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Breeding sheep for scrapie resistance

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SUMMARY – Scrapie disease belongs to Transmissible Spongiform Encephalopathies as well as Bovine Spongiform Encephalopathy or human Creutzfeldt-Jakob Disease. Scrapie is a natural disease which was first described in 1732. For years sheep breeders fought scrapie by eliminating sick animals and culling related animals. Evidence for a genetic resistance to scrapie has been observed in different experiments conducted in the UK since the sixties, in particular the "*Sip*" gene, *Sip* for scrapie incubation period. In 1991, the relationship between the polymorphism of the *PrP* gene and the incidence of natural scrapie in several breeds was described. From 1990 to 1996, different studies showed that three codons of the gene have an action on the susceptibility to natural scrapie. The analysis of an epidemic in a French experimental farm detailed the level of susceptibility of the alleles and genotypes. In France, actions started in 1995 in dairy sheep breeds and in 1999 in meat sheep, through the survey of the frequencies of alleles and genotypes of the *PrP* gene in 25 breeds. In meat sheep, a few breed societies started a selection programme in 2000. In 2002 a large national programme concerning all the French breeds is starting under the coordination of the Ministry of Agriculture and Fisheries.

Key words: Sheep, genetics, Transmissible Spongiform Encephalopathies, scrapie, *PrP* gene.

RESUME – *"Sélection des ovins pour la résistance à la tremblante". La tremblante du mouton appartient à la famille des Encéphalopathies Spongiformes Transmissibles au même titre que l'Encéphalopathie Spongiforme Bovine ou la Maladie de Creutzfeldt-Jakob chez l'homme. La tremblante est une maladie naturelle rapportée depuis 1732 en Angleterre. De tous temps les moutonniers l'ont combattue en éliminant les animaux atteints et en réformant les animaux apparentés aux animaux touchés. L'existence d'une susceptibilité d'origine génétique a été mise en évidence dès les années soixante par différentes expérimentations en Grande-Bretagne, et notamment le gène "*Sip*", pour scrapie incubation period. En 1991, la relation entre le polymorphisme au gène* PrP *et l'incidence de la tremblante a été décrite dans plusieurs races. De 1990 à 1996, plusieurs études ont montré que trois codons du gène gouvernent la sensibilité à la tremblante. L'analyse de l'épidémie survenue dans un troupeau expérimental en France a permis de situer les niveaux de sensibilité des différents allèles et génotypes. En France, des actions de sélection vers la résistance ont débuté dès 1995 en races laitières. L'inventaire des fréquences des allèles et génotypes au gène* PrP *a été réalisé en 1999 pour 25 races allaitantes. A la suite de quoi quelques actions de sélection ont débuté en 2000. Fin 2001, un programme national concernant toutes les races françaises est lancé sous l'impulsion du Ministère de l'Agriculture et de la Pêche.*

Mots-clés : *Ovin, génétique, Encéphalopathies Spongiformes Subaigües Transmissibles, tremblante, gène* PrP.

Introduction

Scrapie disease belongs to Transmissible Spongiform Encephalopathies as well as Bovine Spongiform Encephalopathy (BSE) or human Creutzfeldt-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 susceptibility to scrapie was observed in different experiments made in the UK. It was then related with the polymorphism of the *PrP* gene which 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. Some French results are presented here. Selection programme for scrapie resistance based on *PrP* polymorphism is then discussed.

Pathology and diagnosis of scrapie

Symptoms of scrapie were first reported in 1732 in England, neuro-degeneration lesions were described in 1898 by Benoist and Morel, transmission by inoculation was demonstrated in 1936 by Cuillé and Chelle (from Schelcher *et al.*, 2002). These observations lead to the name Transmissible

Spongiform Encephalopathies (TSE), of which scrapie was the first described disease. Other TSE like human Creutzfeldt-Jakob Disease was described in 1920, Chronic Wasting Disease for wild ruminants in 1980, Bovine Spongiform Encephalopathy in 1986. All have in common a very long incubation period: several months or years.

Scrapie is known by many sheep breeders, even if its prevalence is low. It can be considered as endemic in many countries. Symptoms appear usually on animals between one and five years old. Scrapie is suspected following four groups of nervous symptoms: social behaviour modifications, locomotor incoordination, pruritis, trembling. Slimming can also be observed. A sick animal often does not present all the symptoms but only one or two of them. This may be related with the presence in the field of different strains of scrapie. The clinical signs can last from two weeks to several months, the outcome being always the death of the animal (Hunter, 1997; Schelcher *et al.*, 2002).

A sure diagnosis can be given *post mortem* after histopathological examination of the brain. Lesions with formation of vacuoles indicate positive diagnosis. Neurones reveal an aggregated protein called prion protein PrP. This form associated with scrapie is the abnormal form (PrP^{Sc}) of the protein, the normal one (PrP^{c}) is present in the neurones and in different organs. A complementary diagnosis can be given by the detection of PrP^{Sc} by different techniques like immunohistochemistry, western blotting or ELISA test (Schelcher *et al.*, 2002).

Incidence

In France, declaration of scrapie cases became compulsory since June 1996. Survey of affected sheep flocks in the South-East reported 76 flocks with cases between 1990 and 1995 (Calavas *et al.*, 2002). Then the national survey detected about 50 flocks per year from 1996 to 2000 and 34 flocks in 2001. 60% of these flocks are located in the same geographic area, suggesting strong epidemiological relationships.

When his flock is declared affected, the owner cannot sell any reproducer during two years after the last positive animal. The animal health policy between 1996 and 2002 took into account the prevalence by year-of-birth. When it was higher than 10%, animals had to be slaughtered. The new policy enacted in March 2002 takes into account the genetic susceptibility to scrapie, the resistant animals being kept alive.

Four positive goat flocks have been reported during the same period (Calavas *et al.*, 2002). In absence of evidence for a genetic resistance in goats, the policy requires the slaughtering of the whole flock.

Disease experts think that the true incidence is under-reported. In the UK where declaration is notifiable since January 1993 and compulsory slaughter scheme for sheep and goats displaying clinical signs of scrapie is active since July 1998, about 500 cases were reported each year from 1996 to 2000, 280 in year 2001 (DEFRA, 2002). One way to enforce under-report is the active surveillance programme started in 2002 by the European Union with tests in abattoirs and fallen stocks. In France 60,000 sheep and 40,000 goats all with age over 18 months will be tested this year.

Genetic control

Genetic determinism

Possible genetic control of the scrapie was demonstrated in studies conducted by Alan Dickinson at the Neuropathogenesis Unit of Edinburgh (review by Hunter, 1997) by the selection of two Cheviot lines after subcutaneous inoculation of a source of experimental scrapie obtained from pooled brains of scrapie sheep, one line showed a short incubation period of about 300 days, the other line had a long incubation period of about 1000 days. The gene was called *Sip* for scrapie incubation period, with two alleles sA et pA for short and prolonged. Allele sA was partially dominant with this source of experimental scrapie but seemed to be recessive with natural scrapie.

Polymorphisms of the *PrP* gene was found to be associated with differences in experimental scrapie (Goldmann *et al.*, 1991) and then in natural scrapie in the UK (Hunter *et al.*, 1994), in the

Netherlands (Belt *et al.*, 1995), in France (Clouscard *et al.*, 1995). Polymorphisms were observed at ten codons but only three codons play a role in the control of the susceptibility to scrapie: codons 136, 154 and 171. All possible combinations of the 3 codons 136 (A coding for Alanine, V for Valine), 154 (R for Arginine, H for Histidine), 171 (Q for Glutamine/R/H) were not found. The wild allele is probably ARQ (simplified notation for $A_{136}R_{154}Q_{171}$). Four mutated alleles have been detected so far (VRQ, AHQ, ARH, ARR) each of them deriving from the wild allele by a single codon variation (Elsen *et al.*, 1999). Linkage of *PrP* gene to the *Sip* gene was established by Hunter *et al.* (1989).

PrP genotypes and susceptibility

A natural scrapie epidemic appeared in a closed Romanov flock of our Institute in 1993. Based on a size of 650 ewes, about 100 animals died every year between 1993 and 1995 (Elsen *et al.*, 1999). All sheep were genotyped since 1995 and the relation between the *PrP* genotype and scrapie status is presented in Table 1.

Table 1. *PrP* genotype in the exposed animals of the INRA flock of Langlade

Status	*PrP* genotype										Total
	ARR ARR	ARR AHQ	AHQ AHQ	ARR ARQ	AHQ ARQ	ARR VRQ	AHQ VRQ	ARQ ARQ	ARQ VRQ	VRQ VRQ	
Scrapie	0	0	1	2	7	10	3	90	203	121	437
Healthy	137	74	12	106	93	143	100	133	238	32	1068
Total	137	74	13	108	100	153	103	223	441	153	1505

Scrapie cases are mostly associated with the three genotypes VRQ/VRQ, ARQ/VRQ and ARQ/ARQ where 79%, 46% and 40% respectively animals died. Alleles ARR and AHQ gave a nearly dominant resistance to the scrapie agent.

The age at death (Table 2) was the lowest for VRQ/VRQ: 705 days (about 25 months or two years and a month), 114 and 113 days more for ARQ/VRQ and ARQ/ARQ with a higher variation rate for the latest genotype, and very different of that of ARR/VRQ which was 1810 days (about 60 months or five years) with nine animals of this genotype.

Table 2. Age at death and *PrP* genotype

	Genotypes				
	VRQ/VRQ	ARQ/VRQ	ARQ/ARQ	ARR/VRQ	Others
Number born after 1992	101	146	56	9	10
Age at death (days)					
Mean	705	819	818	1810	1273
Standard error	110	236	421	661	760
Coefficient of variation	15.7	28.8	51.4	36.6	59.6

The scrapie status of the dam influenced the transmission of the disease. When the dam was scrapie positive her lambs were scrapie positive up to 93% and negative to 7%. When the dam was scrapie negative her lambs were scrapie positive up to 20% and negative up to 80%. The genotype of the dam and the genotype of the lamb modulate surely the transmission, a detailed analysis of these relationship is in progress.

Breed survey for *PrP* alleles

Selection on scrapie resistance requires data about the frequencies of the *PrP* alleles in different breeds. A large survey of the European sheepbreeds was implemented by a European Union funded

project from 1998 to 2000. The French breeds surveyed were five dairy breeds and twenty four meat breeds or strains.

Genotyping by RFLP-PCR performed techniques allow the detection of the four alleles: ARR, AHQ, ARQ and VRQ (Elsen *et al.*, 2002). 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.

The sampling of the animals was organized by breed. For milk breeds animals were the Artificial Insemination Centres rams of the dairy breeding schemes. For meat 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 representativeness, these 100 animals were chosen maximizing the number of sires, grandsires and flocks.

The genotype results (Table 3) show considerable variation in the distribution of the four alleles between the different breeds. For the 29 breeds reported, ARR allele frequency ranges from 15.1% in Caussenarde du Lot 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 to 25% in Bleu du Maine.

Table 3. Initial *PrP* allele frequencies in 29 French sheep breeds or lines

Breed	Purpose	†n	*PrP* alleles			
			ARR	AHQ	ARQ	VRQ
Basco-Béarnaise	Milk	149	0.399	–	0.601	–
Berrichon du Cher	Meat, terminal	95	0.805	0.063	0.105	0.026
Bizet	Meat, hardy	53	0.632	0.019	0.349	–
Blanc du Massif Central	Meat, hardy	120	0.250	0.050	0.621	0.079
Bleu du Maine	Meat, prolific	100	0.700	–	0.050	0.250
Caussenarde du Lot	Meat, hardy	106	0.151	0.174	0.604	0.071
Charmoise	Meat, terminal	99	0.313	0.030	0.495	0.162
Corse	Milk	152	0.470	0.039	0.487	0.003
Grivette	Meat, prolific	68	0.441	–	0.544	0.015
Hampshire	Meat, terminal	103	0.602	0.005	0.379	0.015
Ile de France	Meat, terminal	99	0.687	–	0.146	0.167
Inra401	Meat, prolific	310	0.360	0.071	0.458	0.111
Lacaune, milk line	Milk	561	0.545	0.016	0.427	0.012
Lacaune, Gebro line	Meat, terminal	99	0.566	0.010	0.278	0.146
Lacaune, Ovi-Test line	Meat, prolific	100	0.400	0.030	0.505	0.065
Limousine	Meat, hardy	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 (FWI†† black belly)	Meat, tropical, hairy	50	0.180	0.100	0.680	0.040
Mérinos d'Arles	Meat, wool	99	0.359	0.025	0.591	0.025
Mérinos de l'Est	Meat, wool	91	0.159	0.066	0.774	–
Noire du Velay	Meat, hardy	55	0.227	–	0.736	0.036
Préalpes du Sud	Meat, hardy	101	0.441	–	0.559	–
Rava	Meat, hardy	71	0.430	0.007	0.528	0.035
Rouge de l'Ouest	Meat, terminal	96	0.667	–	0.250	0.083
Suffolk	Meat, terminal	98	0.704	–	0.281	0.015
Tarasconnais	Meat, hardy	97	0.325	0.010	0.660	0.005
Texel	Meat, terminal	100	0.270	0.050	0.590	0.090
Vendéen	Meat, terminal	101	0.163	–	0.822	0.015

†n = number of sampled animals.
††FWI = French West Indies.

ARR allele is frequent in most of terminal sires breeds: higher than or close to 60% for six of them (Ile de France, Berrichon, Rouge de l'Ouest, Bleu du Maine, Suffolk, Hampshire), around 30% in Texel. O'Doherty *et al.* (2000, 2001) reported Irish data with convergent figures about Suffolk, Texel, Rouge de l'Ouest, Bleu du Maine breeds.

Hardy breeds show a high frequency of the ARQ allele, arising around 60% with Blanc du Massif Central, Préalpes, Caussenarde, as well as the Merino breeds Mérinos d'Arles and Mérinos de l'Est. 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 four limits pointed by Elsen *et al.* (1997):

(i) Universal resistance against any scrapie strain.

(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.

Concerning (ii), Andréoletti *et al.* (2000) found that 9-months old ARR/ARR lambs are not PrP^{sc} affected while VRQ/VRQ are since they are 4 months old. It was still the case when they were 24 months old. This observation enforces the hypothesis that resistant animals are not healthy carriers.

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 line (1995) and then in the Lacaune meat lines 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.

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:

(i) Eradication of the VRQ allele in the breeding schemes flocks.

(ii) Production of ARR/ARR rams to be used for replacement in affected flocks.

(iii) Selection in favour of the ARR allele as an additional selection goal for all the breeding schemes.

(iv) Generalization of the use of ARR/ARR terminal rams in the commercial flocks.

All these plans intend to limit the risk of outbreak of a crisis which may occur if ovine BSE is found in a believed natural scrapie case. To date it is not the case, the biochemical comparison performed by Baron *et al.* (2000) showed that 21 French natural scrapie isolates differ clearly from that of a BSE experimentally infected sheep. Protection brought by homozygous ARR appear as consistent against both scrapie and artificially induced ovine BSE (Jeffrey *et al.*, 2001).

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 into 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, breeding value estimation (parental evaluation, individual test, progeny test), artificial insemination, purebred, maternal crossing, terminal crossing.

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