AGENCE FRANÇAISE
DE SECURITE SANITAIRE
DES ALIMENTS

The General Manager

Afssa – Referral no. 2006-SA-0099

Maisons-Alfort, 18 août 2006

OPINION
from the French Food Standards Agency
on risk assessment linked to the possible presence of BSE in sheep

In a letter dated 15 March 2006, the French Food Standards Agency received a request from the Department of Trade and Industry, the Department of Health and the Department of Environment, Food and Rural Affairs to assess the risk linked to the possible presence of BSE in sheep.

I- Context

This request follows the detection of two cases of transmissible spongiform encephalopathy(TSE) in sheep (case 05-825 and case 06-017) after a first line BSE/scrapie discrimination test showing a non-distinct profile (case 06-017) or a subtly distinct profile (case 05-825) from that found in sheep experimentally infected with BSE . A first opinion from the Agency on this subject was delivered on 1 March 2006.[1]

As part of the additional risk management strategy to be considered should these cases be confirmed, the Agency was asked in particular to:

- assess the relevance of the classification of the entire intestine as specified risk material (SRM)for animals born before 2002;
- update the risk assessment relating to milk produced by sheep;
- evaluate the different screening programmes in abattoirs for small ruminants under 18 months.

In a letter dated 27 March 2006, Afssa requested that the TSE scientific steering committee (SSC) and its epidemiological Working Group on Animal TSEs:

(i). Classify the expected benefits of the various measures relating to SRM (intestine) removal, screening programmes, milk collection in affected livestock, genetic selection, flock register schemes;
(ii). Propose, if necessary, a sampling scheme for the surveillance of TSEs in sheep under 18 months;
(iii). Propose recommendations on the rapid TSE test to use and the type of sample to be analysed;
(iv). Estimate the potential human exposure risk to the sheep BSE agent.

II- Expertise

The TSE SSC delivered the following opinion on 11 July 2006:

"The present opinion mainly relies on the animal TSEs Epidemiological Working Group report, enclosed in appendix I.

1. ***Where the expected benefits of consumer protection, surveillance and control of sheep TSEs measures are concerned:***

   a. *To include sheep intestines, regardless of animal age, in the list of SRM*

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[1] Avis de l'Afssa sur l'évaluation des risques liés à des cas d'EST atypiques dans l'espèce ovine en date du 1er mars 2006. *Afssa's opinion on the risk assessment of atypical TSE cases in the ovine species dated 1 March 2006.*

The matter of the risk associated with sheep intestines must be analysed taking into account (i) the early presence of PrPres in the intestine of infected animals, (ii) the effect of deliming on intestine infectivity and (iii). The modes of transmission of scrapie

(i) Manifestation of PrPres in the intestine:
The data previously mentioned by the Committee show the presence of PrPres in gastrointestinal tract inherent lymphoid tissue 21 days after oral contamination (60 days for tonsils[2]), in gastrointestinal tract inherent nerve tissue between 3 and 4 months after oral contamination and in the brain from 10 months[3]. Tests carried out on the brain stem do not allow the detection of recently infected animals carrying pathological PrP in the intestinal lining.
Taking into account the replication precocity of the agent in the intestine, rapid tests on the tonsils would not determine exclusion of the entire infected intestine from the food chain.
Furthermore, according to M Jeffrey[4] ARR/ARR animal intestines are able to absorb PrPres from an inoculum during experimental contamination. It is therefore not conceivable to use animal genotype as a basis for including intestine removal in the SRM list.

(ii) Effect of deliming:
Studies already referred to by the Committee[5] [6] have indicated that deliming of the intestines does not fully eliminate the PrPres present (and therefore infectivity) and it appears virtually impossible to quantify the residual amounts.
The work by Koolmees[7] has only studied the evolution of gastrointestinal tract inherent lymphoid tissue during this process. The presence of PrPres has not otherwise been evaluated in this study. Moreover, the author indicates that research on the plexus has not been carried out and that most probably submucosal plexi remain. Several studies confirm the accumulation of pathological PrP in gastrointestinal tract inherent autonomous nervous structures (enteric and myenteric plexi)[8] [9].
As a result, deliming cannot be considered as a technique capable of eliminating structures that may contain pathological PrP

(iii) Scrapie - the modes of transmission:
In sheep, classical scrapie, but also BSE (experimental flock), is the result of horizontal/maternal transmission[10] (we should note that in atypical scrapie, there is no data to support horizontal or vertical transmission).
Even if must also be considered as a potential contamination factor for sheep flocks[11], this route, contrary to bovine BSE, cannot be considered predominant in the transmission of TSEs in small ruminants.
Also, animals born from 2002 cannot be considered less exposed to the BSE agent.
As a result the Committee maintains its recommendation to remove the intestines regardless of animal age and genotype.

[2] Andreoletti O, Berthon P, Marc D, Sarradin P, Grosclaude J, van Keulen L, Schelcher F, Elsen JM, Lantier F. Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie J Gen Virol. 2000 Dec;81(Pt 12):3115-26.
[3] Avis de l'Afssa concernant le dépistage rapide des ESST chez les petits ruminants en date du 19 mai 2003. *Afssa opinion on the rapid detection of TSE in small ruminants dated 19 May 2003.*
[4] Jeffrey M, Gonzalez L, Espenes A, Press CM, Martin S, Chaplin M, Davis L, Landsverk T, MacAldowie C, Eaton S, McGovern G. Transportation of prion protein across the intestinal mucosa of scrapie-susceptible and scrapie-resistant sheep J Pathol. 2006 May;209(1):4-14.
[5] Avis de l'Agence française de sécurité sanitaire des aliments concernant l'évaluation des effets de la technique du délimonage appliquée aux intestins d'ovins au regard du risque d'encéphalopathies spongiformes subaiguës transmissibles en date du 8 novembre 2001. *Afssa opinion on the assessment of the deliming technique used on ovine intestines with regards to TSE risk dated 8 November 2001.*
[6] Données actualisées relatives au protocole « délimonage » chez les ovins - Etude du laboratoire de l'Afssa, Lyon en date du 21 novembre 2002. *Updated data relating to the "deliming" protocol in ovines – Afssa Laboratory Study, Lyon, dated 21 November 2002.*
[7] Koolmees PA, Tersteeg MH, Keizer G, van den Broek J, Bradley R. Comparative histological studies of mechanically versus manually processed sheep intestines used to make natural sausage casings. J Food Prot. 2004 Dec;67(12):2747-55.
[8] van Keulen LJ, Schreuder BE, Vromans ME, Langeveld JP, Smits MA. Scrapie-associated prion protein in the gastrointestinal tract of sheep with natural scrapie. J Comp Pathol 1999 Jul;121(1):55-63.
[9] Andreoletti O, Berthon P, Marc D, Sarradin P, Grosclaude J, van Keulen L, Schelcher F, Elsen JM, Lantier F. Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. J Gen Virol. 2000 Dec;81(Pt 12):3115-26.
[10] Bellworthy SJ, Dexter G, Stack M, Chaplin M, Hawkins SA, Simmons MM, Jeffrey M, Martin S, Gonzalez L, Hill P. Natural transmission of BSE between sheep within an experimental flock. Vet Rec. 2005 Aug 13;157(7):206.
[11] Philippe S, Ducrot C, Roy P, Remontet L, Jarrige N, Calavas D. Sheep feed and scrapie, France. Emerg Infect Dis. 2005 Aug;11(8):1274-9.

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Furthermore, the Committee reiterates that in BSE-infected small ruminants, the removal of SRM as currently defined, as well as the removal of intestines, does not guarantee the absence of infected material in the foodstuff from this animal.
The Committee therefore considers that where TSEs in small ruminants are concerned, the simple removal of SRM does not provide the same level of guarantee as for bovines.

b. To improve screening programmes

Current surveillance is based essentially on three programmes:

(i). passive surveillance (clinical),
(ii). testing on fallen stock for animals over 18 months (through probing between 2002 and 2005, systematic screening as of November 2005, and effective since the beginning 2006) and at the abattoir (through probing between 2002 and 2006, systematic screening as of March 2006, and not effective to date),
(iii). in a more marginal way through the official sanitary control which is not the main objective of the latter.

The Committee reiterates that unlike BSE surveillance in bovines, which is based on individual animal status characterization in turn governing carcass market authorization, the objective of the surveillance of sheep remains identifying affected flocks. Furthermore, in the current use of rapid tests (tests carried out on a sample from the central nervous system), a negative result does not guarantee the non-infectious status of the animal.

Extending the screening programme to animals under 18 months at the abattoir would help detect those animals in the early stages of the illness, between 12 and 18 months (see appendix I: epidemiological working group report and appendix II concerning EC evaluation of rapid tests for small ruminants). However, in the current context of rapid tests (based on the analysis of a brain tissue sample), extending the screening programme to animals under 18 months would have a limited range. In fact, the percentage of potentially positive animals (where the obex is concerned) in this age range is certainly lower than for the currently analysed population.
The implementation of tests on young animals would only make sense if they were carried out on lymphoid tissue samples (see below), as it would then be possible to significantly increase detection of animals in the early stages of infection.

For this reason the Committee considers that it is fitting to improve on the current system before planning any additional measure of monitoring.

In fact, the Committee considers that there are several factors limiting the efficacy of the currently applied programmes:
The lack of reliable, individual identification of small ruminants allowing each animal tested to be associated with the flock(s) from which it originates seems, in the Committee's point of view, to be one of the major problems. Significant irregularities linked to animals otherwise eligible for but escaping screening were revealed by the analysis of available data from previous surveillance schemes (see appendix II of the epidemiological working group report, included with this opinion).

The Committee therefore recommends:

(i). the rapid implementation of continual individual animal identification, an essential prerequisite to flock status surveillance in relation to sheep TSEs;
(ii). improvement in animal exit route controls (implicating once again reliable identification of all animals).

Furthermore, the Committee considers it necessary to submit all positively tested animals, including secondary cases identified in an affected flock, to the BSE/scrapie discrimination test. It cannot be excluded that BSE and scrapie may circulate simultaneously in the same flock.

This step will allow:

(i). to increase i the efficacy of screening for possible BSE cases;
(ii). to document the variability of strains circulating in a single flock.

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c. *To suspend milk collection in a flock as soon as a TSE is suspected and while waiting for the result of the discrimination test.*

The issue of the potential infectivity of milk from sheep affected by TSE has been addressed in the opinion of March 2005. To date, no infectivity in milk or milk products from sheep infected by TSEs has been reported. However pathological PrP was detected in the mammary parenchyma of a ewe, simultaneously infected by scrapie and nodular lymphocyte chronic mastitis, brought on by the Maedi-Visna virus[12]. Studies aiming to assess the presence of PrPres in the milk of small ruminants are underway and may generate data in one or two years
While waiting for these results, the Committee considers that the risk related to the possible presence of an infectious agent in milk cannot be officially excluded, but remains low.
As a result, the Committee does not recommend suspending milk collection in farms affected by a TSE. Furthermore, due to the uncertainties concerning BSE/scrapie discrimination test sensitivity and specificity, on which the Committee has expressed its reservations many times, there is no question of basing this suspension on the result of a discrimination test.

d. *To continue with genetic selection.*

Genetic selection, aiming in particular to increase the frequency of ARR allele in sheep livestock, was initially developed on the basis of field observation showing strong or even total resistance of the animals carrying this allele, in particular in the homozygote state, to classical scrapie[13;14 ;15 ;16 ;17 ;18]. One of the aims of this programme is to contribute to the consumer protection by reducing the risk of exposure to sheep TSEs. The question of continuing with this programme must take into account the diversity of sheep TSEs: classical scrapie, atypical scrapie and BSE.

(i) Where classical scrapie is concerned, the introduction of the ARR allele remains a very efficient tool in increasing sheep flock resistance to infection, and can only have a positive effect on controlling this type of scrapie;

(ii) Where ovine BSE is concerned, current available experimental data suggests that ARR homozygotous or heterozygotous animals have an extremely low susceptibility to oral exposure compared to animals carrying an ARQ or AHQ allele. ARR/ARR animal resistance to BSE is not however total, as the agent may be passed on via intracerebral transmission during as the agent can be experimentally transmitted via intracerebral inoculation [19]. Finally, recent studies showed an accumulation of PrPres in the spleen of a massively and orally exposed animal to a sheep BSE isolate[20]. Taking these elements into account, the impact of the increase in ARR allele frequency within a genetic selection scheme seems to have a positive impact on the human exposure risk linked to the possible presence of BSE in sheep.

(iii) Finally, data relating to genetic determinism of atypical scrapie-affected sheep, of which the identification is extremely recent, remains limited. The highest incidence is observed in animals carrying AHQ and $AF_{141}RQ$ alleles, either in the homozygote state or in combination with any

[12] Ligios C, Sigurdson CJ, Santucciu C, Carcassola G, Manco G, Basagni M, Maestrale C, Cancedda MG, Madau L, Aguzzi A. PrPSc in mammary glands of sheep affected by scrapie and mastitis. Nat Med. 2005 Nov;11(11):1137-8. .
[13] Elsen JM, Amigues Y, Schelcher F, Ducrocq V, Andreoletti O, Eychenne F, Khang JV, Poivey JP, Lantier F, Laplanche JL. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov Arch Virol. 1999;144(3):431-45.
[14] Hunter N, Moore L, Hosie BD, Dingwall WS, Greig A. Association between natural scrapie and PrP genotype in a flock of Suffolk sheep in Scotland. Vet Rec. 1997 Jan 18;140(3):59-63.
[15] Thorgeirsdottir S, Sigurdarson S, Thorisson HM, Georgsson G, Palsdottir A. PrP gene polymorphism and natural scrapie in Icelandic sheep. J Gen Virol. 1999 Sep;80(Pt 9):2527-34.
[16] Tranulis.MA, OsLand A, Bratberg B, Ulvund MJ Prion protein gene polymorphisms in sheep with natural scrapie and healthy controls in Norway.J Gen Virol. 1999 Sep;80(Pt 4):1073-7.
[17] Acin C ; Martin-Buriel I , Goldmann W, Lyahyai J, Monzon M, Bolea R, Smith A, Rodellar C, Badiola JJ, Zaragoza P. Prion protein gene polymorphisms in healthy and scrapie-affected Spanish sheep.J Gen Virol. 2004 Jul;85(Pt 7):2103-10.
[18] Billinis C, Psychas V, Leontides L, Spyrou V, Argyroudis S, Vlemmas I, Leontides S, Sklaviadis T, Papadopoulos O. Prion protein gene polymorphisms in healthy and scrapie-affected sheep in Greece. J Gen Virol. 2004 Feb;85(Pt 2):547-54.
[19] Houston F, Goldmann W, Chong A, Jeffrey M, Gonzalez L, Foster J, Parnham D, Hunter N. Prion diseases: BSE in sheep bred for resistance to infection. Nature. 2003 May 29;423(6939):498.
[20] Andreoletti O, Morel N, Lacroux C, Rouillon V, Barc C, Tabouret G, Sarradin P, Berthon P, Bernardet P, Mathey J, Lugan S, Costes P, Corbiere F, Espinosa JC, Torres JM, Grassi J, Schelcher F, Lantier F. Bovine spongiform encephalopathy agent in spleen from an ARR/ARR orally exposed sheep J Gen Virol. 2006 Sep;80(Pt 4):1043-6.

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other allele. The ARR allele susceptibility seems to be similar to that of the $AL_{141}RQ$ and VRQ alleles. In terms of human exposure, there is no data currently available for assessing the risk related to atypical scrapie. Given the available but rare data on genetics it is not possible to estimate the impact of genetic selection in atypical scrapie. However, selection does lead to the elimination of AHQ and $AF_{141}RQ$ alleles, which in atypical scrapie is positive. Selection of the ARR allele does not induce additional risk.

In conclusion, in the context where atypical scrapie, classical scrapie and BSE should co-exist, the Committee remains in favour of the continuation of genetic selection.
Moreover, it is recommended that:

(i). genotype testing carried out within the health policy and "National scheme for genetic resistance improvement" regulatory frameworks be extended to the PrP gene codon 141;
(ii). all identified cases of scrapie are subjected to complete sequencing of the PrP exon 3 gene. This last step will identify in a proactive manner any mutations linked to particular susceptibility or resistance to the various scrapie strains, including those which may emerge in the future.

e. To move towards a scheme of qualification

The implementation of a reliable individual animal traceability and identification scheme constitutes an essential prerequisite to the establishment of a flock register scheme.
The current exhaustive and active surveillance programme (results of tests carried out in quartering and in the abattoir) as well as genetic selection, provide the basis for flock registration where BSE and classical scrapie are concerned. On the other hand, in view of the current state of knowledge and the uncertainties that remain, it seems difficult to define an appropriate scheme of qualification for atypical scrapie. If, in the end, it was a question of an arising illness without a controllable exposure factor that would affect all genotypes, which seems to be the case, a flock scheme of qualification would be impossible to set up.

For a given flock, the level of insurance provided by a flock scheme of qualification , according to the number of tests carried out and taking into account the genetic structure of the flock, should be estimated. It is also fitting to estimate from which level of genetic resistance to BSE and classical scrapie the circulation of TSEs becomes impossible, and testing, in turn, pointless. A flock scheme of qualification would allow the access to data such as the precise evaluation of current TSE incidence in affected flocks and their genetic structure.
Lastly, such a procedure would raise the public health safety level and at the same time progressively relieve French sheep flock surveillance. However, appropriate control measures must be taken in order to ensure that the framework is maintained (in controlling flock re-exposure).

Where prioritising these measure is concerned:

In the short-term, the Committee does not recommend suspending milk collection in farms affected by scrapie, or the implementation of testing on sheep under 18 months. It reiterates its recommendation concerning the removal of intestines, regardless of animal age, and recommends maintaining genetic selection.

In the long-term, the Committee believes that the main purpose is the implementation of a scheme of qualification. This purpose can only be achieved if reliable animal traceability and exhaustive surveillance exist (elimination of animals escaping screening). The quality of active surveillance remains governed by those tests capable of detecting all forms of scrapie with the highest analytical sensitivity possible. Testing, on both samples from tissue from the central nervous system (brain stem) and lymphoid tissue (mesenteric lymph nodes) samples, may increase the efficiency of active surveillance by detecting certain forms of TSE earlier. Furthermore, the implementation of a scheme of qualification implies specific modelling integrating flock genetic structure, which cannot be carried out by the TSE SSC alone.

2. *In the case of the sampling scheme using probing at the abattoir for animals under 18 months:*

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As pointed out in 1b, the Committee does not recommend the implementation of screening tests on the obex in animals under 18 months. However if the health authorities wish to implement such a scheme in order to obtain individual carcase identification[21], only systematic screening would increase consumer safety. In any case, tests on the obex must only apply to animals over 6 months. Below this age, detection of PrPres in the central nervous system is highly unlikely.

3. *In the case of tests and screening methods to be implemented:*

a. Analysis of central nervous system samples:

The obex is the anatomic region for the detection of BSE. Atypical scrapie is detected with "satisfactory" sensitivity by several tests on cortex or cerebellum samples (in practice only the cortex can be removed in routine slaughtering or abattoir conditions) but few tests detect the illness on an obex sample (see appendix II of the epidemiological working group report).

Under these conditions, the TSE SSC recommends using tests of the highest analytical sensitivity on the brain stem, for both BSE and atypical scrapie. That is to say the TeSeE Sheep & Goats Biorad and IDEXX tests (AESA assessment of 17 May and 26 September 2006). However, the Committee underlines that recent results indicate that test sensitivity is below that of bioassays on genetically modified mice, over expressing sheep PrP. In other terms, a negative rapid test for atypical samples does not guarantee the absence of infectivity in the tissue tested.

b. Analysis of lymphoid tissue samples:

In active surveillance programmes at the abattoir and during quartering, combining the analysis of a mixture of an obex and lymphoid tissue sample for a single animal is foreseeable, but subject to a feasibility evaluation technique (notably tissue homogenisation conditions). In theory, the association of the two types of samples may help better identify infected animals in which no PrPres accumulation is detectable in the central nervous system (animal in the early incubation stage). The expected advantages of such a scheme would be the early detection of infected flocks. However This would only concern TSE strains for which the infectious agents spread to lymphoid organs (this is the case with several classical strains and the sheep BSE strain for instance) but not for atypical scrapie.

In the framework of the health policy:
-For atypical cases the use of tonsil biopsies would not be of importance as currently available data seems to indicate the absence of the accumulation of PrPres in lymphoid tissue.
-For classical scrapies and BSE, these biopsies would help to identify those animals carrying susceptible genotypes but which are not infected. The importance, in terms of individual status, of this analysis would however be limited (due to the kinetic accumulation of PrPres in lymphoid tissue) and the analysis would have to be repeated in order to confirm the absence of TSE in the flock. The contribution of this scheme to public and animal health would therefore be limited. The use of tonsil biopsies could however be useful in some special cases, for instance in a breed with limited genetic resources.

4. *Exposure of the French population to sheep BSE:*

Taking into account the time limit granted for processing this referral, the Committee was unable to estimate the potential prevalence of BSE in sheep, as it was for goats[22]. This work will be carried out during the next Committee mandate. The analysis will be all the more pertinent as the Committee will have access to the results emanating from the reinforcement of active surveillance which will allow for a more precise estimation."

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[21] Le comité rappelle cependant que cette qualification ne peut être obtenue de façon aussi pertinente que chez les bovins en raison des caractéristiques physiopathologiques des ESST chez les petits ruminants (voir point 1). *The Committee reiterates however that flock register can not be permanently maintained as it can in cattle due to the physiopathological characteristics of TSEs in small ruminants (see point 1).*

[22] Avis de l'Afssa relatif à l'évaluation du programme de surveillance renforcé chez les caprins en date du 22 mai 2006. *Afssa opinion for assessment of the reinforced surveillance programme in goats, dated 22 May 2006.*

III- Afssa opinion

This is the analytical information that Afssa is able to provide in response to the questions on the potential presence of BSE in two sheep.

Afssa draws the attention of the health authorities in particular to the following points:

- the importance, highlighted once again, of classifying all small ruminant intestines as SRM, regardless of animal age and genotype;

- the importance of the individual identification of animals without which the flock scheme of qualification cannot be implemented;

- the necessity to optimize efficiency in current surveillance programmes with the aim of accumulating additional epidemiological data and managing escape from these programmes before planning new ones.

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