, ARR/ARR sheep may be considered as fully resistant against any natural scrapie strain (Elsen, 2001). Moreover this resistance appears to be extended to BSE strain since after experimental contamination with this TSE strain none ARR/ARR sheep showed clinical sign or $PrP^{sc}$ accumulation (Goldmann *et al.,* 1994; Foster *et al.,* 2001; Jeffrey *et al.,* 2001).

**Table 1. *PrP* genotypes (codons 136, 154, 171) in scrapie-affected and healthy control sheep**

| *PrP* Genotype | Lacaune breed | | | Manech blond faced breed | | |
|---|---|---|---|---|---|---|
| | Affected (n=58) 10 flocks | Healthy (n=937) | Odds Ratio (OR)[A] | Affected (n=368) 32 flocks | Healthy (n=3149) 152 flocks | Odds Ratio (OR)[A] |
| ARR/ARR | 0 | 38.7 % (363) | | 0 | 3.1 % (96) | |
| ARR/AHQ | 0 | 2.2 % (21) | | 0 | 0.2 % (5) | |
| ARR/ARQ | 0 | 43.9 % (411) | | 0.8 % (3) | 27.9 % (879) | 0.02 * |
| ARR/VRQ | 0 | 1.2 % (11) | | 0 | 1.2 % (38) | |
| ARQ/AHQ | 0 | 1.0 % (9) | | 0 | 0.4 % (14) | |
| ARQ/ARQ | 86.2 % (50) | 12.4 % (116) | 1.00 | 78.3 % (288) | 62.8 % (1977) | 1.00 |
| ARQ/VRQ | 13.8 % (8) | 0.6 % (6) | 3.90 * | 20.1 % (74) | 4.4 % (137) | 3.78 * |
| VRQ/VRQ | 0 | 0 | | 0.8 % (3) | 0.1 % (3) | 3.78 * |
| | | P[B] | 0.0054 | | P[B] | 0.0001 |

[A]: OR significantly different from 1.00 (P < 0.01) are identified by an asterisk.
[B]: P = global significance (Wald statistics).

**$PrP^{sc}$ CARRYING *versus* *PrP* GENOTYPE**

**Material and methods.** Monitoring of 15 scrapie affected Manech blond faced flocks has been implemented in 1999, with the full *PrP* genotyping (7346 until now). Accumulation of $PrP^{sc}$, the abnormal isoform of the prion protein (PrP) which is the only known molecular marker of scrapie, was investigated by immunohistochemestry as described in Andreoletti *et al.* (2000) in palatine tonsils from 654 adult healthy sheep (between 2 and 6 years old) culled in 2000 and 2001 in these 15 flocks.

**Results and discussion.** In the flocks where scrapie incidence during the last two years was higher than 3 %, about 2 % of ARQ/ARQ sheep and 14 % of ARQ/VRQ and VRQ/VRQ sheep were found positive, *e.g.*, exhibited $PrP^{sc}$ in tonsils (table 2). Conversely, in agreement with kinetic studies carried out in experimental flocks (van Keulen *et al.,* 1999; Andreoletti *et al.,*

2000) none of the 36 ARR/ARR explored sheep showed $PrP^{sc}$ accumulation in their tonsils, suggesting the absence of healthy carriers.

**Table 2. Number of $PrP^{sc}$-affected ewes (in palatine tonsil) according to their *PrP* genotype and scrapie incidence of the flock**

| Scrapie incidence of the flocks (# flocks) | *PrP* genotype of the (culled) ewes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ARR/ ARR | ARR/ AHQ | ARR/ ARQ | ARR/ VRQ | ARQ/ AHQ | ARQ/ ARQ | ARQ/ VRQ | VRQ/ VRQ |
| < 1 % (4) | 0/4 | 0/1 | 0/36 | | 0/1 | **1**/69 | 0/7 | |
| 1-2 % (3) | 0/2 | | 0/25 | 0/2 | | 0/53 | 0/3 | |
| 2-3 % (4) | 0/23 | 0/2 | 0/91 | 0/7 | 0/4 | 0/95 | **2**/15 | |
| > 3 % (4) | 0/7 | 0/3 | 0/88 | 0/9 | 0/3 | **2**/82 | **2**/21 | **1**/1 |
| Total | 0/36 | 0/6 | 0/240 | 0/18 | 0/8 | **3**/299 | **4**/46 | **1**/1 |

## RELATIONSHIPS BETWEEN *PrP* LOCUS AND MILK PRODUCTION TRAITS

**Material and methods.** between 1995 and 2001, 6451 AI rams were *PrP* genotyped (at least at codon 171), so that 15 granddaughter families of heterozygous sires genotyped QR at codon 171 (11 in Lacaune, 3 in Basco-Bearnaise and 1 in Manech blond faced breeds) including 428 sons were presently available. The analysed phenotypes were daughter yield deviations (DYD) for milk, fat or protein yield, fat or protein content, obtained from the national EBV (Barillet *et al.,* 1996). The analysis were performed using the following models :

$$DYD_{ij} = s_i + g_j + e_{ij} \quad \text{(association analysis)}$$

$$DYD_{ij} = s_i + g_{ij} + e_{ij} \quad \text{(linkage analysis)}$$

where $s_i$ was the fixed effect of the sire i, $g_j$ or $g_{ij}$ the *PrP* genotype of the son j respectively between sires (association) or within sire i (linkage), eij the residual assumed to be normally distributed with a zero expectation and a heterogeneous variance $\sigma^2_{ei}/CD_{ij}$. $CD_{ij}$ was the reliability of the proof of the son j.

Moreover, heritability for milk yield was estimated from 2211 first lactation Manech blond faced ewes recorded among the 15 monitored affected flocks, using an animal model including or not the *PrP* genotype of the ewes in the model.

**Results and discussion.** the average EBV reliability of the 428 progeny tested sons ranged between 0.706 and 0.812 according to the dairy trait. With this design, no association nor linkage between the *PrP* locus and milk traits were detected (table 3). Similarly, including or not the *PrP* genotype in the model did not modify the heritability estimate of milk yield which was 0.33 for the Manech ewes.

## CONCLUSION

An increasing amount of results (including those of this paper) has been now accumulating in favour of selecting for ARR allele at the population level. In France, a field project started in 1995 in dairy sheep breeds. In a first step, breeding was based on *PrP* genotyping of AI rams without modification of the current selection scheme: the main objective was more to eliminate

VRQ allele and to prompt using ARR/ARR rams in affected flocks than to select for ARR allele at the population level.

Since 1999, the responsibles of the dairy sheep breeding programs have intensified selection for scrapie resistance, (i) by increasing the *PrP* genotyping in the nucleus up to 15,000 sheep in 2002 (within the framework of a national scrapie plan supported by the Ministry of Agriculture and Fisheries) and (ii) by doubling the number of candidate rams entering breeding centres of young rams and the AI centres. So far the ARR allelic frequency of the AI rams born between 1997 and 2001 increased from 0.53 to 0.68 in dairy Lacaune breed, and from 0.16 to 0.39 in Manech blond faced breed. The objective is that in 2004 all the rams in the AI centres and nucleus flocks will be ARR/ARR in the (dairy) Lacaune breed and at least heterozygous ARR (excluding ARR/VRQ) in the 4 other French dairy sheep breeds. Up to there, monitoring and studies will continue to add knowledge about the difficult questions of increasing the existence of healthy carriers and possible genetic relationships between *PrP* locus and production traits.

**Table 3. Association or linkage analysis between *PrP* locus (codon 171) and milk yield production traits using a granddaughter design**

| Trait | Granddaughter design[A] | Analysis | | |
|---|---|---|---|---|
| | Average EBV reliability of sons (n=428) | Association | Linkage | |
| | | P value | % informative meioses | P value |
| Milk yield | 0.755 | 0.218 | 49 % | 0.608 |
| Fat yield | 0.706 | 0.259 | | 0.272 |
| Protein yield | 0.706 | 0.544 | | 0.616 |
| Fat content | 0.812 | 0.944 | | 0.219 |
| Protein content | 0.812 | 0.316 | | 0.414 |

[A]: heterozygous sires (n=15) genotyped QR at codon 171 of the *PrP* locus

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