55th Annual meeting of the European Association for Animal Production
Bled, September 5th-9th, 2004
Commission on Animal Genetics

BREEDING FOR SCRAPIE RESISTANCE IN FRANCE

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INTRODUCTION

Scrapie, the small ruminants prion disease, belongs to transmissible spongiform encephalopathies (TSE), which also include Creutzfeldt-Jakob disease in Humans and bovine spongiform encephalopathy (BSE) in cattle. There is no scientific evidence to indicate that scrapie poses a risk to human health. But the emergence of BSE in 1986, its link with the new variant of Creutzfeldt-Jakob disease proven in 1996, and the demonstration that BSE can be experimentally transmitted to small ruminants gave reasons to the French Ministry of Agriculture to launch a programme to eradicate scrapie in the whole country. The genetic approach represents a good way to eradicate the disease. Indeed, numerous research works have shown that, in sheep, susceptibility to scrapie is modulated by allelic variations of a major gene encoding for protein PrP : the *PrP* gene (Hunter *et al*, 1996). About 15 alleles are known for the *PrP* gene, but only the 5 more frequent alleles are currently used in France to characterize the *PrP* genotype of sheep and consequently their level of resistance/susceptibility to scrapie. These five alleles can be sorted by increasing susceptibility to scrapie : ARR, AHQ, ARH, ARQ, VRQ. The general picture is that the VRQ allele is associated with a high degree of susceptibility whereas the ARR allele confers resistance with a nearly dominant effect. Therefore, the selection of ARR/ARR genotypes may be a relevant strategy to control scrapie either at the flock level or at the population level. Such an approach requires, however, to answer the following crucial points : resistance of ARR/ARR is universal, ARR/ARR sheep are not healthy carriers, no deleterious effect of the ARR allele on the other traits are observed. All scientific knowledge, accumulated by now, confirm these hypotheses and thus reinforce the idea that improving resistance to scrapie through a selection on PrP genotype is a sound strategy. Hence, France, the UK, the Netherlands and the EU had launched selection programmes for resistance to scrapie at a large scale.

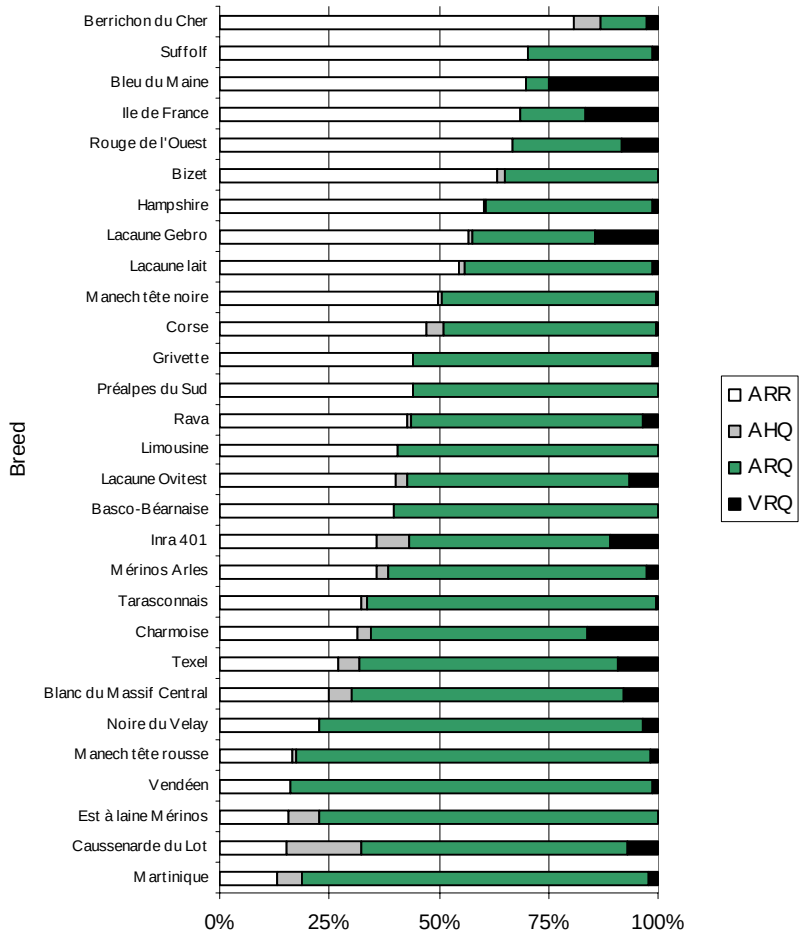
THE GLOBAL FRENCH SCRAPIE ERADICATION PROGRAMME

To eradicate scrapie, the French Ministry of Agriculture had implemented four complementary actions :

- clinical surveillance of the disease since June 1996 : 94 flocks have been detected as scrapie affected in the year 2002 and 43 in the year 2003, by this way.
- active surveillance with tests on slaughtered and quartered animals, in the framework of the EU surveillance programme. In 2002 and 2003, about 60,000 tests were performed on sheep. The abattoir and fallen stock surveys were indicating a crude prevalence of about respectively 0.1% and 0.7% in 2002 ; 0.1% and 0.2% in 2003.
- scrapie eradication programme for flocks which report scrapie, had been implemented since 1996, and had been modified since March 2001 by including *PrP* genotyping to define risky animals.

- a selection programme for scrapie resistance in all the sheep breeds had been launched since October 2001 for 5 years. As a preliminary to selection for resistance, an European survey of the initial allele frequencies in the pure breeds was established in 1999-2000 (CT973305 program). The figure 1 shows the considerable variation in the distribution of the four alleles (ARH and ARQ are pooled) between the different breeds : ARR allele frequency ranges from 13.3% to 80.5% and VRQ allele frequency ranges from 0% to 25% (Elsen et al, 2002).

Figure 1: Initial *PrP* allele frequencies (%) in 29 French sheep breeds or strain (ARQ=ARH)



A SELECTION PROGRAMME FOR SCRAPIE RESISTANCE INCLUDED IN EXISTING BREEDING SCHEMES

Livestock selection has been based in France on official organisations for a long time. This background enabled to rapidly implement a selection programme for scrapie resistance, even if it was necessary to adapt or improve the already existing breeding schemes.

French selection background. Selection of small ruminants in France is based on pyramidal schemes. Each sheep population of a given breed is split into two groups : a selection nucleus (selected population) and the rest of the population (base population). The breeding tools (production and pedigree recording, AI, progeny test, assortative matings,…) are exclusively or mainly used within the nucleus flocks, firstly to create genetic gain, and secondly to organize its diffusion by AI or natural mating towards the base population. This collective and structured organisation has allowed the implementation, for each breed, of breeding schemes, the efficiency of which depends on the more or less wide use of the breeding tools.

Even if the final objective is to improve the scrapie resistance of the whole population, concentrating the selection effort on the registered nucleus flocks participating to the breeding schemes was decided

considering cost-effectiveness reasons : higher global selection efficiency, better quality of the identification system, ability to provide semen or reproducers to the whole population.

Laboratories. In France, genotyping for livestock selection is mainly operated by the Labogena company which associates research and extension activities (among them, markers and genes genotyping and parentage checking). Since 2002, 9 other laboratories have been approved by the Ministry of Agriculture to genotype *PrP* locus for the national scrapie plan, Labogena being considered as reference laboratory for *PrP* analysis.

Quality assurance and circulation of information. The quality assurance and the circulation of genetic data were already organised in the context of livestock selection. When the national selection programme for scrapie resistance started, it appeared important to centralize the molecular information, provided by the different laboratories, in a central database connected to the existing genetic database used for usual selection purposes. The objective was to increase the reliability of the data and to facilitate following utilisation. For instance, linking both databases allows to infer genotypes of unsampled animals, using the information of genotyped related animals.

Management. In France, geneticists and managers of breeding schemes have been working jointly for a long time to carry on research and extension services in a close co-operation. Introducing the *PrP* gene information in the breed selection programmes has been required new abilities for the technicians and managers of breeding schemes, dealing with the way of using *PrP* genotype (planned matings, weight to give to the major gene with regard to the classical merit index, genotyping inference). At a national level, the selection programme for scrapie resistance is defined by a board including the Ministry of Agriculture and people working for research and extension services and breeding associations. This strong collaboration allows a close co-operation between research and development which is very important in a situation where scientific knowledge is still going on and where new knowledge must be included as fast as possible in selection decision.

Thus, the French selection programme for scrapie resistance has been benefited from the general and efficient existing organisations of livestock selection. It represents a key starting point to improve scrapie resistance in the whole population from efficient, rapid and economic points of view.

A SELECTION PROGRAMME WITH GLOBAL OBJECTIVES ADAPTED TO EACH BREED

General objectives. In October 2001, the national scrapie programme board defined 4 objectives to be reached in each sheep breeding scheme. These objectives must be achieved simultaneously, as quick as possible, both to increase resistance to scrapie in the whole population and to control disease in affected flocks :

- Elimination of the VRQ allele.
- Diffusion of ARR/ARR rams or semen to be used for replacement in affected flocks, an objective to be reached as a priority. Indeed, the national scrapie flocks programme obliges scrapie affected flocks to be restocked only with ARR/ARR rams, which allows to strongly protect from scrapie the young replacement ewes.
- Selection for the ARR allele. In the French sheep selection programmes, the *PrP* genotype has been considered as an additional selection criteria, to select for scrapie resistance while maintaining the selection for the production traits and the genetic variability.
- Diffusion of ARR/ARR rams or semen to commercial flocks. Once affected flocks being provided with ARR/ARR rams or semen, providing all the sheep population in order to secure the food chain is the next major objective which should be fulfilled producing ARR carriers.

Given the diversity of sheep breeds and sheep breeding schemes, the practical implementation in a given breed of these 4 objectives implied to consider the breed characteristics and thus to define specific selection programmes for scrapie resistance. The main breed characteristics can be summarized as following :

- initial *PrP* allele frequencies : the figure 1 makes obvious that French sheep breeds have a very different starting situation as regard to scrapie resistance ;

- prevalence of the disease in the breed : even if scrapie occurs all over the country, scrapie affected flocks are concentrated more particularly in a few areas and so far, in a few breeds ;
- weight of the breeding schemes in the whole population : *(i)* dairy breeds where AI is extensively used to spread the genetic progress in the base population ; *(ii)* terminal sires breeds, characterized by a large diffusion of males for non-registered terminal sires populations and also for a part of non-registered hardy populations ; *(iii)* hardy breeds which provide semen and few males to the commercial flocks ; *(iv)* rare breeds, for whom the main objective is to keep the genetic variability ;
- characteristics of breeding schemes : size of the selection nucleus, use of AI, type of genetic evaluation,…

Types of genotyped animals. Whatever the diversity of breed situations, the main shared idea is to concentrate the actions on the males, which play a key-role in the creation and diffusion of the genetic gain. The female genotyping may be needed at different steps of the selection on the *PrP* gene. Two types of females may be genotyped.

*Adult sires***.** The AI rams are early genotyped as a first obligatory step, in order to know, at least, the *PrP* genetic structure of the most important reproducers of the breed. The genotyping of natural mating rams may be a mean to identify and cull the most susceptible rams (with the VRQ allele) as well as to improve more rapidly the frequency of the favourable allele by choosing the replacement from the more resistant rams.

*Candidate young sires***.** Candidate young sires are also genotyped because they represent a key-step for the selection. Indeed, these young males are intended to enter the gathering structures and then, to be used as AI rams or natural mating rams. The sooner in the selection process the genotyping is done, the more important the selection pressure on the *PrP* gene is. That's why, in order to be as efficient as possible, most of sheep breeding schemes are organised with an on-farm genotyping of the young candidate males.

*Young males for affected and commercial flocks***.** Other young males may be genotyped in order to achieve the 2nd and 4th objective of the national selection programme for scrapie resistance. The necessity of quickly providing resistant rams or semen to affected flocks and production flocks, requires to genotype a large number of lambs. In order to optimise the costs, the genotyping should focus on lambs known to be born from at least heterozygous ARR sires or dams.

*Sire dams***.** Their genotyping is interesting to organise assortative matings, particularly when the unfavourable alleles have a high frequency in the population. This genotyping allows to speed up the selection for the good alleles while maintaining the selection on the other traits.

Young females for replacement. Genotyping these young females is needed for the complete elimination of an undesirable allele (case of VRQ for *PrP* in sheep). It is also useful to select for the favourable allele(s). It may be possibly a way to know the genotype of the whole breeding scheme's population. If it is organised during the first years of the selection programme, it may reduce strongly the number of young males to be genotyped.

For breeders involved in breeding schemes, all blood sampling, sending to laboratory, and laboratory testing are free-of-charge concerning target animals as defined by the selection programme for scrapie resistance of a given breed. No restriction exist concerning the use of sires or sire dams, depending on their *PrP* genotype, but some compensations are given if a susceptible sire or sire dam is culled and replaced by another reproducer more resistant and with the same genetic merit for production traits.

FIRST RESULTS

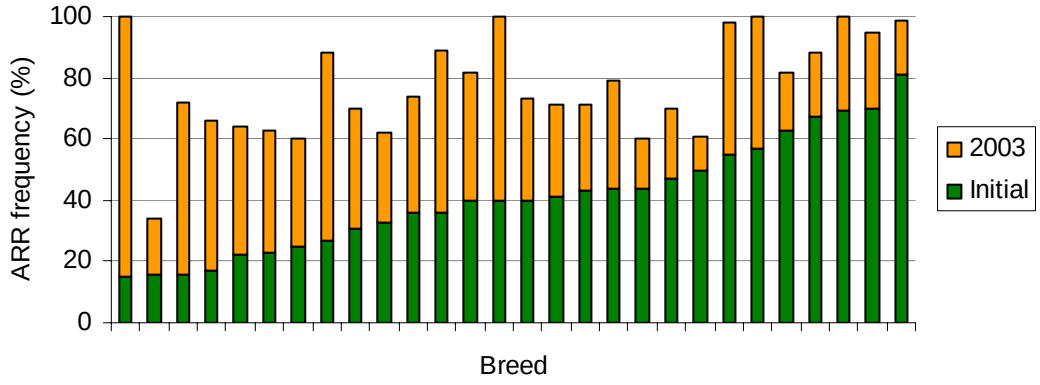
Number of genotyped animals by year. Since the beginning of the selection for the *PrP* gene, about 80-90,000 animals have been genotyped each year, financially supported by the French selection programme for scrapie resistance (table 1). Moreover, around 80,000 animals have been genotyped with private funds, since 2002.

Table 1 : Number of *PrP* analyses in the framework of the French selection programme

	2002	2003	2004
Young rams	28,600	40,900	35,200
AI rams	500	0	0
Natural mating rams	9,500	0	0
Sire dams	15,500	7,700	6,300
Ewe lambs	29,100	41,300	37,000
Total	**83,200**	**89,900**	**78,500**

Evolution of the allele frequencies. The figure 2 shows the evolution of the ARR allele frequency of the candidate young sires compared to the active sires, after two years of selection. On average, the increasing of the ARR frequency is about 40%. It ranges from 11% to 85%, depending on the breed and its selection strategy. In the same time, the very susceptible allele (VRQ) was efficiently eliminated for the young sires in all the selection schemes.

Figure 2 : Evolution of the ARR allele frequency of males after two years of selection



CONCLUSION

Selection on the *PrP* gene represents a sound strategy to improve the scrapie resistance in sheep. The French scrapie eradication programme uses this genetic control with both the aim of protecting the affected flocks and improving the scrapie resistance of the whole population. The French selection programme for scrapie resistance started in October 2001, for 5 years, and is applied in all the sheep breeding schemes. The facts that it was launched within the framework of the organised livestock selection, benefits from numerous existing schemes at the beginning and was adapted to each breed, represent the key points of its efficient and rapid implementation. Nevertheless, in spite of these encouraging results, the improvement of the scrapie resistance of the French sheep population will need a long time. Therefore it is necessary first, from a technical point of view, to keep in mind cost-effectiveness aspects and then, from a scientific point of view, to be aware of new scientific knowledge to be included in selection decision.

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