ALLELE FREQUENCIES OF THE *PrP* GENE IN 29 FRENCH SHEEP BREEDS. POSSIBLE USE IN SELECTION FOR RESISTANCE TO SCRAPIE

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INTRODUCTION

Scrapie disease belongs to Transmissible Spongiform Encephalopathies as well as Bovine Spongiform Encephalopathy or human Creutzfeld-Jakob Disease. Scrapie is a natural disease which was first described in 1732. For years sheepbreeders fought Scrapie by eliminating sick animals and culling related animals. Evidence for a genetic resistance to Scrapie has been observed in different experiments made in the UK, the susceptibility being defined by a shorter incubation period after artificial inoculation of a scrapie isolate (Dickinson *et al.,* 1968). The relationship between the polymorphism for the *PrP* gene and the incidence of natural scrapie in several breeds was gradually established since 1991 (review by Hunter *et al.,* 1997). The *PrP* gene encodes for the PrP protein, the PrP^{sc} isoform of which accumulates in the central nervous system of affected animals.

As a preliminary to selection for resistance, a survey of the initial allele frequencies in the European breeds was established. The French results are presented here. Selection for scrapie resistance based on PrP polymorphism is then discussed.

MATERIAL AND METHODS

Genotyping. The genotype at the *PrP* locus was determined for the three codons for which polymorphisms were known to be linked to scrapie susceptibility (Elsen *et al.,* 1999). RFLP-PCR performed techniques allow the detection of a mutation in each of the three following codons 136, 154 and 171. Four alleles were detected : ARR, AHQ, ARQ and VRQ listed in increasing range of susceptibility to scrapie. The allele ARH described by other techniques was here confused with the allele ARQ. Both give quite the same level of susceptibility, ARQ a little more than ARH.

Breed sampling. The sampling of the animals was organized by breed, or by strain within the Lacaune breed which includes three strains, one dairy scheme and two meat sheep schemes. Data were already available in 1998 for dairy breeds given the genotyping of the artificial insemination centres rams from breeding programmes, and also for the Inra401 breed (Tchamitchian *et al.*, 1986) with a sample of the INRA nucleus flock at La Sapinière. A large survey was conducted in 1999 in more than 20 meat sheep breeds. Animals were sampled among the young rams issued from pedigree flocks and submitted to individual performance test for growth and body composition abilities by every breed society (Perret *et al.*, 1994). One hundred animals for every breed were expected to be sampled. To enlarge the sample representativity, these 100 animals were chosen maximizing the number of sires, of grandsires and of flocks. Specifically to the Martinik (strain related to Black Belly sheep bred in the French West Indies) and Bleu du Maine breeds the number of males was completed by as far as possible unrelated females.

Table 1. Initial PrP allele frequencies in 29 french sheep breeds or strains

Breed	Purpose	n*	*PrP* alleles			
			ARR	AHQ	ARQ	VRQ
Basco-Béarnaise	Milk	149	0.399	-	0.601	-
Berrichon du Cher	Meat	95	0.805	0.063	0.105	0.026
Bizet	Meat	53	0.632	0.019	0.349	-
Blanc du Massif Central	Meat	120	0.250	0.050	0.621	0.079
Bleu du Maine	Meat	100	0.700	-	0.050	0.250
Caussenarde du Lot	Meat	106	0.151	0.174	0.604	0.071
Charmoise	Meat	99	0.313	0.030	0.495	0.162
Corse	Milk	152	0.470	0.039	0.487	0.003
Est à laine Mérinos	Meat	91	0.159	0.066	0.774	-
Grivette	Meat	68	0.441	-	0.544	0.015
Hampshire	Meat	103	0.602	0.005	0.379	0.015
Ile de France	Meat	99	0.687	-	0.146	0.167
Inra401	Meat	310	0.360	0.071	0.458	0.111
Lacaune, OviTest strain	Meat	100	0.400	0.030	0.505	0.065
Lacaune, Gebro strain	Meat	99	0.566	0.010	0.278	0.146
Lacaune, milk strain	Milk	561	0.545	0.016	0.427	0.012
Limousine	Meat	90	0.406	-	0.594	-
Manech blond faced	Milk	315	0.167	0.008	0.806	0.019
Manech black faced	Milk	122	0.496	0.012	0.488	0.004
Martinik	Meat	99	0.133	0.053	0.793	0.021
Mérinos d'Arles	Meat	99	0.359	0.025	0.591	0.025
Noire du Velay	Meat	55	0.227	-	0.736	0.036
Préalpes du Sud	Meat	101	0.441	-	0.559	-
Rava	Meat	71	0.430	0.007	0.528	0.035
Rouge de l'ouest	Meat	96	0.667	-	0.250	0.083
Suffolk	Meat	98	0.704	-	0.281	0.015
Tarasconnais	Meat	97	0.325	0.010	0.660	0.005
Texel	Meat	100	0.270	0.050	0.590	0.090
Vendéen	Meat	101	0.163	-	0.822	0.015

n*= number of animals sampled

RESULTS AND DISCUSSION

The *PrP* genotype results. The genotype results (table 1) show considerable variation in the distribution of the four alleles between the different breeds. For the 29 breeds or strains studied, ARR allele frequency ranges from 13,3 % in Martinik to 80,5 % in Berrichon du Cher breeds, AHQ allele frequency ranges from 0 % for ten breeds to 17,4 % in Caussenarde du Lot,

ARQ allele frequency ranges from 5 % (Bleu du Maine) to 82,2 % (Vendéen), VRQ allele frequency ranges from 0 for five breeds to 25 % in Bleu du Maine.
ARR allele is frequent in most of terminal sires breeds : higher than or close to 60 % for seven of them (Ile de France, Berrichon, Rouge de l'Ouest, Bleu du Maine, Suffolk, Hampshire and Lacaune Gebro), around 30 % in Texel and Charmoise or around 16 % in Vendéen. O'Doherty *et al.*, (2000 ; 2001) reported irish data with convergent figures about Suffolk, Texel, Rouge de l'Ouest, Bleu du Maine and Vendéen breeds.
Hardy breeds show a high frequency of the ARQ allele, arising around 60 % with Limousine, Blanc du Massif Central, Préalpes, Caussenarde, Tarasconnais, as well as with Merino breeds : Mérinos d'Arles, Est à laine Mérinos up to 77 %. Bizet differs from the other hardy breeds with a low frequency for ARQ (35 %) and a higher one for ARR (63 %). The history of this breed located in Cantal and Haute-Loire (Massif Central) departments reports crosses in the late nineteenth century with Dishley rams, such crosses being also reported for Ile de France and Berrichon du Cher, two breeds which also present a predominant frequency of the ARR allele. Dairy breeds display very different frequencies, four of them having a rather high ARR initial frequencies like Lacaune milk strain 54 %, Manech black faced 50 %, Corse 47 %, Basco-Béarnaise 40 % while Manech blond faced has only 17 %.

Selection for scrapie resistance. Selection for resistance can be proposed having in mind the limits pointed by Elsen *et al.* (1997) and recovered by Smits *et al.* (2000) who sumed them up in four remarks : (i) universal resistance with different scrapie strains, (ii) resistant animals may be healthy carriers, (iii) deleterious effect on the selection on other traits, (iv) selection for scrapie resistance is a costly process which must be optimized. For remark (i), current studies on scrapie cases corroborate full resistance of ARR/ARR sheep (Elsen *et al.,* 2002). Concerning remark (ii), additional results on the absence of PrP^{sc} in the tonsils of genotype ARR/ARR sheep in affected flocks (Andréoletti *et al.,* 2000) enlarge the hypothesis of absence of healthy carrier. About effect on other traits (iii), we did not find evidence for an association between ARR allele frequency for meat breeds and the phenotypic mean of the breed for prolificacy and for the average daily gain of the lambs (unpublished data). Roden *et al.*, (2001) found no relationship in Suffolk sheep with the following traits : 8-week weight, scan weight at about 20 weeks, muscle depth, ultrasonic fat and Lean Index Score. Bonnet (1999) found absence of relationship with semen fecundity of Lacaune rams. Association with milk traits is currently studied (Barillet *et al*., 2002).

In France, elimination of the alleles associated with the highest scrapie susceptibility, firstly VRQ then ARQ, began a few years ago in the Lacaune milk strain (1995) and then for the Lacaune meat strains and Inra401 (1998). Manech blond faced in 1999 (Smits *et al.*, 2000) and Caussenarde du Lot in the year 2000 began to select resistant rams to be used for reproduction in affected flocks. In the UK, PrP genotyping was first proposed for Suffolk sheep (Hosie and Dawson, 1996) and then extended to others breeds (Dawson *et al.,* 1998). National scrapie plans have been implemented within the last four years in the Netherlands (Smits *et al.,* 2000) and since the year 2001 in the UK. A French programme co-ordinated by the Ministry of Agriculture and Fisheries arose in October 2001 at the intention of all the sheep breeds. This plan is organised on a five-years schedule and follows up four goals : (1) eradication of the VRQ allele, (2) production of ARR/ARR rams to be used for replacement in affected flocks,

(3) selection in favour of the ARR allele as an additional selection goal for all genetic improvement schemes organised at the breed level, (4) generalization of the use of ARR/ARR rams as terminal sires for the commercial flocks.

CONCLUSION

The genetic variability of susceptibility to Transmissible Spongiform Encephalopathies of small ruminants, mainly explained by the polymorphism of the *PrP* gene offers a very attractive lever of action to control these pathologies. Large scale genotyping tools now exist. Organizations like breed societies are qualified to implement the selection of reproducers for *PrP* genotype. Work is in progress with the French breed societies in order to define specific programmes taking in account : the presence or not of scrapie cases in the breed, the current frequency of the four *PrP* alleles, the specificities of the breeding scheme like size of the selection nucleus, parental evaluation, individual test, progeny test, artificial insemination, purebred, maternal crossing, terminal crossing.

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REFERENCES

Andréoletti, O., Berthon, P., Marc, D., Sarradin, P., Grosclaude, J., van Keulen, L., Schelcher, F., Elsen, J.M. and Lantier, F. (2000) *J. Gen. Virol.* **81** : 3115-3126.
Barillet, F., Andréoletti, O., Palhière, I., Aguerre, X. *et al.* (2002) *These Proceedings*.
Bonnet, E. (1999) "Rapport de stage ISARA INRA-SAGA", 45p.
Dawson, M., Hoinville, L.J., Hosie, B.D. and Hunter, N. (1998) *Vet. Record.* **142** : 623-625.
Dickinson, A.G., Stamp, J.T., Renwick, C.C. *et al.* (1968) *J. Comp. Pathol.* **78** : 313-321.
Elsen, J.M., Barillet, F., Tien, V. and Khang, J. *et al.* (1997) *INRA Prod. Anim.* **10** : 133-140.
Elsen, J.M., Amigues, Y., Schelcher, F., Ducrocq, V. *et al.* (1999) *Arch. Virol.* **144** : 431-445.
Elsen, J.M., Barillet, F., François, D., Bouix, J. *et al.* (2002) *Bulletin des GTV* **13** : 123-128.
Hosie, B.D. and Dawson, M. (1996) *Vet. Record* **138** : 215-216.
Hunter, N. (1997) "The Genetics of Sheep", p. 225-240, Editors L. Piper and A. Ruvinsky, CAB International, Oxon, UK.
O'Doherty, E., Aherne, M., Ennis, S., Weavers, E. *et al.* (2000) *Vet. Record.* **146** : 335-338.
O'Doherty, E., Aherne, M., Ennis, S., Weavers, E. *et al.* (2001) *Res. in Vet. Sc*. **70** : 51-56.
Perret, G., Bouix, J., Poivey, J.P. *et al.* (1994) *Renc. Rech. Ruminants* **1** : 187-192.
Roden, J.A., Haresign, W. and Anderson, J.M.L. (2001) *Proc. Brit. Soc. An. Sc*. **45**.
Smits, M.A., Barillet, F., Harders, F., Boscher, M.Y. *et al.* (2000) *Proc 51st E.A.A.P.*, **S-IV-4**.
Tchamitchian, L., Ricordeau, G., Bibé, B. *et al.* (1986) *Proc. 3rd WCGALP* **9** : 535-540.