1
2

Annex

Request for an opinion on the possible changes to the control measures for sheep and goat herds in which a case of atypical scrapie has been detected

Epidemiology of animal TSEs working group

The question posed by mandate 2006-SA-0195 was examined by the working group on the epidemiology of animal TSEs at its meetings on 26 September, 25 October and 29 November 2006.

1. Context of the request for an opinion and definition of the scope of the expert evaluation

The review of the control measures for small ruminants was undertaken as a result of the TSE Roadmap. It originated from the European Commission's proposal to cease applying control measures to infected herds in which the index case of scrapie is characterised as atypical. These herds would be subject, for a period yet to be defined, solely to systematic screening of all or some of the animals aged over 18 months slaughtered or culled.
The Agence française de sécurité sanitaire (Afssa) [French Food Safety Agency] received a request from the Direction générale de l'alimentation (DGAl) [Directorate General for Food] for an opinion on the option put forward by the Commission and for a definition of the additional measures required to protect public and animal health and to improve the available knowledge of atypical scrapie.

2. Method

The expert evaluation consisted of an analysis of the data from the active surveillance programmes for small ruminant TSEs conducted in France since 2002, of data from the same programmes at European level and of the available scientific literature.

3. Analysis of the subject to be evaluated based on the method defined

3.1. Definition of atypical scrapie

Atypical scrapie is an experimentally transmissible spongiform encephalopathy, found in sheep and goats. It has been defined by EFSA based on phenotypical criteria (EFSA 2005). The proposed definition was based in particular on the results of various diagnostic tests:

- positive rapid tests: used in France, these are the Bio-Rad and Idexx tests (Annex 1: *« Aspects relatifs aux tests rapides de dépistage des ESST chez les petits ruminants »* (Annex 2 of the opinion of 20/07/06 on mandate 2006-SA-0099))
- Western Blot with strong Proteinase K treatment negative,
- Western Blot with moderate Proteinase K treatment positive and showing a lower band below 15 kD,
- variable immuno-histochemistry and histopathology with vacuolisation lesions and more regular immuno-marking in the cerebellum than the brain stem.

The question of whether all cases of atypical scrapie correspond to the Nor98 strain or to several strains has not been completely clarified. However, the most recent results do not enable a distinction to be made between cases of atypical scrapie and Nor98 cases (e.g. in France, Arsac et al., 2006, EID, `http://www.cdc.gov/ncidod/EID/13/1/06-0393.htm`).

3.2. Review of current knowledge of the physiopathology of atypical scrapie

In animals infected with atypical scrapie, the PrP^{Sc} is principally detected in the brain and the cerebellum; it is not always detected in the obex.
To date, it has never been detected outside the central nervous system, neither in sheep (Benestad, Sarradin et al. 2003), nor in murine models (Le Dur, Beringue et al. 2005; 2006); in particular, it has never been detected in the lymphoid system.
This does not exclude the possibility of the presence of PrP^{Sc} at non-detectable levels or of the presence of a form of intermediate PrP (with low resistance and insoluble). A study of the infectivity of the lymphoid formations would be particularly useful.

3.3. Transmissibility of atypical scrapie

The transmissibility of atypical scrapie has been demonstrated in experimental conditions in transgenic VRQ/VRQ mice (Le Dur, Beringue et al., 2005; J.-N. Arsac et al., 2006), and among the experiments currently underway using intracerebral inoculation in sheep, transmissibility following IC inoculation has been reported in a sheep of genotype AHQ/AHQ with an incubation period of approximately 300 days (Marion Simmons, VLA, oral communication, Neuroprion meeting, Porto Carras – Greece, May 2006). Oral transmissibility to sheep has not yet been demonstrated experimentally.
Contagiosity has not been demonstrated in natural conditions. An initial case-control study conducted in Norway (Hopp et al., 2006) did not show any risk factors for the occurrence of atypical scrapie from the introduction of animals or from contacts, in contrast with a similar study conducted in Norway also on cases of classical scrapie (Hopp et al., 2001); it must be noted, however, that the study on atypical cases has limited power due to the number of cases included (n = 28).

3.4. Clinical and age aspects of cases

Clinical cases of atypical scrapie, whose existence has been a matter of debate until recently, has been reported in several European countries: France, Ireland, Italy, Norway, United

Kingdom (current European study). It should be noted that in Norway, clinical cases represent about half of the cases of atypical scrapie (S. Benestad, personal communication).

The clinical signs of atypical scrapie appear to differ from those of classical scrapie (no pruritis, marked ataxia), but remain highly evocative of scrapie (Onnasch, Gunn et al., 2004; Konold, Davis et al., 2006).

Cases of atypical scrapie are, it would seem, on average older than cases of classical scrapie (current European study, (Gavier-Widen, Noremark et al., 2004)). This age difference implies that atypical scrapie could have a longer incubation period. Some of the animals infected with atypical scrapie may therefore be eliminated before the appearance of clinical signs. This partial knowledge requires analysis based on the age of the animals tested and their different genetic susceptibilities.

3.5. Genetic susceptibility

In sheep, the genetic determinism of atypical scrapie differs from that of classical scrapie. As well as the effect, as in classical scrapie, of the mutations described on codons 136, 154 and 171 of the PrP gene, there is a mutation on codon 141 (L: Leucine → F: Phenylalanine), a mutation only observed to date with the ARQ allele. The AHQ and AFRQ alleles are associated with a greater susceptibility to atypical scrapie.

A recently published study (Moreno, Laurent et al., 2006) showed that in France, the odds ratio associated with the carriage of AFRQ and AHQ alleles was respectively 14.1 $[8.9\text{-}22.4]_{5\%}$ and 8.0 $[4.4\text{-}14.8]_{5\%}$, with reference to the ALRQ allele. In this study, the allele frequencies in the sheep population (denominator) were estimated from a limited sample of animals: the frequency of codon F_{141} in the population was extrapolated from a limited sample of animals in which the 4 codons had been genotyped; the frequency of the different alleles was extrapolated from two larger studies of the genotype of the three codons. These data were also exploited to describe the situation in France in the EFSA opinion on the genetic resistance programme (EFSA, 2006). In this study, the odds ratio of the other alleles was not significantly different from the reference allele (ALRQ). In other European countries, estimation of the risks associated with the alleles is different; however, the increased susceptibility conferred by the AFRQ and AHQ alleles is seen (EFSA 2006). In the various studies available, the ARR allele seems to confer some susceptibility and the VRQ allele seems to be protective, but none of these effects is statistically significant (Moreno, Laurent et al. 2006).

3.6. Organisation of the control of small ruminant TSEs

The control measures for small ruminant TSEs were put in place in response to the risks from classical scrapie and bovine spongiform encephalopathy, before the phenomenon of atypical scrapie had been discovered. The control measures for small ruminant TSEs include surveillance systems, the management of outbreaks and selection for genetic resistance.

TSE surveillance comprises an element of passive surveillance, provided by the clinical surveillance network. In France, the intensity of clinical surveillance of scrapie has reduced sharply since active surveillance has been put in place (Table 1).

Table 1: Number of suspicions and cases of scrapie detected by clinical surveillance (classical and atypical combined)

	Sheep		Goats	
Year	Suspicions	Cases	Suspicions	Cases
2002	91	91	1	1
2003	56	41	5	3
2004	32	12	0	0
2005	22	14	0	0
Total	**201**	**158**	**6**	**4**

Active surveillance has been in place since 2002 at abattoirs and rendering plants, in accordance with a Community requirement (Regulation 999/2001). This programme is designed to test every year a minimum number of animals aged over 18 months selected at random firstly from the population of animals slaughtered for human consumption (surveillance at abattoirs) and secondly from the population of animals found dead or euthanased on the farm and collected for rendering (surveillance at rendering plants). The change in the risk perception has led to a progressive increase in the scope of the surveillance programmes (Table 2).

Table 2: Sampling objectives for sheep and goats for the active surveillance of scrapie at abattoirs and rendering plants

	Sheep		Goats	
Year	Abattoir	Rendering plant	Abattoir	Rendering plant
2002	10%	10%	25%	25%
2003	10%	10%	25%	25%
2004	2%	4%	0%	10%
2005	2%	4%	50%	100%
2006	2% then 100%	100%	100%	100%

As part of the active and passive surveillance, obex samples are collected and then sent for analysis using a rapid test in the approved Department laboratories. Non-negative samples are sent to the National Reference Laboratory (AFSSA Lyon) for confirmation and typing (index cases only). The typing of positive samples is the only way of characterising atypical cases.

The broad outlines of the control measures are as follows (cf. Annex 2):

- in sheep flocks, the selective destruction of animals described as genetically susceptible and highly susceptible, and restocking with resistant animals;
- in goat herds, destruction of all animals, with the option of joining an experimental protocol (with sampling of tonsils in particular) providing exemption from complete slaughter.

3.7. Summary of current epidemiological information on atypical scrapie in France

1. Number of cases and routes of detection

The number of cases of atypical scrapie detected between 1 January 2002 and 30 September 2006, all species and all surveillance programmes combined, is 195 (Tables 3 and 4).

Table 3: Number of cases of atypical scrapie in sheep (1 January 2002 to 30 September 2006)

Programme	2002	2003	2004	2005	2006	**Total**
Rendering plant	4	7	2	5	57	**75**
Abattoir	11	21	7	8	50	**97**
Clinical surveillance					2	**2**
Official control prog.		1	2			**3**
Control measure			1	2	5	**8**
Total	**15**	**29**	**12**	**15**	**114**	**185**

Table 4: Number of cases of atypical scrapie in goats (1 January 2002 to 30 September 2006)

Programme	2002	2003	2004	2005	2006	**Total**
Rendering plant	1	2		3	1	**7**
Abattoir				2		**2**
Official control prog.			1			**1**
Total	**1**	**2**	**1**	**5**	**1**	**10**

Active surveillance

Active surveillance is the principal means of detecting cases of atypical scrapie: 52% of cases are detected at abattoirs and 41% at rendering plants. The prevalence of atypical scrapie found by the active surveillance programme at abattoirs and rendering plants is given later in the document.

Clinical cases

In 2006, two cases were diagnosed in the context of clinical surveillance.

Official control programme

The official control programme (CSO) has resulted in the detection of four cases of atypical scrapie on four farms (Table 5); these are the only cases in which CSO surveillance has led to the detection of a TSE. The atypical cases on these four farms were detected following the performance of a relatively low number of tests capable of detecting this form of TSE (between 7 and 24 depending on the farm). However this observation must be placed in context, since the animals included in the CSO (up to 2005) were cull animals, therefore generally older. This data should also be compared with the total number of animals tested since 2002 as part of the CSO.

On these farms, a precise analysis is required of the parameters for implementing the CSO: implementation sequence, case genotyping, etc., which requires additional data to be available.

Finally, farms included in the CSO are listed at *département* level by the veterinary services. The lack of any compilation of *département* data at national level means that the total number

of farms is unknown. Nor do we know to what extent the tests carried out as part of the CSO are systematically recorded.

Table 5: Farms included in the CSO on which a case of atypical scrapie has been detected

			Surveillance prior to the discovery of the atypical case		Tests following the discovery of the first atypical case (control measure)
Outbreak	Species	Number	Duration (in months)	Number of tests (n; % Biorad + Idexx)	Number of tests (% Biorad or Idexx)
1	sheep	ND	13	17 (6 = 35%)	133 (20%)
2	sheep	ND	21	24 (24 = 100%)	165 (60%)
3	sheep	820	19	7 (6 = 85%)	139(85%)
4	goats	528	20	18 (3 = 16%)	97 (50%)

Control measures

The control measures do not require the typing of positive cases detected in outbreaks after the index case. However, of approximately 2,000 positive isolates from the control measures, some were typed, either for research purposes (approximately 80 isolates) or due to a classification error in the national database (19 isolates). The presence of secondary cases of atypical scrapie and the possible co-existence of atypical and classical scrapie have therefore very rarely been documented and are the subject of a discussion at the end of this document.

2. Detection capacity

Tests used

Of the 195 cases of atypical scrapie, 14 were initially detected by the Idexx Herdcheck test (out of approximately 17,500 tests) and 181 by one of the Bio-Rad tests (out of approximately 600,000 tests) (Platelia/TeSeE or TeSeE Sheep/goat).

In sheep, the tests capable of detecting atypical scrapie (Idexx and Bio-Rad) represent a total of 41% of the tests carried out at rendering plants and 61% of the tests carried out at abattoirs (Table 6). In goats, the use of tests capable of detecting atypical scrapie is lower, with respectively 49% and 15% of tests.

Table 6: Number and type of tests per surveillance programme (1 January 2002 to 30 September 2006)

	SHEEP			GOATS		
	Bio-Rad + Idexx	Total number of tests	Bio-Rad+ Idexx / Total number of tests (%)	Bio-Rad + Idexx	Total number of tests	Bio-Rad+ Idexx / Total number of tests (%)
Rendering plant	96,414	234,033	41	56,182	113,744	49
Abattoir	91,287	149,567	61	30,700	192,277	15

Almost all (633/652) of the scrapie cases detected by active surveillance were typed, either at the time of detection (incident cases) or retrospectively (historic cases).

Number of tests

Overall, during the surveillance period, the proportion of tests capable of detecting atypical scrapie has increased (Figures 1 and 2).

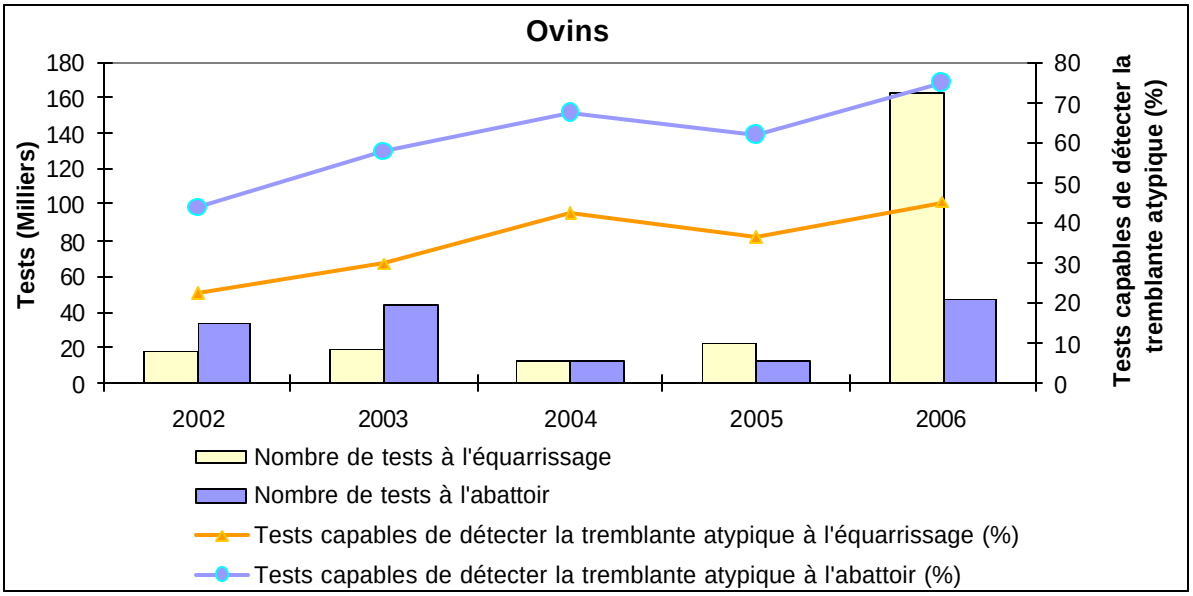

Figure 1: Number of tests and proportion of tests capable of detecting atypical scrapie in sheep from 1 January 2002 to 31 July 2006

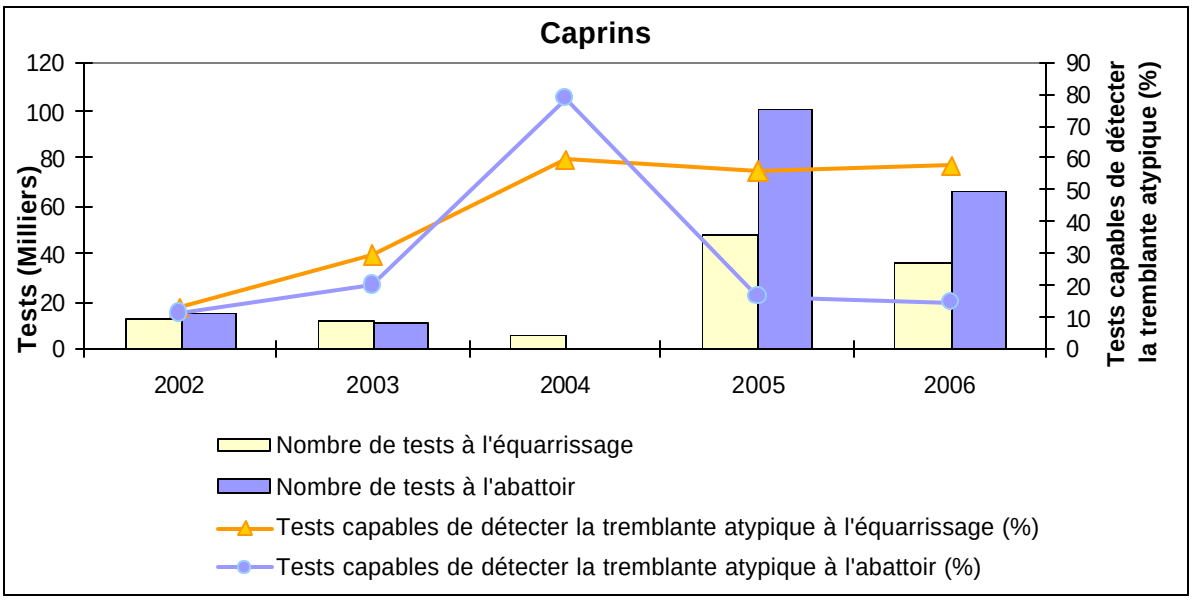

Figure 2: Number of tests and proportion of tests capable of detecting atypical scrapie in goats from 1 January 2002 to 31 July 2006

3. *Estimate of prevalence in France*

The calculation of annual prevalence relates to the surveillance period from 01/01/2002 to 31/07/2006 for which there are data available from DGAl on surveillance and data on the typing of isolates. The total number of cases for this period is 140. It must be noted that the increase in the number of tests in 2006 did not result in any change in the level of raw prevalence detected but did enable greater accuracy in the estimation of this prevalence (reduction in the width of confidence intervals) for atypical and classical scrapie.

Annual raw prevalence of atypical scrapie is calculated as the ratio of the number of cases of atypical scrapie to the number of tests capable of detecting atypical scrapie. The exact confidence intervals are calculated (binomial rule) for a type I risk of 5%.

The prevalence of classical scrapie is given for comparison. This is calculated as the ratio of the number of cases of classical scrapie to the total number of tests performed. The exact confidence intervals are calculated (binomial rule) for a type I risk of 5%.

In all cases, the assumption is made that the different tests have identical sensitivity: on the one hand, Biorad and Idexx for atypical scrapie, and on the other hand, all tests for classical scrapie.

Since the sampling sites (rendering plants and abattoirs) use *département* laboratories which do not use the same tests, the populations used to calculate the prevalence of atypical and classical scrapie can differ; some caution must therefore be exercised when interpreting the differences.

By the same token, a comparison of prevalence over time should take account of changes in the surveillance programmes: bias in the selection of animals, quality of sample storage, geographical variations, a change to exhaustive testing.

Prevalence at rendering plants

In sheep, raw prevalence of atypical scrapie at rendering plants, estimated at 0.069 %, is lower overall than classical scrapie, estimated at 0.132 % (Table 7). It appears relatively stable over time, in contrast to classical scrapie (Figure 3). The higher prevalence of classical scrapie at rendering plants in 2002 has been partially attributed to the inclusion in the surveillance programme of cases culled as part of control measures (Morignat, Cazeau et al. 2003).

Table 7: Raw prevalence of classical and atypical scrapie in sheep at rendering plants

Year	Atypical prevalence (%)	Atypical CI 95%	Classical prevalence (%)	Classical CI 95%
2002	0.103	[0.028; 0.264]	0.595	[0.486; 0.72]
2003	0.124	[0.05; 0.256]	0.217	[0.156; 0.294]
2004	0.038	[0.005; 0.139]	0.171	[0.106; 0.262]
2005	0.062	[0.02; 0.145]	0.086	[0.052; 0.134]
2006	0.067	[0.049; 0.088]	0.076	[0.063; 0.09]
Total	**0.069**	**[0.054; 0.088]**	**0.132**	**[0.117; 0.147]**

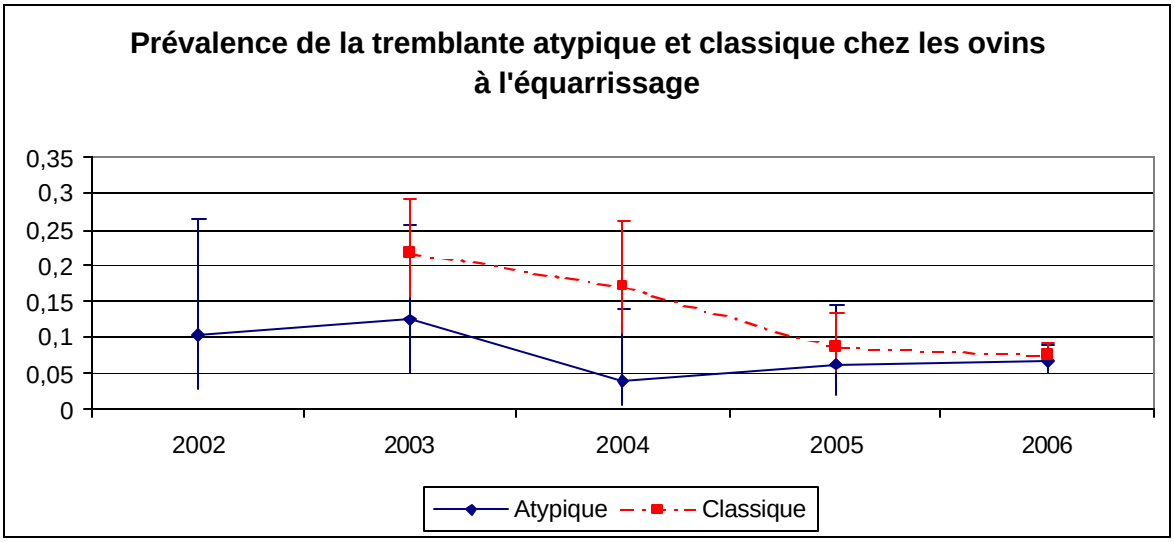

Figure 3: Raw prevalence (as a %) of classical and atypical scrapie in sheep at rendering plants (the prevalence of classical scrapie is not shown for 2002 due to classification errors in animals subject to control measures)

In goats, the raw prevalence of atypical scrapie at rendering plants is estimated at 0.012% and is not significantly different from the prevalence of classical scrapie, estimated as 0.019% (Table 8 and Figure 4). For both forms, prevalence is lower than that observed in sheep.

Tableau 8: Raw prevalence of classical and atypical scrapie in goats at rendering plants

Year	Atypical prevalence (%)	Atypical CI 95%	Classical prevalence (%)	Classical CI 95%
2002	0.064	[0.002; 0.355]	0.065	[0.028; 0.129]
2003	0.057	[0.007; 0.206]	0.033	[0.009; 0.086]
2004	0	[0; 0.109]	0.018	[0; 0.098]
2005	0.011	[0.002; 0.033]	0.008	[0.002; 0.021]
2006	0.005	[0; 0.027]	0.014	[0.005; 0.032]
Total	**0.012**	**[0.005; 0.026]**	**0.019**	**[0.012; 0.029]**

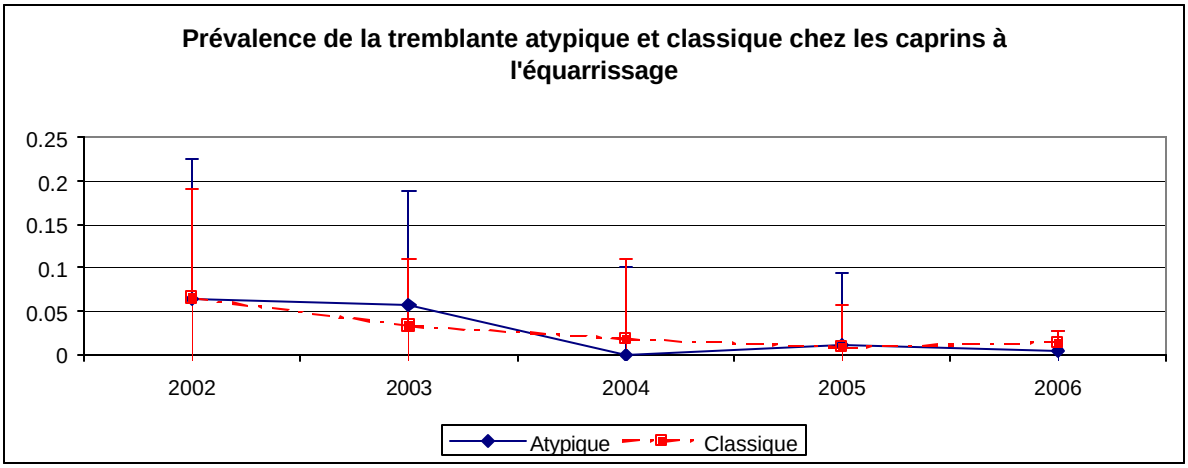

Figure 4: Raw prevalence (as a %) of classical and atypical scrapie in goats at rendering plants

Prevalence at abattoirs

In sheep, the raw prevalence of atypical scrapie at abattoirs, estimated at 0.073%, is slightly higher than classical scrapie, estimated at 0.033% (Table 9). It appears relatively stable over time, as does classical scrapie (Figure 5).

The prevalence of atypical scrapie is very similar in rendering plants and abattoirs, although in abattoirs the tests for detecting atypical scrapie are used on average 1.5 times more often than in rendering plants.

Tableau 9: Raw prevalence of classical and atypical scrapie in abattoirs

Year	Atypical prevalence (%)	Atypical CI 95%	Classical prevalence (%)	Classical CI 95%
2002	0.075	[0.037; 0.134]	0.047	[0.027; 0.077]
2003	0.082	[0.051; 0.125]	0.043	[0.026; 0.067]
2004	0.083	[0.034; 0.172]	0.056	[0.023; 0.116]
2005	0.106	[0.046; 0.208]	0.025	[0.005; 0.072]
2006	0.057	[0.035; 0.088]	0.009	[0.002; 0.022]
Total	**0.073**	**[0.057; 0.093]**	**0.033**	[0.024; 0.043]

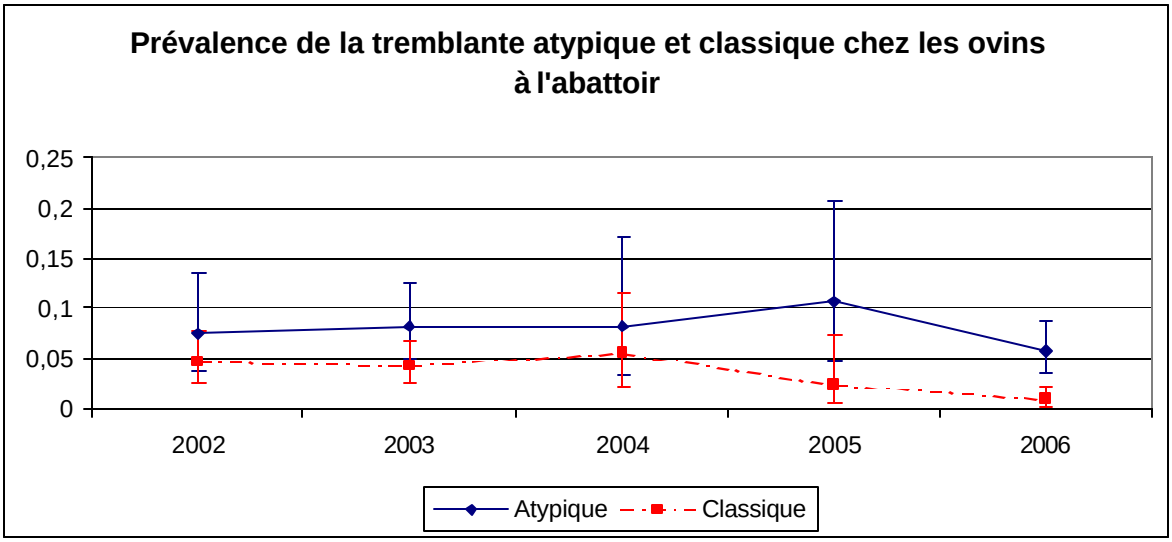

Figure 5: Raw prevalence (as a %) of classical and atypical scrapie in sheep at abattoirs

In goats (Table 10 and Figure 6), the raw prevalence of atypical scrapie in abattoirs is estimated at 0.007%, which is comparable to the prevalence of classical scrapie, estimated at 0.002%, and much lower than that observed in sheep.

Tableau 10: Raw prevalence of classical and atypical scrapie in goats at abattoirs

Year	Atypical prevalence (%)	Atypical CI 95%	Classical prevalence (%)	Classical CI 95%
2002	0	[0; 0.223]	0.007	[0; 0.037]
2003	0	[0; 0.167]	0.018	[0.002; 0.064]
2004		no tests		
2005	0.012	[0.001; 0.044]	0	[0; 0.004]
2006	0	[0; 0.038]	0	[0; 0.006]
Total	**0.007**	**[0.001; 0.024]**	**0.002**	**[0; 0.005]**

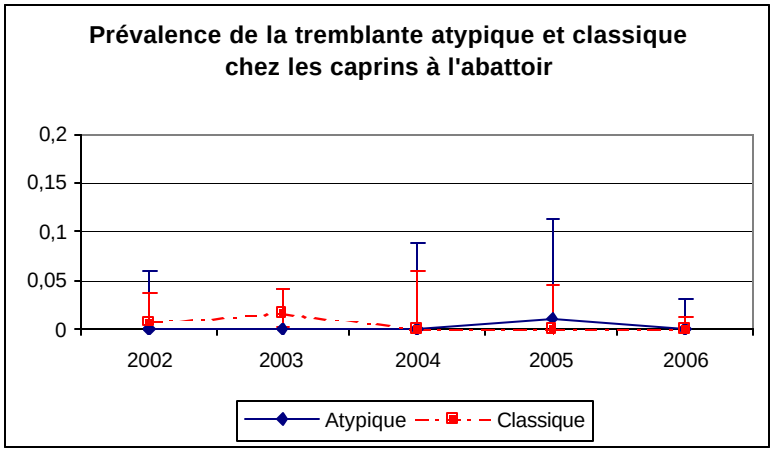

Figure 6: Raw prevalence (as a %) of classical and atypical scrapie in goats at abattoirs

Overall, the prevalence of atypical scrapie is low but comparable to the prevalence of classical scrapie. Furthermore, the annual incidence of the disease does not seem to be changing significantly.

4. Existence of secondary cases and intra-herd prevalence

The existence of several cases of atypical scrapie in a single herd would argue in favour of an acquired disease (exposure to a common factor and/or contagiosity), the probability that several independent cases could co-exist on the same farm being in theory very low [1].

Similarly, the co-existence of cases of atypical and classical scrapie in the same herd raises the issue of the independence of these two diseases from each other[2].

If this co-existence were not random, it could be interpreted as the exposure to common risk factors, if any exist and/or as two phenotypical manifestations of exposure to the same agent strain.

Table 11 shows the outbreaks in which several cases of scrapie have been diagnosed, including at least one case of atypical scrapie. Several farms may be involved in a single outbreak. The data are sometimes incomplete and research is underway to describe these outbreaks in more detail; finally, these data require individual verification with the veterinary services concerned and a statistical interpretation at this stage would be premature.

[1] If these cases are independent, the probability of the co-existence of cases = atypical prevalence² * number in the herd screened using a test capable of detecting atypical scrapie.

[2] If these diseases are independent, the probability of coexistence de cases = prevalence classical scrapie * atypical prevalence * number in the herd screened using a test capable of detecting atypical scrapie).

Table 11: Farms on which several cases of TSE have been diagnosed including one case of atypical scrapie (period 1 January 2002 to 31 September 2006)

No.	Department	Isolate of the index case	Number of positives	Number of atypical	Not typed (Biorad-Idexx)	Control measures Biorad + Idexx	Control measures all tests	Active surveillance and all tests	Active surveillance Biorad + Idexx tests
1	64	?	4	1	1	117	229	20	4
2	80	classical	4	1	1	116	124	105	21
3	64	classical	31	1	4	31	107	155	63
4	12	atypical	3	1	0	5	71	114	81
5	46	not atypical	18	1	0	311	1024	1451	743
6	46	atypical	2	2	0	15	21	12	6
7	48	atypical	5	1	0	20	238	55	25
8	64	classical	37	1	2	181	270	185	185
9	12	classical	3	1	0	21	69	264	215
10	9	atypical	2	1	0	359	372	90	46
11	64	atypical	2	2	0	28	28	48	30
12	87	classical	23	1	0	0	171	851	8
13	64	classical	8	2	0	87	112	16	57
14	46	classical	2	1	0	5	6	86	110
15	12	classical	2	1	0	16	16	166	61
16	64	atypical	3	2	0	107	107	30	13
17	12	atypical	6	1	0	30	30	95	55

In 4 outbreaks, two cases of atypical scrapie were diagnosed (including 3 index cases) and in 14 outbreaks there was co-existence of atypical and classical scrapie.

Detection of one case of atypical scrapie led to the demonstration of secondary cases of classical scrapie in 6 outbreaks.

If these diseases are independent of each other, the probability of finding cases of classical scrapie in outbreaks of atypical scrapie should be the same as the prevalence of classical scrapie on farms with equivalent risk factors (geography, farming system, etc.), which is not quantifiable at the present time. Similarly, if the diseases are independent, the rate of detection of atypical scrapie in animals in an outbreak of classical scrapie should be comparable to the prevalence of atypical scrapie for equivalent factors of genetic susceptibility (in the absence of other known risk factors).

The lack of typing of cases subject to control measures and the absence of an unequivocal link between the animals tested, the herds of origin and the outbreaks in which they are recorded as part of the control measures are an hindrance to this comparison.

It is possible that the number of outbreaks with several cases of atypical scrapie and the number of outbreaks in which the index case is classical and in which there are atypical cases have been under estimated. In fact:

- the isolates detected during control measures are not typed systematically;
- not all the animals from the same outbreak slaughtered under the control measures are necessarily tested with a test capable of detecting atypical scrapie;
- only some of the animals susceptible to atypical scrapie are tested pursuant to the Order of 27 January 2003 laying down the control measures for sheep scrapie, which requires the slaughter of animals susceptible to classical scrapie. The result is that females of the genotype ARR/ARR, ARR/ARQ and ARR/AHQ, and ARR/ARR males are kept. Their status in terms of atypical scrapie therefore remains undefined.

The intra-herd epidemiological situation can therefore be considered unknown.

5. Situation in Europe

Declaration of cases of atypical scrapie to the European Commission has only been compulsory since 2005. An initial descriptive survey at European level recorded numbers of cases in 21 countries (Noremark and Hopp 2006). Atypical scrapie has been reported in 15 European countries, with some of these countries detecting no cases or only very few cases of atypical scrapie despite active surveillance programmes (Denmark, Finland, Portugal, Switzerland). This survey is entering a second phase, currently underway and intended to describe and compare the prevalence of atypical and classical scrapie through active surveillance.

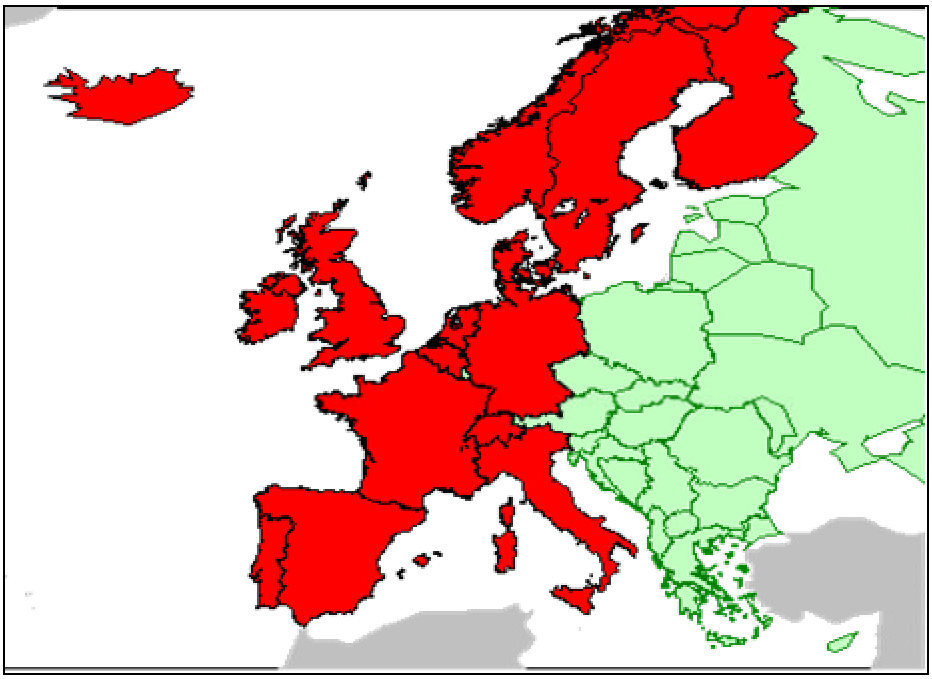

Figure 7: Map of countries in which at least one case of atypical scrapie has been diagnosed (in red) from (Noremark and Hopp 2006) (as at 30/10/06)

As in France, it seems that in the other European countries, observation of secondary cases of atypical scrapie is limited, since the systems in place to detect them differ quite widely from one country to another and vary from systematic surveillance for 5 years (Portugal) to the complete absence of measures (United Kingdom). In Germany, secondary cases of atypical scrapie have been detected a number of times (in 7 of 83 outbreaks) on large farms (over 500 animals) (A. Buschmann, personal communication).

It seems that atypical scrapie is a rare and cosmopolitan disease occurring with very low rates of attack in the outbreaks. These characteristics are not really indicative of an acquired disease, however, the data are too fragmented to be able to exclude the hypothesis of an acquired disease with low rates of attack.

It must also be noted that in certain countries, the surveillance programmes have found only atypical cases (in particular in Portugal); a comparative study at European level of the prevalence of the classical and atypical forms of scrapie would therefore be very useful.

Knowledge of the development of the disease over a longer period and a description of the epidemiological situation in herds would help to provide arguments in favour of one or other of these hypotheses.

3.8. Risks from atypical scrapie

The risks from atypical scrapie concern firstly public health and secondly, animal health.

Risks for public health

At the present time, the zoonotic risk from classical scrapie remains hypothetical. As atypical scrapie has a different pathology, the risk from it requires separate assessment, which is outside the remit of this mandate and would require more extensive knowledge, notably on the physiopathology of the disease.

In the current state of knowledge, the following elements must be taken into account:

- the zoonotic potential of atypical scrapie is unknown, but prudence requires the consideration that a risk might potentially exist;
- although the PrPres seems to be located in the central nervous system (CNS) in atypical scrapie, there are as yet very few physiopathology studies and in some experimental models, isolates showing low levels of PrP^{Sc} seem to have a high infectious titre;
- the capacity to detect infected animals is poor unless a suitable test and sampling method are used.

Risks for animal health

The information available implies that intra-herd prevalence of atypical scrapie is markedly lower than in classical scrapie. Moreover, since the average age of atypical cases is close to the standard culling age of the animals and clinical signs are rarely expressed, the economic

impact of the disease on farms can be assumed to be negligible, other than the impact of the control measures currently in place.

However, because the origin and the risk factors for the disease have not been clarified, it is possible that an epidemiological development in the disease (already underway or in the future) might lead to a reassessment of the risk.

3.9. The gaps in our knowledge of atypical scrapie epidemiology

Information on atypical scrapie is extremely fragmented and crucial questions remain to be answered, first and foremost on its transmissibility in natural conditions.

A number of elements are required over time to supplement the information currently available:

- the capacity to monitor prevalence in the population, either by implementing an exhaustive detection programme or by a sampling-based programme implemented meticulously, with tests capable of detecting atypical scrapie and the systematic use of typing on all positive samples;
- information on intra-herd prevalence and its development over time, which could provide information on the spontaneous nature of atypical scrapie. This would require the use of ad hoc tests, the systematic implementation of typing on all positive samples, accurate traceability of animals from infected farms, and the monitoring of infected herds with systematic testing of all animals leaving them (dead or sold);
- an evaluation of the independence of the occurrence of the classical and atypical forms (study of the infected farms, geographical study) which could provide information on its association or independence of occurrence as regards classical scrapie;
- research into risk factors for the presence of atypical scrapie on farms, which might also provide information on the transmissible or spontaneous nature of the disease;
- clarification of the relative susceptibility to infection of different genotypes of sheep. To achieve this, there is a need to increase the total number of animals tested, and also to implement typing at the 4 codons of interest, as a minimum in cases, outbreaks and in a representative sample of the sheep population;
- in non-epidemiological terms, research into the pathogenesis of the disease in small ruminants, and in particular into the distribution of infectivity.

3.10. Analysis of the current control measures

Impact of the slaughter measures

The current control measures require the slaughter of animals genetically susceptible to classical scrapie. As regards atypical scrapie, the justification for this type of slaughter measure as a means of protecting animal health is a matter of debate, since the risks for animal health seem limited. However, the slaughter measures can be conceived as a means of protecting public health. We note here that the control measures currently applied in outbreaks of atypical scrapie in sheep permit the sale of animals positive for this disease: animals resistant or semi-resistant to classical scrapie are not destroyed and they are not systematically tested prior to consumption.

Moreover, as regards sheep, current slaughter measures do not cover all susceptible animals and do not therefore constitute a method of eradicating the outbreak.

The following animals from an outbreak of atypical scrapie, slaughtered for human consumption, are not specifically tested for atypical scrapie: (i) sheep resistant to classical scrapie and (ii) goats from herds joining the exemption protocol. SRM removal is therefore the sole systematic protection against a zoonotic risk from atypical scrapie. The effectiveness of this measure can be defined as good if infectivity is restricted to the central nervous system, otherwise the measure's effectiveness becomes partial and unknown.

Consequences of genetic selection in sheep

The application of genetic selection as required by the regulations leads to the indirect elimination of the genotypes most susceptible to atypical scrapie.

By using the genotype data presented above, it is possible to calculate the ratio of the risk of atypical scrapie in a population from which animals susceptible to classical scrapie have been eliminated to the risk of atypical scrapie in the original population (Table 12); this odds ratio is (27 / 15 576) / (55 / 23 720) = 0.75 $[0.47\text{-}1.19]_{5\%}$. This OR is not significantly different from 1 but this may originate from a lack of power due to the low numbers involved in the calculation. Application of genetic counter-selection measures for animals susceptible to classical scrapie would result, based on this reasoning, in a risk reduction of 25%; this is a not insignificant result but would be a partial control measure. The effect of this measure would be strengthened in the long term by the selection of ARR/ARR animals.

Whatever the case, the full application of genetic counter-selection measures in an outbreak would result in the risk becoming asymptotically similar to the risk of atypical scrapie from the genotype ARR/ARR, for which there is currently no precise estimate (OR for the ARR haplotype = 1.3 $[0.8 - 2.2]_{5\%}$, prevalence for the ARR/ARR genotype ~ 0.1% (6/ 6040) (according to Moreno, Laurent et al. 2006)).

Table 12: Breakdown of the numbers of animals used to estimate genetic susceptibility per allele based on susceptibility as defined in the Order of 27 January 2002 (from EFSA, 2006)

	Atypical	Total
genotypes susceptible to classical scrapie	28	8,144
genotypes not susceptible to classical scrapie	27	15,576

The genetic selection measures applied in outbreaks of atypical scrapie do not therefore enable a strong reduction in risk in the short term, but create a trend towards a lower level of risk in the long term, to be defined.

Impacts of movement restrictions

The current health measures prohibit the movement of animals (sales and contacts) until the measures have been fully applied.

This ban is in reference to a risk of horizontal transmission of the disease which has not been proved in atypical scrapie.

However, this measure does guarantee the option of monitoring animals from the herd. Moreover, it limits the risk of environmental contamination with nervous system tissue from infected animals which have died on pasture and been left there (e.g. in transhumance). The existence of a risk from a contaminated environment, probable in classical scrapie, is not known for atypical scrapie.

Impacts of the measures suspending the CSO

Suspension of the Official Control Programme (CSO) for three years from farms infected with atypical scrapie constitutes a safeguard against the diffusion of the genetic susceptibility factor (PRNP gene or other unidentified effect) within the breed notably through ewes carrying AFRQ or AHQ alleles. There are no at-risk categories defined for goats.

In fact, although this is not a system objective, suspension of the CSO therefore helps control risk, a consistent but strict control for a disease whose risks are uncertain.

3.11. Evaluation of the European Commission proposal

The European Commission's proposal comprises two elements:

- ceasing to apply control measures to infected herds in which the index case of scrapie is classified as atypical;
- conducting systematic (or sample-based) testing for two (or three) years of culled or slaughtered animals aged over 18 months with a possible ban on sales outside the Member State in which the outbreak was detected.

This proposal:

- imposes no restriction on the trade in animals from outbreaks of atypical scrapie or on contacts between these herds and other herds. Taken to its extreme, the sale or transfer of the whole infected herd could result in all the animals avoiding surveillance;
- does not explicitly recommend the use of a test enabling the detection of atypical scrapie for monitoring herds;
- does not lay down any genetic selection measures (destruction of animals carrying susceptibility alleles, ban on the introduction of animals carrying susceptibility alleles).

Consequently, on the assumption that the disease is transmissible, it would not enable control of the disease within the outbreak, or of its dissemination; it would therefore not prevent a possible spread of the disease but would permit an estimation of the risk that such a spread

could occur. The means proposed for this risk estimate are limited, however (inappropriate test conditions, censored observations).

In terms of public health, it introduces an additional element of protection to SRM removal with the removal from consumption of animals tested positive (for two (or three) years after the index case).The effect of this measure would be limited solely to outbreaks where atypical scrapie has been detected, i.e. those where the animals are tested with an ad hoc test.

4. Conclusion and proposed opinion

- Ensuring protection of public health

The principal measure of consumer protection, in terms of TSEs, is SRM removal, prior to any tests, and therefore with no distinction as to the type of TSE with which the animals may be infected.

At the present time the risk to public health from atypical scrapie remains unknown. However, this uncertainty has led to the proposal of certain precautionary measures which may be reduced or even lifted to reflect developments in knowledge.

As a precaution, the working group is recommending the screening, using a test capable of detecting atypical scrapie, of all animals which have reached the age when testing is required[3], sold for human consumption and from a farm infected with atypical scrapie and their removal from consumption if the test is positive[4]. Moreover, if one of the tested animals shows classical scrapie in the discriminatory test which must systematically be used, the control measures for classical scrapie should then be applied.

- Ensuring protection of animal health

The principal question surrounding this issue relates to the transmissibility of atypical scrapie between animals in natural conditions. Current data do not enable a definitive answer, but allow the assumption that this natural transmissibility is probably limited, if it exists (low prevalence of atypical scrapie in the populations tested, apparently low intra-outbreak prevalence, absence of PrPres detectable outside the central nervous system).

Nevertheless, it has already been shown that in certain herds, two or even three cases of atypical scrapie have been detected; the probability of this occurring in the context of a spontaneous disease is low but not non-existent, but we are unable to analyse it properly since we do not have sufficient accurate and complete data on the outbreaks.

We must therefore allow the possibility that the disease is transmissible naturally and put in place a system to protect the other herds, based on the assumption that transmissibility is low.

[3] The choice of testing age is based on knowledge of the physiopathology of the disease. Current knowledge shows no need to change this age as compared with the age used for classical scrapie.

[4] We note that the European Commission proposal to permit the consumption of animals testing negative solely in the Member State in which a given outbreak has been detected has no rational basis in terms of public health protection, if the intention is to achieve homogenous management of this aspect at European level.

In this context the minimum measure to be taken would be a ban on the sale of breeding stock to other herds during the surveillance period. However, occasional contacts between herds, such as during transhumance, could be left unrestricted.

The second aspect concerning animal health is the use of the genetic selection tool. The lack of current data makes it impossible to come to any definitive conclusions on the respective susceptibility of different genotypes to atypical scrapie, other than for sheep, in which the AHQ and AFRQ alleles are more susceptible. A programme of genetic selection could be based on a number of different strategies:

- the preventive elimination of the most susceptible genotypes; we do not yet know whether these are infected more often within the outbreak or in the ordinary population;
- a ban on the most susceptible genotypes from breed selection schemes;
- the introduction of resistance alleles, yet to be defined.

The scope of such a programme would be limited by the fact that the risks to animal and public health are low, if they exist, and that all genotypes are affected by atypical scrapie. Until more accurate data are available, it is therefore reasonable, for the time being, not to initiate the genetic selection system in herds infected with atypical scrapie. This position could change with the acquisition of new information or if it was felt worth implementing an appropriate genotyping system in areas of outbreak (cf. below).

- **Improving knowledge of atypical scrapie**

The recommendations in this area arise from what has been said previously. It is essential for more accurate information to be available on the prevalence of atypical scrapie in herds in order to be able to estimate its power of transmissibility, to complete our comprehension of genetic susceptibility and of the risk factors for its presence in herds, and to improve our understanding of its physiopathology.

In the context of the surveillance of infected farms, it is extremely important that the following measures are implemented, as these would contribute to improving our epidemiological knowledge, the only means of enabling a reasoned amendment of the control measures:

- systematic testing of all dead or slaughtered animals in the herd aged over 18 months [5];
- putting in place a sampling system suitable for atypical scrapie research;
- imposing the choice of a screening test capable of detecting atypical scrapie;
- imposing typing which differentiates between classical and atypical scrapie in any positive sample;
- genotyping the animals from the herd at the four codons of interest.

[5] We note that the European Commission proposal to perform tests on a sampling basis on animals in outbreaks of atypical scrapie has no rational basis:

- in terms of a public health objective, this would mean not testing all the animals intended for consumption and therefore permit the consumption of positive animals from these herds;
- in terms of a knowledge improvement objective, the would severely hinder the estimation of prevalence in these infected herds, given the very low prevalence observed from the data available.

Furthermore, in order to ensure the data acquired are suitable for scientific exploitation, they must imperatively be accompanied by the putting in place of a specific individual identification system for these herds, and linked to information on year of birth and bloodline.

- **Herds under surveillance and duration of surveillance**

The definition of the herds to which the measures will apply and the duration of the surveillance in these herds are questions requiring an answer:

- in terms of the first question, the information available argues for applying the control measures solely to the herd in which the case was born[6]; or the one in which it spent its first year;
- in terms of the second question, whether one accepts the hypothesis of an infectious disease or the hypothesis of a disease with a toxic origin, prudence requires surveillance of the herd for a period enabling the majority, if not all, of the animals potentially exposed to be tested as they leave the herd[7]. This period could then be reviewed with a view to reduction, as new information emerges.

5. Annexes (references, sources, etc.)

Arsac J.-N., D. B., C. Feraudet, F. Debeurme, M. Perdriau, S.L Benestad, J. Grassi, and T. Baron (2006). Molecular signature of atypical scrapie as observed in field isolates and after transmission in an ovine transgenic mouse model (TgOvPrP4). PRION 2006, Turin.

Benestad, S. L., P. Sarradin, et al. (2003). "Cases of scrapie with unusual features in Norway and designation of a new type Nor98." The Veterinary Record 153: 202-208.

EFSA (2005). "Scientific Report of the European Food Safety Authority on the Evaluation of Rapid post mortem TSE Tests intended for Small Ruminants." The EFSA Journal 31: 1-17.

EFSA (2005). "Opinion of the Scientific Panel on Biological Hazards on classification of atypical Transmissible Spongiform Encephalopathy (TSE) cases in Small Ruminants1." The EFSA Journal 276: 1-30.

EFSA (2006). "Opinion of the Scientific Panel on Biological Hazards of the European Food Safety Authority on the Breeding programme for TSE resistance in sheep." The EFSA Journal 382: 1-46.

Gavier-Widen, D., M. Noremark, et al. (2004). "Recognition of the Nor98 variant of scrapie in the Swedish sheep population." J Vet Diagn Invest 16(6): 562-7.

Hopp, P., M. J. Ulvund, et al. (2001). "A case-control study on scrapie in Norwegian sheep flocks." Preventive Veterinary Medicine 1622: 183-198.

[6] This measure is based on the hypothesis of an acquired disease (infectious or due to exposure to subacute toxicity); this hypothesis, since cases are generally older, implies a longer incubation period and a younger age at infection (unless the hypothesis becomes one of susceptibility to infection increasing with age, which has not, to our knowledge, been observed in an infectious disease), or exposure to a toxic agent for a longer period.

[7] Surveillance and checks for five years, similar to the system in place in Portugal, seems a satisfactory duration.

Konold, T., A. Davis, et al. (2006). "Atypical scrapie cases in the UK." Vet Rec 158(8): 280.

Le Dur, A., V. Beringue, et al. (2005). "A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes." PNAS 102(44): 16031-16036.

Morignat, E., G. Cazeau, et al. (2003). Surveillance active de la tremblante chez les petits ruminants. Analyse du programme 2002. Synthèse. AFSSA Lyon: 16.

Moreno, C. R., P. Laurent, et al. (2006). Resistance to atypical scrapie linked to codons 136, 141, 154, 171 of PrnP Gene in France. Neuroprion 2006, Turin, Italy.

Onnasch, H., H. M. Gunn, et al. (2004). "Two Irish cases of scrapie resembling Nor98." The Veterinary Record 155: 636-637.

Noremark, M. and P. Hopp (2006). Reported occurence of Atypical Scrapie in Europe. 11th Symposium on Veterinary Epidemiology and Economics, Cairns.

ANNEX 1: ASPECTS OF THE RAPID TSE TESTS FOR SMALL RUMINANTS

In 2005, the European Food Safety Authority (EFSA) evaluated the performance of 9 diagnostic tests for detecting TSE in sheep. This comparative study, the only one currently available, was published in the form of two reports, on 17 May and 26 September 2005 respectively.

The tests were first evaluated for their capacity to detect different forms of TSE in the central nervous system (CNS) of sheep, more specifically in brain stem fragments for classical scrapie and BSE isolates (the most suitable part of the CNS for sampling as trepanation is unnecessary) and in cortex fragments for atypical scrapie isolates.

All the tests showed a specificity of almost 100% (capacity to detect as negative 1000 samples from New Zealand sheep considered healthy);

All the tests except one (FujiRebio) are recommended by the EFSA for use in detecting classical scrapie. In fact, the 8 tests adopted showed diagnostic sensitivity of almost 100% (capacity to detect as positive 219 samples from sheep infected with scrapie at the clinical stage) for detecting classical scrapie. In terms of analytical sensitivity (detection of high dilutions of a positive sample, mimicking the detection of samples from animals in the incubation stage), the test performances in descending order were as follows: Bio-Rad TeSeE Sheep & Goat > IDEXX > Enfer = Institut Pourquier = InPro CDI > Bio-Rad TeSeE > FujiRebio > Prionics LIA (the analytical sensitivity of the Prionics Western Blot test was not evaluated).

All the tests evaluated correctly identified the three brain stem samples from sheep experimentally infected orally with the BSE agent, in the clinical stage of the disease, and are therefore recommended for this purpose by EFSA.

All the tests, except the Prionics LIA and FujiRebio tests, identified the three cortex samples from sheep infected with the Nor-98 strain of atypical scrapie and are therefore recommended by EFSA for analysing samples of cortex or cerebellum. However, solely the two Bio-Rad and the IDEXX texts demonstrated sufficient analytical sensitivity with these isolates (all the other tests detected only the pure samples) and are therefore the only tests recommended for analysis of a brain stem sample. In fact, in atypical scrapie, the concentration of PrPres is higher in the cerebellum and the cortex and lower in the brain stem, in contrast with classical scrapie isolates.

In the absence of a strong argument refuting the pathogenicity of atypical isolates in humans, the Expert Committee considers that, within a large scale screening programme, the tests used for detecting TSE in sheep must be capable of detecting all TSE isolates in these species. Consequently, all the tests evaluated by EFSA, with the exception of the FujiRebio and the Prionics LIA, may be used, on condition that they are used simultaneously on both the brain stem and on a sample of cortex or cerebellum (the only areas permitting the detection of atypical scrapie strains by 4 of these tests). In practice, the only alternative to the brain stem is the cerebellum which can also be sampled (in more or less satisfactory conditions) through the foramen magnum. Cortex sampling, which requires trepanation, is difficult and dangerous to implement. If the tests are carried out on the brain stem only (as stated in the DGAL memo N2006-8079 dated 27 March 2006 recommending the use of the brain stem in first line sampling), which again is the only suitable sampling region given that trepanation is not required and sampling can be carried out safely, the Committee considers that only the Bio-Rad Sheep and Goat and IDEXX tests are to be recommended, as only

these tests can detect all strains of scrapie in this type of sample. Otherwise, using the cerebellum as the only sampling site, notwithstanding the technical problems arising in sampling on this region of the anatomy, would greatly reduce surveillance sensitivity with regard to the BSE agent (due to the weak expression of PrPres in the cerebellum in BSE cases) and must not be retained as an alternative.

In the second phase, EFSA evaluated test performances using the lymphoid organs to demonstrate TSEs in sheep. Solely the mesenteric ganglions and the spleen were tested and using certain tests only (Bio-Rad TeSeE, Bio-Rad TeSeE Sheep and Goat and IDEXX on the mesenteric ganglions and the spleen, the Institut Pourquier test and the Prionics WB test on the mesenteric ganglions only). EFSA recommends that these tests only be used on the peripheral organs for which they have been evaluated.

Within a large scale post mortem screening programme for TSE in sheep, the lymphoid organs would be useful in detecting infected animals earlier, before the central nervous system becomes infected or presents a measurable quantity of PrPres. Studies undertaken at the National Veterinary School in Toulouse suggest that the use of these peripheral organs doubles the number of positive animals compared with the use of CNS tests only (Andreoletti, personal communication). However, it must be emphasised that TSE screening programmes for small ruminants are carried out with the principal aim of detecting and eliminating infected flocks (population logic) than with the aim of eliminating individual animals to protect the consumer (individual logic). As a result, the use of lymphoid organs permits the identification of infected flocks only a few days or weeks earlier than the tests on the CNS alone, so the same flocks will be identified anyway but later on, provided the tests are carried out systematically.

Therefore, when systematic screening is used, all the flocks will be detected sooner or later by tests on the CNS, so these tests alone should be used. On the other hand, when screening by sampling, the combination of tests on the CNS and the lymphoid organs would enable an improvement in screening network performance.

In practice, among those lymphoid organs tested within the EFSA evaluation, the mesenteric lymph nodes are the easiest to sample. It should be noted that the possibility of using other lymphoid organs, such as the retropharyngeal lymph nodes or the tonsils, is worth studying for traceability reasons, in view of the fact that these organs are located in the head with the CNS.

Source: Mandate 2006-SA-0099 – Report of the CES ESST – July 2006

ANNEX 2 – SCRAPIE CONTROL MEASURES

The control measures for scrapie are precisely described in the Orders of 27 January 2003 laying down the control measures for goat and sheep scrapie. These measures can be summarised as follows:

When a case of scrapie is confirmed, one of two scenarios applies:

- either the infected animal has lived on the same holding since birth and up to at least 6 months before the suspicion of scrapie was established;
- or the infected animal has lived on several holdings since birth.

In the first scenario (the infected animal has always lived on the same holding) the birth farm is placed under an APDI [Arrêté prefectorale de declaration d'infection – Prefectoral Infection Declaration Order] and subject to eradication measures.

In a sheep flock, eradication measures require the euthanasia and selective destruction of animals described as genetically susceptible (sheep carrying two ARQ, ARH or AHQ alleles; rams carrying at least one ARQ, ARH or AHQ allele) and highly genetically susceptible (sheep carrying at least one VRQ allele). Euthanasia must be carried out as soon as possible, but it may be delayed for a maximum period of two breeding seasons in flocks where there is a high proportion of susceptible animals.

In a goat herd, all animals are euthanased and destroyed as soon as possible. Certain herds may, however, be placed in an experimental protocol (notably with sampling of tonsils) and exempted from slaughter.

In both sheep and goat flocks, animals from herds subject to an APDI and which have lived, when aged less than one year, with the infected animal when it was aged less than one year, are deemed to be at-risk from scrapie. These are found and eliminated (measure restricted to susceptible animals or highly susceptible for sheep).

Once the eradication is complete and the APDI has been lifted, the herds are subject to health surveillance for three years. During this time, restocking with sheep is only permitted with animals described as genetically resistant (homozygote ARR/ARR rams exclusively; other sheep carrying at least one ARR allele and no VRQ alleles).?

In the second scenario (the infected animal has lived on several holdings) the at-risk farms are placed under an APMS (Arrêté Préfectoral de Mise sous Surveillance - Prefectoral Surveillance Order] and subject to intensive surveillance measures for 3 years.

The following are deemed at risk:

- the birth holding of the suspect animal;
- all holdings where the suspect animal has lambed or kidded when a female is concerned.

The at-risk herds must:

- declare all movements (entry and exit) of live animals;
- transport all animals aged over 12 months intended for consumption directly to the abattoir;
- declare all deaths of animals over 12 months old;
- deliver all dead animals over 12 months old to a rendering plant, even if their weight is less than 40 kilograms.

A rapid test for scrapie is performed on all animals aged over 12 months transported to the abattoir or collected by a rendering plant.

If a case of scrapie is detected during the surveillance, a survey is conducted and the at-risk herds concerned are placed under an APDI or an APMS. If no cases are found after three years, the intensive surveillance is suspended.