Appendix I

TSE in the French sheep population

Current situation – prioritization of measures for improving surveillance with a view to protecting public health

Working group report on animal TSE epidemiology

July 2006

Context

Following the confirmation, in February 2006, of two cases of sheep TSE by a single biochemical strain typing test where one case showed a non-distinct profile and the other case a subtly distinct profile from the BSE agent, the authorities requested that Afssa formulate an opinion on the relevance of moving forward with the current risk management scheme for sheep TSEs while awaiting results from the bioassays in mice currently underway. It is within this context that Afssa delivered an opinion on March 1 2006. On the one hand, this opinion aims to clarify the limits to be set on the interpretation of the two cases in question and on the other hand propose recommendations for the flocks from which the two cases originated.

Afssa was asked once again, in March 2006, to give an opinion on the relevance of maintaining, in 2006, systematic screening of sheep at rendering plant, and of extending screening to the abattoir for cull sheep over 18 months and with the removal of SRM applying to the entire intestines of animals born before 2002. If it was deemed appropriate, measures concerning animals under 18 months at the abattoir could be brought in addition. Taking into account these elements and the questions in the inter-ministerial referral, Afssa has asked the TSE SSC and the Working Group for animal TSE epidemiology to look into the four following issues:

- the prioritization of the various consumer protection measures;
- the sampling scheme regarding probing at the abattoir of lambs and sheep under 18 months;
- the optimization of screening tests;
- the exposure of the French population.

Preamble

As a preamble to this document, it seems important to recall that cow and sheep TSE pathogeny and epidemiology are very different. This dictates two very different approaches for the protection of both public and animal health.

Where BSE surveillance in bovines is concerned, the current scheme aims to define individual animal status and at the same time take action for public and animal health: it helps ensure public health protection in eliminating risk material and destroying the carcases of animals testing positive. It also aims to protect animal health in helping to detect affected farms and to control the disease. This double objective can be attained due to the characteristic pathogeny of BSE in bovines, where the central nervous system is affected but not the carcase.

In sheep, the situation is very different. If BSE existed naturally in sheep, protecting the consumer from the possible presence of the BSE agent in sheep would not be possible solely through the removal of risk material and the destruction of carcases of animals testing positive. Such is the result of BSE and classical scrapie pathogeny in sheep. The central nervous system is affected only after the other organs and as spreading of the agent is not limited to specified risk material, other elements of the carcase may be contaminated by the BSE agent even though tests in the brain may not prove to be positive. Scientific data supports more and more frequently the fact that the prion accumulates in muscle tissue and blood (at levels a lot lower however than those found in the central nervous and lymphoid systems). The intraspecific transmission of BSE in sheep has been demonstrated experimentally and would more than likely be repeatable under natural conditions.

The protection of public health with regards to sheep BSE must therefore follow a different logic than that implemented for bovine BSE. Any scheme should be organised on a flock scale rather than on the scale of the individual animal. In this context the use of rapid tests must be considered, either for detecting farms affected by TSE and in which measures to fight the disease would allow for consumer protection, or on the contrary for determining whether the population is TSE-free. The scientific basis for such a qualification scheme is difficult to establish given that it is not known exactly how the TSE agent spreads, especially sheep BSE, and given that it is a disease with a long incubation period.

Finally, if BSE and classical scrapie behave in the same way in sheep (in particular where pathogeny and genetic susceptibility are concerned), a newly described form of TSE, named atypical scrapie, behaves differently. This heralds major implications in terms of diagnosis (preferred accumulation zone of the different PrPres) and control.

Aims of the report

The present document aims to provide scientific and technical support to the TSE SSC for replying to the referral relating to the assessment of the risk linked to the presence of TSE in sheep. To this end, the animal TSE Epidemiological Working Group has analysed the current surveillance system and the French situation with regards to sheep TSE. It proposes prioritizing the measures aimed at improving TSE detection in the sheep population in view to protecting public health and controlling these diseases in animals.

1. Analysis of the current surveillance scheme

The current surveillance scheme for sheep TSE is based on four programmes:

- clinical surveillance of sheep on a national level (since 14 June 1996, date on which it became compulsory to declare scrapie in small ruminants);
- the official sanitary control (OSC), implemented in 1998 and reviewed in 2004 and which concerns intra-community exchanges between sheep and goat breeders and genetic material trading (semen, embryos) in France and in Europe;
- the active surveillance scheme at rendering plant, operating since 2002, in accordance with European regulation CE 999/2001; this programme was implemented under the form of a yearly survey from 2002 to 2005; it became systematic after a memo from the food directorate dated 22 November 2005;
- the active surveillance scheme at the abattoir. This was implemented in the same way as the previous scheme in 2002, and became systematic after a memo from the food directorate dated 27 March 2006.

Furthermore, the health policy contributes to disease control through the cleansing of infected and detected flocks, and the implementation of genetic selection.

Reinforced surveillance at the abattoir and during the slaughtering of animals over 12 months from farms at risk following the detection of a case of scrapie (farms under APMS follow-up) contributes to two active surveillance schemes as described above since 2003[1].
Finally, since January 2006, reinforced surveillance of flocks benefiting from zootechnic identification in the genetic sense is requested in a memo from the Ministry of Agriculture, Fisheries and Food. Such surveillance, founded on EC provisions, concerns a certain percentage of the animals sent to the abattoir. No population is involved to date.

- **The contribution of surveillance schemes to the detection of TSE sources of outbreak**

A source of outbreak is a sheep farm in which a case of TSE has been detected for the first time during a surveillance programme.

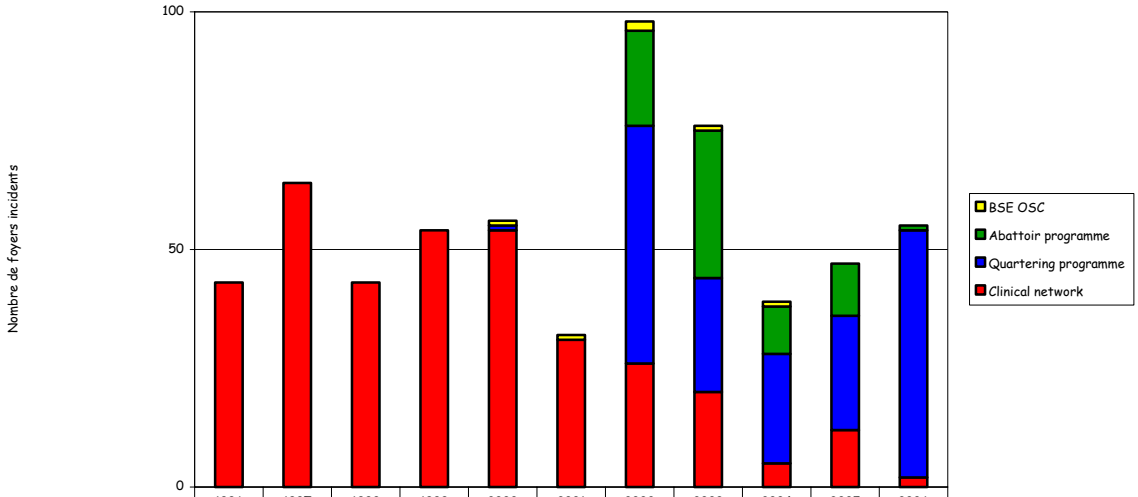
The simple analysis of the distribution of sources of outbreak detected since 1996 according to the surveillance programme (cf. infra graph), draws the following lessons:

- following what was described for BSE; clinical surveillance means some sources may go unrecognized. Additional sources are detected as soon as other surveillance programmes are implemented. Furthermore, as for BSE, the increasing importance of active surveillance significantly reduces the efficiency of clinical surveillance through a now well-described communicating vessels effect (from 2002 to 2005 respectively 67, 69, 37 and 45 clinical suspicions of scrapie over the whole country for a population of approximately 7 million adult sheep; that is to say one suspicion each year in around 60 out of the 80 000 existing farms (Agreste update 2003));

- the OSC does not contribute significantly to the detection of sources of outbreak as by definition it concentrates on those farms requesting certification and which are therefore in principle TSE-free. In any case here neither the farmer nor the veterinary have identified any clinical cases of TSE. Besides this it is only implemented in a limited number of sheep farms[2]. However since 1998 the OSC has helped detect 6 sources of outbreak (source: Afssa scrapies control data-1 March 2006). Finally, the proportion of animals undergoing testing is very low (1% of animals over two years per flock and per year up until 2005).

Annual distribution of the number of sources of outbreak per programme *(01/06/1996 to 01/03/2006; source - Afssa scrapies' control data)*

[1] The number of animals testing positive within this framework is recorded in the DGA1 databse whereas negative tests are not.
[2] The number of farms is not counted on a national level; the OSC only approaches farms selecting various sheep breeds. In the best case scenario (if 100% of selector farms were within the OSC, that is to say 2 300 farms), the OSC would cover 2.8% of the 80 000 sheep farms and 14% of the 6 750 000 ewes and ewe lambs.



	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
BSE OSC					1	1	2	1	1		
Abattoir programme							20	31	10	11	1
Quartering programme					1		50	24	23	24	52
Clinical network	43	64	43	54	54	31	26	20	5	12	2

Active surveillance programmes using probing have detected a certain number of sources of outbreak since 2002. However, on a yearly basis, sheep identified as positive at rendering plant and at the abattoir originated from different farms (for the traceable animals), except on two occasions in 2002 and 2004, during which an animal identified as positive at rendering plant and one at the abattoir originated from the same flock. The virtual separation between flocks diagnosed at rendering plant and at the abattoir can be explained by the fact that when a positive case is diagnosed at either of these points, the population of origin is no longer subjected to ordinary surveillance measures. It is subsequently monitored within the health policy framework (and the cases detected are counted separately). Beyond this, a programme based on a sample of animals, be it of several thousand animals[3], can only detect a fraction of affected flocks for the following reasons:

- the prevalence of scrapie on animal level is low (from 1 to 2 ‰ of adult animals slaughtered or quartered);
- the prevalence of infected flocks is probably also low. As a reminder, modelling carried out in Norway estimated that at best, such a programme only detected 9% of affected flocks[4].

- **Limits linked to the conception and the implementation of the current exhaustive detection programme**

[3] Surveillance programmes only test a fraction of the affected population (on the basis of a population of 250 000 quartered sheep per year (source: DGA1), 6.9%, 7.5% and 4.9% of the sheep population quartered in 2002, 2003 and 2004 respectively; on the basis of 650 000 adult sheep slaughtered per year (source: DGA1), 5.2%, 6.8% and 1.9% of the slaughtered sheep population in 2002, 2003 and 2004 respectively.

[4] Hopp P, Webb CR and Jarp J. Monte Carlo simulation of surveillance strategies for scrapie in Norwegian sheep. Preventive Veterinary Medicine 2003;61:103-25.

The exhaustive active surveillance programme as described in the two memos mentioned above is limited in its design and its implementation, resulting in TSE-affected sheep flocks going unrecognized.

Concerning the programme outline:

- exhaustive sampling at rendering plant is not totally enforced, as "on busy days on certain sites, a maximum of 500 carcases for sampling can be applied". Furthermore in areas where carcases are "collected in bulk in collective containers (free depot notably in the PACA region) [these] must be made the subject of sampling where possible…". It is clear that measures to ensure the exhaustiviteness of sampling at rendering plant have not been implemented;
- samplers are asked "to do their best" to take a sample from the cerebellum in addition to from the brain stem. Two types of sampling spoons exist, one of which is more appropriate for cerebellum sampling. It would be fitting to assess their respective efficacy and to only use the most effective in this case. On this occasion, it is also advisable to propose a protocol and organise training for the personnel involved in sampling;
- the type of first line test used is done so on the initiative of the departmental laboratory. This may contribute to underestimating the prevalence of atypical scrapie forms, cf. Appendix 1;
- in these conditions, cerebellum samples are only of interest in defining more effectively the classical/atypical status of certain other samples, for instance from the brain stem, on which this distinction is not easily made. On the other hand, atypical scrapie only detectable on the cerebellum will go undetected, except if a combined analysis of the obex/cerebellum is carried out.

Concerning programme implementation:

- the probing programmes at rendering plant and at the abattoir implemented between 2002 and 2005 witnessed a heterogeneous rate of sampling on a geographical scale thus distorting prevalence estimation. Analysis of the exhaustive detection scheme for goats in 2005 indicates that exhaustiveness was not attained. It is highly probable, although undocumented, that this lack of exhaustiveness is not due to a lack of sampling of animals at the rendering plant or at the abattoir but to the disappearance of animals before arrival at these destinations. We can rationally estimate that this hypothesis will be the same for sheep;
- the proportion of non-traceable sheep[5], which is very likely underestimated due to preferential recruitment of traceable animals, was 9.7%, 7.4%, 6.9% and 6.1% in 2002, 2003, 2004 and 2005 respectively at both the rendering plant and the abattoir. The carcase identification process implemented in November 2005 and aiming to ensure identification of the farm from which the animals were collected, did not seem to contribute to a reduction in the proportion of non-traceable animals[6]. However it may be that the effect of these measures

[5] The animal's farm of origin is estimated from individual animal number (automatic extraction of the first 8 figures of the individual number typed into the DGA1 TSE database. This individual number corresponds to the number marked on one of the seals carried by slaughtered or quartered animals;a seal may have been put in place by each farm in which the animal resided).
In this way the numbers only reflect the farm where the animal was born if:

- the animal has never changed farms (or been handed over to a wholesaler before going to the abattoir),
- the number has been correctly copied by the sampling personnel and correctly typed (difficulties linked to the varying format of small ruminant individual numbers.

In a certain number of cases which are impossible to estimate, this automatic extraction may lead to a secondary sheep population, a wholesaler or nothing. It may also lead to populations which no longer exist.

[6] The proportion of unidentified animals (coded XXXXXX in the database increased significantly in the first quarter of 2006, compared to 2005 (8.3% vs 6.1% - chi deux, p<10-6).

was compensated by the fact that unidentified animals were more effectively included in the programme. Nevertheless the final result is that a significant quantity of animals remains non-traceable.

2. Analysis of current knowledge

- **Analysis of active surveillance data for sheep since 2002**

Total TSE prevalence within active surveillance programmes

	2002	2003	2004	2005	2006*
Abattoir	31/33 764 0.92 ‰	45/ 44 408 1.02 ‰	15/12427 1.21‰	12/12208 0.98‰	3/3130 0.96‰
rendering plant	121/ 17 434 6.94 ‰	33/ 18 674 1.77 ‰	22/12294 1.79 ‰	38/22196 1.71 ‰	92/95479 0.96 ‰
Abattoir/rendering plant prevalence ratio	7.5	1.75	1.5	1.75	1

*data at the date of 31 March 2006

The significant difference in total prevalence at rendering plantrendering plant between 2002 and 2003 is partly explained in the imputation of animals to programmes in 2002: certain animals included in the rendering plant category were those animals tested for scrapie in the framework of the health policy and on farms on which a case had been detected[7]. However even in correctly reassigning these animals, the difference in prevalence remains significant. We cannot exclude that some animals at rendering plant may present TSE-compatible symptoms even since the implementation of active surveillance programmes. For 2006 the prevalence trend at rendering plant site must be followed up in particular. A seasonal effect may explain the low prevalence rate for the first quarter of 2006.

Globally, prevalence at the quartering site appears considerably homogenous from 2003 to 2005. Caution should be exercised nevertheless in comparing these total prevalence rates, given the aberrations revealed in the implementation of these programmes: non-random sampling of animals at the abattoir and at rendering plant, geographic heterogeneity of the probing rate and the use of tests of varying reliability. If we take into account these aberrations, estimated prevalence differs appreciably, cf. simulation table below[8].

Total and simulated prevalence of TSE in active surveillance programmes

		2002	2003	2004	2005
Abattoir	Total prevalence	0.92 ‰	1.02 ‰	1.21‰	0.98‰
	Simulated Prevalence*	1.66 ‰	1.05 ‰	2.15‰	ND
rendering plant	Total prevalence	6.94 ‰	1.77 ‰	1.79‰	1.71‰
	Simulated Prevalence*	13.55 ‰	2.73 ‰	2.81‰	ND

[7] Morignat E, Cazeau G and Calavas D: Active surveillance of scrapie in small ruminants – Analysis of the 2003 programme, Afssa Report, 2004.
[8] *Ibid*. Ibid.

* simulated prevalence corresponds to an estimation of the prevalence of all TSEs, including the atypical forms of scrapie.

If we only take into consideration years 2003 and 2004, the relationship between total prevalence at rendering plant and at the abattoir (respectively 1.75 and 2) is very different from that observed for BSE in bovines (20, 19, 25 and 17.4 respectively for bovine BSE in 2001, 2002, 2003, 2004). Where BSE is concerned, animals diagnosed as positive at the rendering plant are for the main part in the clinical phase of the disease. Animals diagnosed as positive at the abattoir seem for the main part to be in the preclinical phase[9] . This difference cannot be explained by the existence of atypical forms of scrapie. If we take out the atypical cases, the prevalence ratio between the rendering plant and the abattoir is of 2.55 in 2003 and 3.98 in 2004. This difference in ratio between bovines and sheep may be explained by the differing pathogeny. The accumulation of PrPres at a detectable level in the central nervous system appears earlier and more progressively in sheep. This explanation is not however likely to suffice when accounting for such a difference. Another explanation may lie in the fact that scrapie symptomatology is sometimes irregular, making identification of clinical cases difficult. This applies on the farm or during *ante mortem* inspection at the abattoir. The proportion of clinical cases among the cases detected at the abattoir may be higher than that for bovines. It would be fitting to explore the latter more specifically.

- **Analysis of detection and strain typing test characteristics**

Test validation

A scientific report from the European Food Safety Authority (EFSA) was delivered in June 2005 on the evaluation of 6 Rapid *post mortem* TSE tests intended for small ruminants[10].

- The 6 tests (Bio-Rad, TeSeE, Bio-Rad TeSeE sheep/goat, Enfer TSE Test v2.0, Institut Pourquier Scrapie Test, Prionics Check LIA Small Ruminant, Prionics Check WB Small Ruminant) perform satisfactorily in terms of sensitivity and diagnostic specificity in detecting classical scrapie, but there are significant differences in analytical sensitivity between the tests;
- all the tests, except for Prionics Check LIA Small Ruminant, detect atypical cases on cortex samples used for the study and are therefore recommended for cortex or cerebellum analysis;
- only the Bio-Rad tests detect the atypical cases effectively (Nor-98) on a brain stem sample;
- all 6 tests detect sheep BSE on the brain stem but there are significant differences in analytical sensitivity between the tests.

EFSA delivered a second report in September 2005[11] relating to the evaluation of three other tests (Fujirebio, IDEXX Herdcheck and InPro CDI-5):

- the Fujirebio test showed insufficient diagnostic sensitivity and was not recommended for approval. Furthermore, it was unable to detect atypical scrapie on any of the three samples;
- the IDEXX and InPro tests perform satisfactorily in terms of diagnostic sensitivity and specificity for classical scrapie and BSE, but the analytical sensitivity of the IDEXX test is higher (in fact similar to that observed with the

[9] Cazeau G, Ducrot C, Collin E, Desjouis G and Calavas D. Questionnaire analysis of BSE cases in France detected by active surveillance and the reasons for non-notification. The Veterinary Record 2004;154(5):133-6.

[10] EFSA Scientific report (2005)31,1-17 on the Evaluation of Rapid *post mortem* TSE Tests intended for Small Ruminants.

[11] EFSA Scientific report (2005)49,1-16 on the Evaluation of Rapid *post mortem* TSE Tests intended for Small Ruminants.

TeSeE sheep/goats test), this test was considered as having the best analytical sensitivity within the first evaluation of TSE tests for small ruminants.

It must be noted that according to the evaluation, the IDEXX test can also be recommended for detecting atypical scrapie on the brain stem whereas the InPro CDI-5 test cannot be used on a cortex or cerebellum sample.

The scrapie/*BSE-like* biochemical strain typing tests (named strain typing tests hereafter) derive from *rapid* tests. These tests are only applied to first line cases confirmed as positive by a *rapid* test and cannot by definition detect *BSE-like* cases among positive TSE cases.

Strain typing test specificity

Little is known about strain typing test specificity. When a sample is shown as positive by a strain typing test, this sample then undergoes a *ring trial*. This is organised on a European level by the European Community Reference Laboratory (CRL), with the implementation of several strain typing tests. At this stage the status of certain samples cannot be clearly established (either certain tests were unable to be applied, or discordant results arose between strain typing tests. To date, several sheep samples (source: strain typing network), of which the British sheep isolate CH1641 enter into this case). The samples passing the *ring trial* are declared *BSE-like*. These samples then undergo a bioassay in mice. To date the only published *BSE-like* case was confirmed as being non-distinguishable from BSE by these bioassays (therefore a specificity of 100% for one case). The possible flaws in strain typing test specificity are dealt with later in the bioassays in mice (the false *BSE-like*/BSE positives are eliminated at this stage). The real prevalence of BSE in sheep can only therefore be known on the outcome of these bioassays. The prevalence of *BSE-like* cases may be greater than that of BSE, but the proportion is unknown[12].

Strain typing test sensitivity

Strain typing test sensitivity is currently estimated at 100%. This sensitivity threshold was based on results from 19 experimentally BSE-infected ruminants (14 sheep within the CRL validation programme for the five tests proposed, 3 sheep from Afssa experiments in Lyon and 2 goats from experiments in Scotland). Where only one "first line" strain typing test is implemented, that is to say used on positive samples, the confidence interval (CI) generates a lower limit of 82.35% (exact binomial law) for this sensitivity.

BSE signature and anatomical regions

We consider today that the biochemical and immunohistochemical BSE signature in sheep is preserved after several passages in this species, and this regardless of animal genotype. Among the 14 sheep isolates included in the strain typing test validation phase, 10 isolates originated from second passages of BSE in sheep. We can also note that these 14 isolates originated from sheep of the same ARQ/ARQ genotype as did the 3 isolates from Afssa experimentation in Lyon. To date there is no published data concerning the stability of this signature in a third passage in sheep, or concerning the BSE signature in sheep of other genotypes. Taking into account that identification of BSE in sheep will be based on the detection of *BSE-like* cases by strain typing tests, identifying a clear signature is essential[13]. This is particularly crucial when we

[12] Once an ESB-like case is detected by one of the typing tests, the sample undergoes a ring trial which includes other typing tests. The biochemical typing method was established from 5 tests (3 Western Blot, 1 ELISA and 1 immunohistochemical) which all identify the BSE strain by characterizing PrPres sensitivity using a protease but with different antibody techniques. The ring trial specificity with regards to the sample's BSE status is superior to the result given by the first line typing test. To calculate the lower limit of the confidence interval, the level of independence for the tests used must be taken into account.

[13] This hypothesis is also essential in planning different health policy measures for classical scrapie sources and BSE agent sources.

consider that exposure of the sheep population to the BSE agent took place between 1980 and 1990, and has diminished in line with the measures taken since then, and that BSE cases diagnosed in sheep today would be the result of subsequent transmission in the sheep population. It is therefore of the utmost importance to have access to additional data relating to this hypothesis: stability in a third passage and beyond that, stability regardless of genotype.

Strain typing tests have only been validated on the central nervous system and more specifically on the brain stem (on 14 sheep within CRL test validation). Furthermore, during a previous assessment phase, two other animals were tested in a satisfactory manner by the 4 tests. These tests concerned primary transmission and in this case the tests were carried out on the brain stem, the prefrontal cortex, the occipital cortex and the parietal cortex. We must therefore consider that these tests were validated on brain stem samples with a limited result range. This indicates that these tests can also be applied to the cerebral cortex. There is no current data to support their use on cerebellum samples. While waiting for additional data concerning the cerebellum, only the brain stem can be used for confirming *BSE-like* cases in the sheep population. The same comments are valid for lymphoid tissue samples as no strain typing tests have been validated for this tissue. This is to be taken into account for instance within the hypothesis that analysis could be carried out on a combination of both a brain stem and lymphoid tissue sample from the same animal. This may contribute to increasing test sensitivity for the detection of TSE-infected animals.

- **BSE and scrapie pathogeny in sheep**

Current knowledge, based on a limited number of experimental cases, leads us to think that BSE pathogeny in sheep is similar to that of classical scrapie, in terms of the kinetic accumulation of PrPres and spreading of this marker in the organism. Furthermore, we also acknowledge that genetic susceptibility factors are similar in BSE and classical scrapie.

We will therefore pose the hypothesis that the proportion of *BSE-like* cases is equivalent among the positive cases confirmed by a rapid test at the rendering plant and at the abattoir.

On the other hand, the pathogeny of atypical cases seems to differ, in particular in the absence of PrPres marking in the lymphoid system. In this way, the analysis of a combined brain stem and lymphoid tissue sample from the same animal could lead to atypical cases going unrecognized, especially as in these cases, PrPres concentration in the brain stem is generally inferior to that observed in classical scrapie.

3. *Prioritization of measures for improving TSE detection in the sheep population*

The prioritization of those measures aimed at improving TSE detection in the sheep population, with the final aim of protecting public health, was assessed taking into account all forms of TSE described to date in the French sheep population (classical and atypical scrapie), or potentially present (BSE).

This prioritization can only be qualitative due to the absence of several parameters (prevalence of affected flocks, distribution of animals testing positive per flock, flock genotype structure, prevalence of positive presence in the nervous system vs. lymphoid tissue, etc.). Furthermore, if

we assume that these parameters are known, a quantitative approach would require modelling. This would have to be carried out within a research programme, and not within this working group.

The first on the list of priorities is to improve the current system – exhaustive detection at the rendering plant and at the abattoir –, in the aim of comprehensive detection of TSE sources[14], before conceiving any additional measures.

3.1 Improve the current detection system

It is first of all advisable to document the results of the recent reinforcement of recommendations for sampling unidentified animals, for instance through checks at the rendering plant. Furthermore, despite marking by the knacker on carcase dispatch, there is still a significant proportion of animals arriving at the cutting premises unmarked (8.3% in the first quarter of 2006). An improvement in the system would consist in taking measures to reduce this proportion.

First step – Ensure animal identification and traceability

It is essential to implement without delay reliable sheep identification, following bovine identification, allowing traceability to the farm of origin.

This is essential, notably:

- where the epidemiological analysis scheme is concerned, in order to be able to correctly estimate prevalence on a national level and to better estimate "flock" prevalence;
- where the risk management scheme is concerned, for improving health policy efficiency through more effective traceability of animals back to affected flocks;
- in the perspective of a national flock qualification scheme. This would enable the attribution of negative tests at the abattoir and at the rendering plant to the flock of origin (step already implemented in the OSC framework).

Second step – Aim for the overall inclusion of sheep of 18 months and over in active surveillance programmes and for full-scale correct documentation of animal evasion/disappearance

Where the rendering plant is concerned, the following aberrations can be identified:

- carcases not collected by the rendering plant (carcases not found in pasture, burying on site, vulture feeding…);
- carcases collected in conditions unfit for sampling to be carried out (collection containers in South East France…);
- carcases collected but not sampled (carcase preservation conditions, age estimation error,…)

[14] And to apply therein control measures adapted to the type of TSE detected. This issue does not come within the framework of this referral.

Where the abattoir is concerned, it would be fitting to control on-farm slaughtering and clandestine slaughtering.

A faultless system would require on-farm checks to ensure that all sheep 18 months and over, dead or due to be slaughtered, are sent to the rendering plant and to the abattoir to be tested. This would reach beyond screening of the animal sub-population currently being sent.

Third step – Selection of a test and sample type for detecting all forms of TSE

The obex is the anatomic site of choice for detecting BSE. Atypical scrapie is detected with "satisfactory" sensitivity by several tests on cortex or cerebellum samples but few tests detect the disease on an obex sample.

Under these conditions, the working group recommends using tests of the highest analytical sensitivity on the brain stem, for both BSE and atypical scrapie.

A second solution consists in combining two tests (a test on the obex for detecting atypical scrapie and subsequently for detecting BSE, and a suitable test for detecting atypical scrapie on the cerebellum). This could increase detection sensitivity for atypical cases compared to the previous solution. An increase in detection sensitivity is not quantifiable on the basis of current knowledge (for this we would need to be able to analyse both obex and cerebellum samples on a sufficient number of sheep affected by atypical scrapie). It would be fitting to provide breeders with a clear and adapted protocol (cf. Annexe 2) and ensure training.

3.2 Additional detection measures or checks

Additional measures can be foreseen if the three proposals are actually implemented.

Fourth measure – Take into account the possible coexistence of several TSE in a flock

The coexistence of a classical scrapie strain and an atypical scrapie strain on the same farm has already been observed, but the frequency of this coexistence is to date undocumented. Currently, differential screening (between classical scrapie and atypical scrapie) is only carried out on the index case of affected flocks. This does not allow for the detection of a possible second form in the flock. Also, systematic differential screening applied to secondary cases as found within the health policy, would allow for an estimation of the frequency of association between classical and atypical scrapie and to define a profile for affected farms (this data would be useful for the implementation of a flock qualification scheme).

In applying the same reasoning, the simultaneous presence of the *BSE-like* strain and other TSEs on the same farm cannot be excluded. Currently only index cases are made the subject of a BSE/scrapie strain typing test. It would be fitting to systematically apply this strain typing test to secondary cases found in health policy slaughtering. This measure would be likely to improve consumer safety.

Fifth step – Testing sheep under 18 months?

Testing animals under 18 months requires taking into account physiopathogenic and epidemiological considerations.

Physiopathogenic considerations

In sheep of the ARQ/ARQ genotype, which do not present any particular resistance to BSE after oral administration of the BSE agent at 6 months, the PrPsc is detectable by immunohistochemistry in lymphoid organs 4 months after inoculation. Spreading of the infectious agent in the nervous system comes later, between 10 months (animals of 16 months) and 16 months after inoculation (animals of 22 months)[15].

The same pathogenic scheme was recently observed in experimentally orally inoculated new-born ARQ/ARQ sheep: detection of PrPSc in lymphoid organs after 4 months and in the central nervous system after 10 months[16]. The age limit at which PrPSc begins to accumulate in the central nervous system in this experimental model is inferior to 10 months and more than likely superior to 6 months (O. Andreoletti, unpublished information).

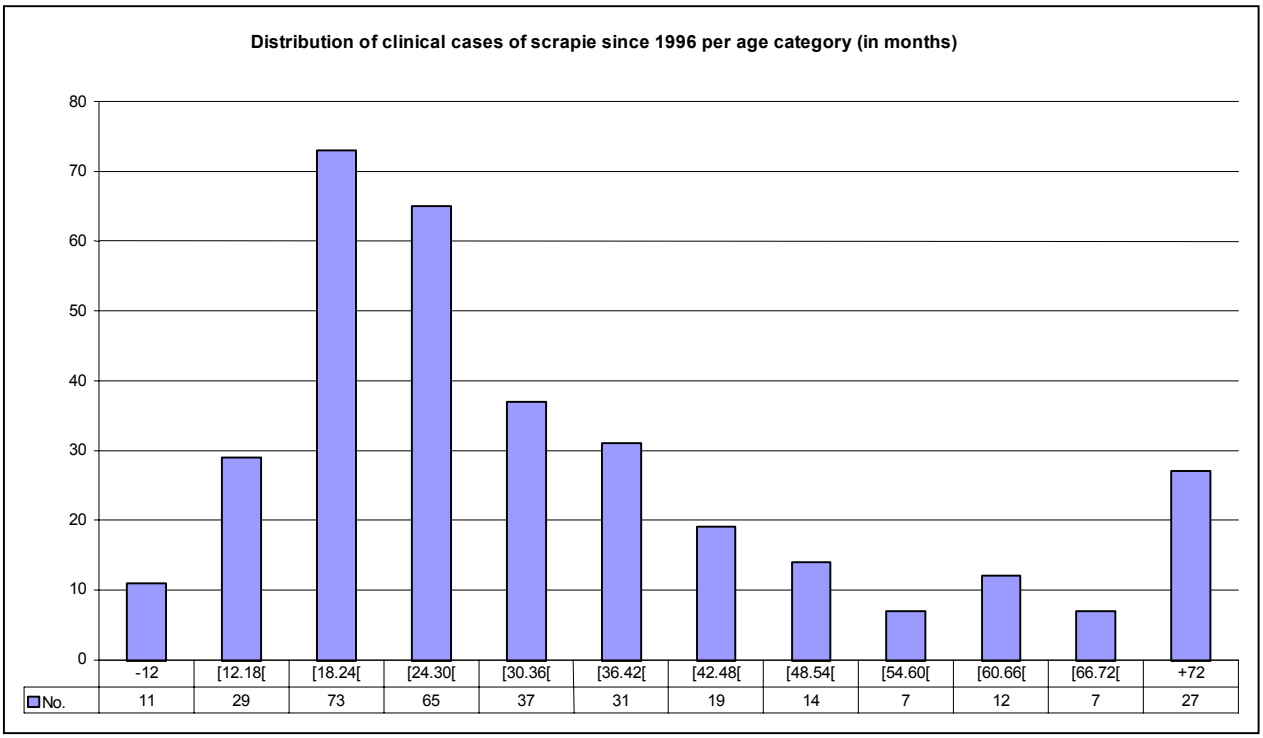
This data indicates that on a theoretical basis, the BSE agent could be detected in infected animals under 18 months. However, it also reveals the pointlessness of a PrPSc detection test on central nervous system samples for animals under 6 months.

Epidemiological considerations

Under natural conditions, the kinetic spreading of the scrapie infectious agent varies according to several parameters (strains, animal genotype). It is therefore difficult to estimate the prevalence of scrapie in animals under 18 months. Data from clinical surveillance since 1996 shows a significant number of cases in animals under 18 months. Cf. graph below.

[15] Bellworthy et al, Vet Rec 2005:

[16] Andréoletti et al J. Gen Virol. 2006



The application of data from analysis on young animals in stamping out policy may allow us to estimate the prevalence of age-related scrapie in infected flocks.

In this way testing younger animals than those currently tested (from 18 months) within an active surveillance programme, considering that infection mainly occurs in younger subjects, would contribute to the detection of more cases and earlier detection in flocks recently infected by a TSE. However, the inclusion of such a population in which the probability of positive detection is considerably low due to the kinetic accumulation of PrPres, would lead to a significant increase in the cost of the programme. Furthermore, in the detection of BSE cases, it is likely that the risk of direct contamination of sheep flocks through feed is nowadays residual (in particular given the interdiction of bone and meat meal (BMM) and certain fats in November 2000). If BSE is currently present in sheep livestock, we can assume that the flocks concerned were for the most part contaminated either through feed before 2000, or through secondary contamination from flocks already contaminated through feed. Under these conditions, and taking into account the characteristics of the rapid tests currently used (analysis on a central nervous system sample), it seems reasonable to limit the programme to animals over 18 months.

Sixth measure - Analysis of lymphoid tissue samples?

The possible implementation of analysis on pooled samples was analysed by the Afssa TSE Committee in its opinion of 25 March 2005[17]. The following points issue from this:

" *– the mixture of brain samples is possible but with a slight loss in sensitivity in detecting infected animals. Where lymphoid tissue samples are concerned, pooling does not seem possible without a great loss in efficacy. Pooling brain and lymphoid tissue samples from a single animal is a hypothesis to be studied,*

[17] Afssa opinion on the risk assessment for TSEs in small ruminants, the strengths and weaknesses of the current scheme and possibilities for development – Opinion of December 2001 updated in March 2005.

- pooling should affect the detection of atypical cases to a greater extent as they are often associated with lower concentrations of PrPres,
- due to expenses which cannot be pared down (sampling, transport and extraction before the test is carried out) and which represent more than 50% of the total cost of tests, the savings made only correspond to a fraction of total costs".

For a move towards exhaustive detection of TSE sources in the sheep population, we cannot recommend pooling brain or tissue samples from several animals. However analysis of brain and lymphoid tissue samples from a single animal deserves to be explored. It would be fitting to evaluate the loss in sensitivity of such an analysis in the case of BSE and atypical scrapie. It would also be fitting to study the practical aspects of the implementation of such an analysis (in particular in sample homogenisation) and if it was appropriate to propose a protocol to this end for first line laboratories.

Combining the analysis of the obex and lymphoid tissue for a single animal may allow for the identification of cases in which PrPres presence in the central nervous system is weak (animals in the incubation phase, preceding neuroinvasion for instance). The expected advantage of such a scheme would be the early detection of sources. However this would only concern TSE isolates of which the infectious agent is disseminated in the lymphoid organs: the case of the BSE strain for instance but not atypical scrapie.

Seventh step – Flock register for TSE in sheep?

The tools implemented for improving detection of TSE in the sheep population may serve as a basis for a flock qualification scheme for BSE and classical scrapie. However, in view of current knowledge, we cannot comment on the pertinence or the interest of such a scheme with regards to atypical scrapie. If, in the end, it was a question of an illness arising without a controllable exposure factor and one that affects all genotypes, which seems to be the case, a flock qualification scheme would be impossible to set up.

As for any such scheme it must concentrate on obtaining TSE-free status but also on regaining this status should it be lost.

Results from tests carried out at the rendering plant and at the abattoir could serve as the basis for a qualification scheme, where animals of the sheep population concerned are identified and the different routes of evasion correctly documented. An analogue approach to that proposed by the EU for registering country BSE status could be used. We can also note that Australian studies which under stretch this approach concentrate on the flock and not the country[18]. This approach must be improved in order to take into account the information available on the genetic profile of the farm concerned. It remains that the conception and validation of such a qualification scheme (notably for taking into account genetic profile) is not the work of the animal TSE epidemiological working group.

Such a scheme of qualification must also ensure the control of registered livestock fallen prey to re-exposure.

[18] Cannon RM (2002). Demonstrating disease freedom–combining confidence levels. Preventive Veterinary Medicine, 52 (3-4): 227-249)

In all events, a move towards a flock qualification scheme must take into account the diversity of sheep farming, for which the risk of TSE, and the practical difficulties linked to the implementation of such a move, varies according to breed, region, breeding practices, etc. The working group has given some thought on these aspects (cf. Annexe 3) which may reveal useful on the subject.

Several factors may limit the possibility of maintaining such a scheme:

- the introduction of and contact between sheep from populations of different statuses;
- the factors encouraging prion dissemination from one farm to another (placenta transport by dogs for instance);
- TSE-specific factors (risk from feed, if it still exists, through contaminated fodder or feed storage premises);
- and other difficulties linked to farm sanitary control (auditing of causes of death, control evasion at the point of slaughter, problems in register follow-up…).

Where status is lost, it must be underlined that one of the main factors limiting status recovery for the farms concerned is the risk that the prion may persist in the environment. TSE pathogeny in sheep encourages the dissemination of the prion to the outside and any farm having met with scrapie may be susceptible to re-infection from the environment.

It will also be necessary to weigh up all of these factors limiting TSE registration and according to the genetic profile of sheep flocks.

Finally, given the pathogeny of TSE in the sheep species and its wide strain variability, "carcase" certification for those destined for the food chain and a combination test on nervous system and lymphoid tissue, would not ensure a high level of protection for the consumer, as it did for BSE in bovines. Such a system could not in any case replace those detection and control measures for flocks previously described.

In summary, the current exhaustive and active surveillance programme provides the basis for the implementation of a flock register based on the results of tests carried out at the rendering plant and at the abattoir. The reliable identification of animals/flocks must be added to this scheme. It is also necessary to implement steps for controlling flock re-exposure. For a given flock, the level of insurance provided by a flock qualification scheme, according to the number of tests carried out and taking into account the genetic structure of the flock, remains to be estimated. It is also advisable to estimate from which level of genetic resistance to BSE and classical scrapie the circulation of TSEs becomes impossible, and testing, in turn, pointless. These studies are currently being developed in a research project (RNA sustainable development programme, ACDUQ project). One of the first repercussions of the project will be to identify and document the missing parameters and data to then develop the model, either through the implementation of experimental studies or the collection of information on flocks. Lastly, such a procedure would raise the public health safety level and at the same time progressively relieve the French sheep flock surveillance scheme.

Appendix 1 : Active surveillance in small ruminants since 2002 : distribution of tests and cases (classical or atypical) per year, species, programme and type of test

			2002					2003					2004					2005					1st quarter 2006			
Species	Scheme	Test	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical
Goats	Abattoir	CHEMIO_ENFER											2					100					9			
		IDEXX																					6			
		LIA_PRIONICS						1										2826					1056			
		WB_PRIONICS	13233					9008					14					81165					25752			
		EL_SD_BIORAD	1651	2	2			1986	3	2		1	2													
		TESEE_BIORAD						229					57					16406	2		2		4768			
Goats	Pt of slaug	CHEMIO_ENFER											43					139					90			
		IDEXX																					227			
		LIA_PRIONICS						39					313					3357					2521			
		WB_PRIONICS	10786					8603					1950					17547					7046			
		EL_SD_BIORAD	1569	13	8	1	4	2447	6	4		1	485	3	1	2										
		TESEE_BIORAD						1068					2909					26941	13	9	3	1	12656	3	2	1
Goats	Policy	LIA_PRIONICS											164					38					209			
		WB_PRIONICS	1340										21					210					11			
		EL_SD_BIORAD						1157					1													
		TESEE_BIORAD						4					1186		8			680		20			33			
Goats	Clinique	EL_SD_BIORAD						1					1		2											
		TESEE_BIORAD											4					3		1						
Goats	CSO	NC											NC			1										
Species	Scheme	Test	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical	NA	No. samples	No. Cases	No. Classical	No. Atypical
Sheep	Abattoir	CHEMIO_ENFER											21					162					36			
		IDEXX																					35			
		LIA_PRIONICS						193					1160					1064					187			
		PRIOSTRIP																3								
		WB_PRIONICS	19081					18646					2852					3413					976			
		EL_SD_BIORAD	14699	31	16	10	3	22665	45	19	21	2	214	15	7	7										
		TESEE_BIORAD						2970					8211					7566	12	3	9		1896	3	1	2
Sheep	Pt of slaug	CHEMIO_ENFER											120					222					1047			
		IDEXX																					2738			
		LIA_PRIONICS						274					1803					5784					21347			
		WB_PRIONICS	13691					13146					5178					8133					36967			
		EL_SD_BIORAD	3883	121	104	3	12	4981	33	22	7	4	645	22	20	2										
		TESEE_BIORAD						671					4583					8057	38	32	5		33380	93	66	24
Sheep	Policy	CHEMIO_ENFER																					6			
		IDEXX																					19			
		LIA_PRIONICS						205					922					2330					333			
		WB_PRIONICS	3217					12361					5009					3377					539			
		EL_SD_BIORAD	292					3445					19					1								
		TESEE_BIORAD						316					4311		243			4105		303	2		1258		13	
Sheep	Clinic	LIA_PRIONICS											3					5					1			
		WB_PRIONICS	90					36					10					5					1			
		EL_SD_BIORAD	1					14										1								
		TESEE_BIORAD						2					13		13			33		23			15		9	1
Ovins	OSC	NC						NC			1		NC			3										
TOTAL						14					29				294	15				391	21				91	28
																							TOTAL ATYPICAL			107

APPENDIX 2

PROTOCOL PROJECT FOR THE REMOVAL OF THE BRAIN ISTHMUS IN SMALL RUMINANTS FOR TSE DETECTION

1- Preliminary definitions

The brain isthmus, also called the brain stem, is an extension of the spinal cord. It rests on the base of the skull and is surmounted by the cerebral hemispheres and the cerebellum. It is made up of the medulla oblongata, the pons and cerebral peduncles of the mesencephalon.

The obex is situated where the caudal medullar meets the brain stem, on the separation of the fasciculus gracilis. It is a thin, triangular grey lamina linking the two restiform bodies.

2- Aims

In small ruminants, screening for scrapie, in its classical and atypical forms, as well as for sheep BSE, forms different or collective aims which can be attained through the use of separate brain material samples (SMR) or combined samples according to the first line screening tests used.

Two sampling protocols can be proposed:
- brain stem protocol (as carried out to date);
- brain stem and cerebellum protocol.

3- In practice

3.1- Preliminary comments

1. At the rendering plant sampling must be carried out by a team of sanitary veterinaries in order to ensure sampling regularity and continuity.

2. At the abattoir, this can be carried out by veterinary service agents under the responsibility of the veterinary inspector.

3. At the rendering plant a carcase identification procedure must be implemented imperatively. The knacker would place a ring marked with a number on the carcase on collection. This number would be noted on the manufacturer's pre-printed pick-up form. Finally, a copy of this form will be given to the veterinary sampler.

4. Atlanto-occipital disarticulation will be carried out at the rendering plant but the head must remain attached to the carcase in order to determine the sex and the breed of the animal. If the atlas remains on the cephalic side, its removal is necessary before proceeding with sampling on the foramen magnum.

3.2- Brain stem sampling methods in small ruminants

These have been adapted from sampling methods on bovines but anatomical differences require certain adjustments.

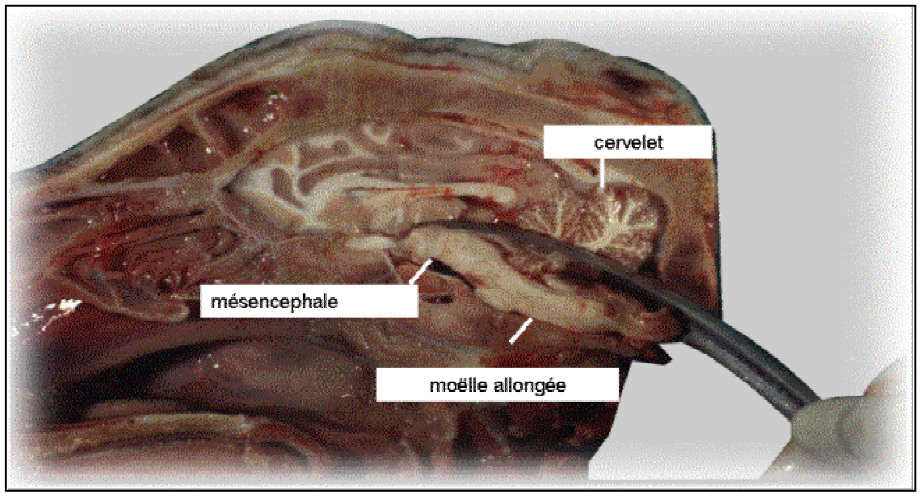
The head is placed on the nose and forehead. The foramen magnum is clearly visible with the end of the brain stem at its centre. The dura mater is clearly visible. Whitish in colour and tough, it is separated from the medulla by the subarachnoid space. The pia-mater is attached to the medulla. The subarachnoid space in small ruminants is larger on the ventral wall, compared to in bovines.

The removal spoon for sampling on small ruminants should be inserted on the ventral side of the brain stem with the back of the spoon turned upwards at an angle of about 60°. Gentle rotation movements will sever the cranial nerve endings and the cerebellum peduncles on the dorsal side of the stem, allowing for the extraction of the analysis material.

3.3- Brain stem and cerebellum material sampling methods

The technique described by a Norwegian team differs from that mentioned previously in the positioning of the animal's head (lying on its jowl), in the material used (a more curved spoon) and the insertion of the spoon on the dorsal side of the medullar. In these conditions a small amount of cerebellum can be obtained. (cf. photo below).

(Metal spoon designed at the National Veterinary Institute of Oslo, Norway, Bjørn Bratberg)



An adjustment could be proposed. A fairly narrow (grooved), curved spoon with moderately sharp edges, and more supple than that used until now, would allow for work to be carried out in the usual conditions (that is to say via ventral penetration). Once inserted at the base of the stem, the spoon is readjusted to a vertical position (gently in relation to spoon suppleness), in a rotating movement, allowing for extraction of the cerebellum material in the grooves.

Trials could be carried out according to the various hypotheses, but we must remember that assessment conditions must come as close as possible to those observed in everyday sampling practice. The main reason for this is that the state of preservation of small ruminant carcases does not always allow for complete differentiation of the macroscopic elements in the diagnosis of the extracted anatomical parts.

4- Conclusion

The projected aims would govern the sampling technique for the brain isthmus in small ruminants. Exclusive detection of scrapie and BSE does not require any particular adjustment with regards to the measures already in place.
Screening for atypical cases of scrapie lies either in the use of a single test on the brain stem or the use of certified tests on the cerebellum and an additional test on the brain stem, taking into

account that sampling conditions at the rendering plant do not guarantee efficient differentiation of the required anatomical parts.

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APPENDIX 3

FACTORS LIMITING THE MAINTENANCE OF A SCRAPIE QUALIFICATION SCHEME

This approach concerns classical scrapie and sheep BSE if it were to be detected. The factors are based on epidemiological knowledge of sheep TSE and on the current contribution of genetics concerning sheep susceptibility to classical scrapie.

1- General factors limiting the maintenance of such a scheme

1-1 Introduction of and contact between animals

Any introduction of animals originating from non-qualified flocks (the main risk of contamination lies in the introduction of potential excretor sheep especially at lambing times). Possible excretion by faecal route must also be taken into consideration even if it has not been officially demonstrated, which implies the surveillance of ram movement.
This may concern purchase of females (renewal or increase of livestock), purchase or loan of rams, chance meetings (such as in fairs or markets), mixing with other livestock of unknown status, organised grouping (transhumance). In this last case flock register may complicate transhumance management if other diseases were already involved.

1-2 Difficulties linked to sanitary precautions

Hygiene and safety measures of the qualified flock with regards to: stray dogs (transport of the placenta or sick lambs from non registered farms), slaughterhouse truck movement, animal transport inducing the regulation and enforcement of correct storage methods for carcases and of the implementation of a disinfectant mat.

1-3 Difficulties linked to the sanitary control of flocks

- Precise auditing of the number deaths and the causes of death and culling is currently impossible. This requires i) an "official" inventory of all the animals present at the beginning of the campaign; ii) exhaustive recording of deaths currently foreseen in the farm register based on receipts from the rendering plant; iii) recording of the cause of death. Nowadays this is rarely carried out (breeders rightly arguing that they do not know and may make a mistake).

  These problems could be overcome if controls were carried out on a significant number of dead and slaughtered sheep.

- Control evasion at the point of slaughter: low mortality, vultures, badly covered zone, identification problems, end of week, difficult access, disposal, etc.

- Flock qualification follow-up: veterinaries are rarely present in sheep farms and brucellosis controls are only carried out every three years in meat production. The technical supervision of sheep farms (very precious as it is regular and based on breeder-technician trust) is reducing due to lack of funds.

2- Scrapie/TSE specific factors limiting the scheme: *the risk from feed*

Does the risk of exposure through feed really exist given the measures in place?

Following the interdiction of bone meal for all ruminants in 2000 in Europe, residual contamination may be a result of contamination of storage premises (storage bodies, factories and farms), from the initial contamination of fodder originating in contaminated farms (we know that the prion can persist in soil for a considerable amount of time, and this hypothesis was touched on to try to explain the recontamination of farms after eradication of the disease in Iceland).

Genetic structure of the breed

Work on genetics began in 2001 within the national genetic improvement programme for resistance to scrapie. The first part of the programme concerns farms undergoing selection and serves as approval of rams for insemination.
This national programme is reinforced by other genetic selection programmes within the regions and departments. One of the aims of these programmes is to be able to produce slaughter lambs by using only resistant rams. Work on genetics is carried out on a voluntary basis. This programme implies that the breeder must make a commitment to type his ram stock and eliminate those considered susceptible and to keep and buy only resistant types.

The application of the health policy in scrapie-infected farms would also allow for the genotyping of all adult animals in the population and thus eliminate the susceptible animals.
It is difficult to foresee that acquiring flock qualification does not take into account the national programme and European directives.

Previously, on 12 October 2005, a circular from the IEPD, based on appendix II of the decision of 13 February 2003 (2003/498/CE) specified the access methods for breeders to zootechnical certification for resistance to scrapie and this on three levels:

I. populations composed entirely of ARR/ARR,
II. populations whose progeny comes exclusively from ARR/ARR rams,
III. populations whose progeny comes exclusively from ARR/XXX rams (excluding VRQ),

It would also be interesting to assess the progression of the genotyping selection programme breed per breed.

The flock qualification scheme would require controllable and precise knowledge for each farm from the date on which only resistant rams were used (fights and inseminations) for renewing livestock and lamb sale. Maintaining such a scheme could only take into account ram renewal and insemination follow-up (and the commitment to renew only with ARR/ARR males and at least ARR/XXX females).