The French Ovine Scrapie Plan: Results And Prospects

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Introduction

Scrapie, the small ruminants prion disease, belongs to Transmissible Spongiform Encephalopathies (TSE) which also includes Creutzfeldt-Jakob disease in Humans and Bovine Spongiform Encephalopathy (BSE) in cattle. In 1989, Hunter *et al.* identified a major gene, the *PrP* gene, encoding the prion protein (*PrP*). The ovine *PrP* coding region is highly polymorphic, but only 3 codons (136, 154, and 171) are currently linked to the level of resistance/susceptibility to ovine classical scrapie and BSE (Goldmann *et al*. (1991), Hunter *et al.* (1993)). In French breeds, 4 alleles are routinely used in selection (sorted by increasing susceptibility to classical scrapie): ARR, AHQ, ARQ, and VRQ (Elsen *et al.* (1997), Elsen *et al.* (2002), Palhière *et al*. (2002a)). ARR/ARR sheep are highly resistant to classical scrapie and BSE, reducing dramatically zoonosis risk. From now, only 3 ARR/ARR animals contracted classical scrapie in natural conditions (Groschup *et al.* (2007)). Moreover, the use of the ARR allele decreases the within flock infection pressure, since placentas from ARR heterozygous lambs are not infectious (Andréoletti *et al.* (2002)). In France, at the end of the 1990's, a representative sampling of each breed (composed of young sires) was *PrP* genotyped to estimate the initial allele frequencies, i.e. the *PrP* starting point for each breed (Palhière *et al.* (2002b)). Then, in October 2001, the French Ministry of Agriculture has launched a genetic scrapie plan in order to reinforce scrapie resistance in the national sheep livestock. Innovative logistics connected to the existing genetic database has been implemented in the framework of this plan. The present paper presents results and prospects of this French ovine scrapie plan.

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I] The French ovine breeding program for scrapie resistance

Given the BSE crisis in cattle, European Commission Decision 2001/999/EC introduced breeding programmes for resistance to TSEs in sheep (classical scrapie and BSE strain), in the aim to reduce the hazard to public health from the theoretical acquisition of BSE by sheep. The French Ministry of Agriculture, research institutes, extension services organizations, and sheep breeding associations decided to launch in October 2001 the national breeding program for scrapie resistance. A steering committee including these different protagonists has been created to manage the plan.

Means of action and objectives of the national scrapie plan (2002-2009). The plan was first launched for 5 years (2002-2006). To consolidate the results, it has been extended for 3 extra years (2007-2009). The Ministry of Agriculture has funded the plan, including management and PrP genotyping costs, with an European co-funding since 2004. The main strategy of the plan was to concentrate the selection effort on breeding schemes considering their higher global selection efficiency and the existence of a genetic database. In October 2001, the steering committee defined 4 objectives: (i) elimination of the VRQ allele; (ii) diffusion of ARR/ARR rams or semen to be used for replacement in the affected flocks; (iii) selection for the ARR allele while maintaining genetic level of production traits and genetic variability; (iv) diffusion of ARR/ARR rams or semen to commercial flocks.

Genotyping strategies. Each year, a quota of genotyping has been designed for each breed, given the global quota allocated by the Ministry. The key point was to focus on the PrP selection of the candidate young sires since representing the future adult sires. To eliminate the VRQ allele, it has been decided to genotype ewe lambs kept for replacement in suckling breeds, because the frequency of this allele was significant. In dairy breeds, genotyping of sire dams has been implemented to optimize assortative matings. Males for diffusion to commercial flocks can also be genotyped depending of each breeding scheme.

Database organisation for the national scrapie plan. 19 laboratories have been accredited for PrP genotyping. The genotype results have been stored in a national database (INMOLE) including until now around 670 000 genotypes. Then INMOLE has been connected to the existing national genetic database via an interface. This interface is being updated once a week, including compatibility controls between molecular data. To give value to the pedigree information and the molecular one, a predicted genotype method has been developed in 2004. PrP predictions give early genotype information what makes easier the choice of the animals to be PrP genotyped in the nucleus flocks. Prediction information is quite reliable, since less than 1% incompatibility has been observed between prediction and PrP genotyping (on 198 280 animals). PrP predictions have also been stored in the molecular interface. Predictions and the improvement of the PrP genetic structure of the nucleus flocks has allowed to reduce over years the number of PrP genotypings, particularly in suckling breeds, as shown in Figure n°1.

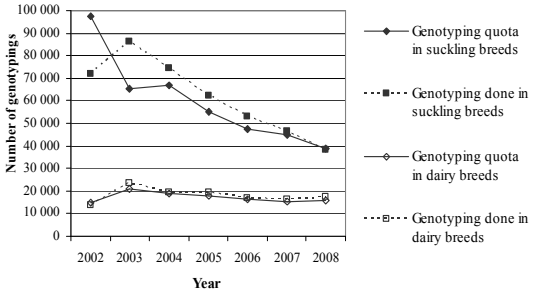

Figure 1: Quotas and genotypings done in the national scrapie plan

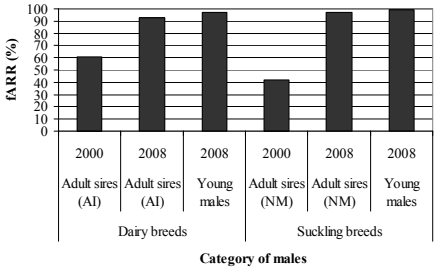

Figure 2 : Male ARR frequency in the selection nuclei

II] Main results of the national ovine scrapie plan

Selection of the ARR allele in the male population. Figure 2 shows the evolution of the ARR frequency in the male population of the nucleus flocks representing the main vector of diffusion: AI rams for dairy breeds and natural mating (NM) rams for suckling breeds. In adult sires, the increase of the allele ARR was significant: +30% and +45% respectively in dairy and suckling breeds. This average hides differences according to the initial PrP allelic frequencies. PrP selection effort on males is also demonstrated by the fact that ARR frequency of young males born in 2008 is higher than one of adult sires present in 2008.

Elimination of the VRQ allele. The VRQ allele was mainly present in suckling breeds. In dairy breed the VRQ initial frequency was less than 2% (Palhière *et al.* (2002b)). Thus in suckling breeds an important effort has been done to eliminate VRQ carriers: since 2003 less than 4% of ewe lambs kept for replacement were VRQ carriers in the most initially disadvantaged breeds. In 2008, no ram from the nucleus flocks were VRQ carriers compared to 8 % in 2002. For the female pathway, elimination of the VRQ allele is expected in a near future provided that the selection pressure is maintained.

Impact of the scrapie plan on genetic merit and genetic variability. In 2004, a survey has estimated the plan consequences on genetic merit and genetic variability in 4 breeds (Brochard *et al.* 2006). No major impact was noticed. The appraisal of the plan carried out in 2009 provided the same conclusions. Nevertheless, a slight stagnation of the genetic gain has generally been observed during the first 2-3 years of the plan. In a few breeds, the genetic merit has been decreasing at the beginning of the plan because of radical decisions in the starting PrP selection. On the contrary, no impact on genetic variability has been noticed, probably due to the rules efficiently applied for families management within each breed.

III] Prospects of the national scrapie plan

Diffusion of scrapie resistance to the whole population. In 2008, 96% AI in dairy breeds were done from homozygous resistant rams, while in suckling breeds 89% rams for diffusion were ARR/ARR sires. The number of rams diffused from the nucleus to the commercial flocks is lower than the diffusion potential: in 2008, 7800 resistant rams were diffused in meat breeds whereas the diffusion potential was 10300 (half is predicted). This point is crucial for continuing the plan: thanks to the plan and the use of PrP predictions, nucleus flocks are today able to diffuse ARR/ARR rams (or semen) with a high genetic merit.

An extended national (classical) scrapie plan (2010-2012). Since April 2008, the steering committee focused on the conception of different scenarios for continuing the plan: the most relevant scenario including the full male diffusion from nucleus to commercial flocks has been accepted by the Ministry of Agriculture in August 2009. Thus the national (classical) scrapie plan was extended for 3 years, 2010-2012: 50 000 genotypings are yearly planned and allocated according to the next principles: (i) genotyping of rams needed for the selection nucleus; (ii) genotyping of young males for diffusion to commercial flocks when then can not be predicted; (iii) increase of the number of females with a known PrP genotype in the nucleus flocks (select resistant females and increase the prediction potential).The Ministry of Agriculture is planning to promote the use of ARR/ARR rams (or semen) in all

the nucleus and commercial flocks. Consequently, ram inventories are needed for all flocks and they could be included in the existing genetic national database.

ARQ allele preservation. Different scrapie strains, classical, atypical, and BSE, have been identified (Bruce (2003)). On the assumption of the apparition of a new type of TSE resistant against the ARR allele, it would be relevant to preserve a bi-allelic variability, i.e. the ARQ allele. Six French breeds, presenting now a proportion of ARR/ARR females between 60 % and 80 % in their nucleus flocks, have to face the question. The ancestral ARQ allele is still present in their commercial flocks but these animals have a lower genetic merit than in the selected population. Furthermore, the ARQ allele has additional polymorphism, either resistant against atypical strain as $AL_{141}RQ$ allele (Moreno *et al.* (2006), or resistant against classical or BSE strains as $AT_{137}RQ$ and $ARQK_{176}$ alleles as demonstrated both in experimental challenge or natural contamination in Sarda breed (Vaccari *et al*. (2009), Maestrale *et al.* (2009)). Thus these last Sarda breed results have to be confirmed in French breeds. For the moment regarding the 6 French breeds concerned by the fixation of the ARR allele in a near future in their nucleus flocks, solutions are envisaged to produce ARR/ARQ rams with the same genetic merit than ARR/ARR rams of the nucleus flocks.

Conclusion

The national scrapie plan is a unique example of a major gene large selection. The close cooperation between partners and the existence of an organised genetic database have played a key-role in the plan success. The quotas of 530 000 genotypings (2002-2009) and the strategies designed for the nucleus flocks of each breed have allowed to achieved the plan objectives: (i) The VRQ allele has been eradicate in the male pathway and widely decreased in the female pathway; (ii) A large number of ARR/ARR rams (or semen) are available for diffusion from nucleus to commercial flocks; (iii) the ARR selection didn't have a negative impact on genetic merit and genetic variability. The French scrapie plan has been extended for 3 years in 2010 with the main objective to diffuse the scrapie resistance to the whole sheep population.

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