A SCRAPIE EPIDEMIC IN A CLOSED FLOCK OF ROMANOV

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SUMMARY

Information from a scrapie epidemic in a closed INRA Romanov flock is presented. Performances, pedigree, histopathological diagnoses and *Prn-P* genotypes were recorded since the outbreak of the epidemic (in 1993). Major influence of the polymophisms at codons 154 and 171, as well as a side effect of polymorphism at codon 136 are shown. A possible relationship between gastrointestinal parasitism and scrapie is discussed.
Keywords: Sheep, scrapie, epidemic, *Prn-P*

INTRODUCTION

The Cheviot flock of the Neuropathogenesis Unit selected into two lines differing for susceptibility to artificial scrapie since 1962 (Dickinson et al, 1968) made a considerable contribution to the understanding of genetic variability of this trait : the susceptibility is controlled by a major gene which is very closed to, if not confounded with, the *Prn-P* locus (Hunter et al, 1992). *Prn-P* polymorphism has been related to natural scrapie in different breeds (Hunter et al, 1993; Laplanche et al, 1993; Westaway et al, 1994; Belt et al, 1995; Clouscard et al, 1995; Hunter et al, 1996).

A new source of information is available with a recent scrapie epidemic which killed more than 300 animals in 3 years in an INRA Romanov flock, considered free from this disease before the outbreak of the epidemic in 1993. Genealogy and production traits of all animals from this flock have been recorded for a long time. All suspected scrapie individuals (with the few exception of the first animals showing illness) have been analysed for histopathological signs of encephalopathy. DNA of all the animals present after 1993 has been collected. First analyses showing relation between *Prn-P* genotypes and scrapie susceptibility are presented.

MATERIAL AND METHODS

Animals. The flock was founded in 1971 by INRA at Langlade near Toulouse. A total of 20 founder Romanov ewes were imported simultaneously from another INRA Romanov flock. From 1977 to 1993, about 600 ewes formed this closed flock devoted to genetic analyses on reproduction and meat traits.

First signs of scrapie appeared in April 1993, in a subflock (P) devoted to an experiment about host resistance to gastro-intestinal parasitism : these animals, born in 1991, were orally challenged in 1992 with the nematode *Teladorsagia circumcincta*. In the main flock (NP), the first cases of scrapie were observed in August 1993. Then, it was decided to send all animals with clinical signs to the Toulouse Vet school for histopathological diagnosis and to collect DNA from all living animals for genetical studies.

Analyses. First diagnoses from clinical symptoms (mainly pruritus and incoordination of gaits) were made by the persons in charge of animals husbandry. Final diagnoses were obtained after histopathology of the brain. Vacuolation of specific neuro-anatomical regions were observed. Individuals were classified as "scrapie" if the histopathological diagnosis was positive, "unaffected" in the other cases.

Prn-P genotypes were described for the three codons the polymorphisms of which were known or suspected to be linked to scrapie sensitivity : 136, 154 and 171. Analyses were performed using RFLP-PCR techniques and aimed at detecting the following variations : Alanine/Valine (A/V) (codon 136), Arginine/Histidine (R/H) (154), Arginine/Glutamine (R/Q) (171).

RESULTS AND DISCUSSION

Observation of scrapie. A total of 307 animals died from confirmed scrapie between April 1993 and the 1st of May 1997 in the flock, the youngest scrapie individual dying at 352 days of age. Table 1 describes the incidence of scrapie in the successive cohorts.

Table 1. Number of animals exposed (during at least 1 year) to scrapie and number of affected animals according to year of birth since the appearance of the disease in 1993

Number	1986	1987	1988	1989	1990	1991	P	1992	1993	1994	1995	Total
Exposed	25	23	59	66	78	106	75	122	120	141	56	871
Scrapie	3	6	10	28	20	17	35	57	57	63	11	307

In the second part of 1993, the epidemic killed 57 (12 pcent) animals, then 112 (28 pcent) in 1994 and 93 (27 pcent) in 1995, mostly less than 3 years old. The pressure of scrapie was maximum in the P subflock, with an average mortality of 63 percent in 1994. The epidemic pressure decreased in 1996, with 33 deaths, this lower figure being largely explained by the decrease of flock size and proportion of susceptible genotypes, due to natural selection.

***Prn-P* genotype frequencies** (table 2). The genotype considering altogether the 3 codons was known for 1323 individuals (944 exposed). Not considering the variant histidine at 171, only 4 haplotypes exist in the population, as previously found by Laplanche et al (1993) in Romanov : ARQ, ARR, AHQ, VRQ (here, an allele will be noted without the codon number, *e.g.* ARQ for $A_{136}R_{154}Q_{171}$). Note that the last 3 haplotypes are derived from the first changing only 1 codon, suggesting that the first one could be ancestral.

Table 2. Genotype frequencies observed each 1st of January since 1993

Genotype	1993	1994	1995	1996
ARR/ARR	3	4	6	7
ARR/AHQ	6	8	10	15
ARR/ARQ	48	51	49	55
ARR/VRQ	38	39	38	44
AHQ/AHQ	2	3	8	6
AHQ/ARQ	44	49	68	64
AHQ/VRQ	25	30	39	37
ARQ/ARQ	121	136	117	74
ARQ/VRQ	174	154	108	41
VRQ/VRQ	48	42	13	4

At the outbreak of the epidemic, allele frequencies did not differ between the main flock (NP) and the subflock (P) ($X^2 = 13.8$).

Effect of *Prn-P* genotype on incidence of scrapie. The effect of the *Prn-P* genotype is shown in table 3 where only the animals exposed at least 1 year to the disease were considered.

These observations confirm on a large number of animals the influence of the *Prn-P* genotype on natural scrapie susceptibility as already shown in other breeds and other locations (see references quoted in the introduction). But, unlike Hunter et al (1996), we observed a major influence of the genotype at codons 154 and 171, and a side effect of the genotype at the codon 136. The H_{154} and the R_{171} alleles give a reduced susceptibility to scrapie, mostly observed here at the heterozygous state due to the allele frequencies. In animals without these "protective" alleles, A_{136} gives a lower susceptibility than V_{136}.

Table 3. Incidence of scrapie according to the *Prn-P* genotypes

	NP flock			P subflock		
Prn-P genotype	Exposed	Scrapie (%)		Exposed	Scrapie (%)	
ARR/ARR	47	0	(0)	0	0	(0)
ARR/AHQ	37	0	(0)	0	0	(0)
AHQ/AHQ	11	1	(9)	0	0	(0)
ARR/ARQ	93	1	(1)	3	0	(0)
AHQ/ARQ	89	2	(2)	2	0	(0)
ARR/VRQ	76	0	(0)	2	0	(0)
AHQ/VRQ	49	0	(0)	5	1	(20)
ARQ/ARQ	171	67	(39)	13	10	(77)
ARQ/VRQ	252	128	(51)	13	12	(80)
VRQ/VRQ	66	48	(73)	8	8	(100)

Consistently, the least squares means for the *Prn-P* genotype in a linear analysis of variance model (n=248 observations, r^2=0.93) considering also the year of birth and sex of the scrapie animals showed that most susceptible genotypes (in the sense of table 3) were also the genotypes with the shortest incubation period (table 4).

Table 4. Age at death of scrapie animals, compared to ARQ/ARQ animals

Prn-P genotype compared to ARQ/ARQ	ARR/ARQ	AHQ/ARQ	ARQ/VRQ	VRQ/VRQ
Mean age at death (days)	646	40	-63	-173

A second original observation is the apparent effect of parasite infection on the susceptibility to scrapie. The epidemic started in the P subflock where the age at death was lower as compared to the main flock (lsmeans : -455 days) and, most important, the effect of the *Prn-P* 136 genotype was largely erased, all the genotypes with $R_{154}Q_{171}/R_{154}Q_{171}$ showing high incidence of scrapie. Experiments have been designed to test both hypotheses that parasites may be cofactor increasing scrapie sensitivity or vector of the transmissible agent itself.
Even if these results, as others, are very encouraging, using polymorphisms at *Prn-P* to increase resistance to scrapie cannot be proposed on a large scale before getting more insurance about the universality of the genetic resistance, and about the possibility that resistant animals are not disseminating the infection. Observations have to be performed on multiple sites and experiments to be organized for answering these questions.

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