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OPINION

of the French Food Safety Agency regarding the consequences of two new scientific studies on animal health control measures for atypical scrapie

DIRECTOR-GENERAL

The French Food Safety Agency (AFSSA) submitted a request by letter, dated 4 February 2009, to the Scientific Panel of specialists on Transmissible Subacute Spongiform Encephalopathies (*CES ESST*) for an opinion on animal health control measures for atypical scrapie.

Review of the Internal Request.

Since identifying cases of atypical scrapie in sheep flocks, the TSSE Scientific Panel has delivered two Opinions^{1,2} concerning animal health control measures to be applied to flocks affected by this disease.

- In its January 2007 Opinion¹, the TSSE Scientific Panel reviewed changes to health control measures for small ruminants which had been proposed by the European Commission. In particular it proposed that, for flocks affected by atypical scrapie, animals with genotypes susceptible to classical scrapie no longer be slaughtered.

Based on its expertise, the Panel acknowledged that the animal health control measures applied to flocks affected by classical scrapie were not suitable for cases of atypical scrapie. It therefore made recommendations to be taken into account for flocks in which a case of atypical scrapie had been detected, including in particular:

- a monitoring period of 5 years: all slaughtered or rendered should undergo testing to detect cases of atypical scrapie.
- for sheep, genotyping should be carried out on the entire group of animals in the flock at the four codons of interest (136, 141, 154 and 171) of the PrP gene.
- a ban on the sale of breeding stock, while under surveillance, to farms which have a different status, due to the lack of data regarding potential transmission under natural conditions.

The Panel was divided in its opinion about what should be done with sheep carrying alleles indicating an increased risk for atypical scrapie.

A majority opinion of the Panel recommended that animals which had the AHQ and AFRQ alleles, genotypes conferring an increased susceptibility to atypical scrapie, not be sold for consumption. Animals carrying these alleles were to be destroyed at the end of their economic life.

¹ AFSSA Opinion dated 15 January 2007 regarding changes to animal health control measures in sheep and goat herds in which a case of classical or atypical scrapie has been detected

² AFSSA Opinion dated 21 April 2008 concerning two draft orders establishing animal health control measures regarding subacute spongiform encephalopathy transmissible by sheep and goats (Request 2007-SA-0406).

A minority opinion of the Panel considered that withholding animal carriers of at least one AHQ and AFRQ allele from human consumption was not justified, even though genotyping at the four codons of interest was justified for the purposes of acquiring knowledge, and subsequent adaptation of the measures to the risk.

- On 21 December 2007, AFSSA then received a request regarding two draft orders related to animal health control measures for sheep and goats². These draft texts were a compromise between changes to the community regulations, the recommendations of the Panel mentioned above, and the actual situation in the field (feasibility and socio-economic constraints).

In response to this request², the Panel deemed a three year period for monitoring farms affected by atypical scrapie to be an acceptable compromise. Nevertheless, it suggested that after sufficient time to implement this surveillance (with regard to the number of herds monitored) the situation be reviewed and the animal health control policy be modified if necessary.

- Quite recently, AFSSA Lyon and INRA [French National Institute for Agronomic Research] in Theix analysed the data derived from their programmes for monitoring small ruminants between 2002 and 2007³. The results indicate that the prevalence of secondary cases of atypical scrapie in outbreaks of atypical scrapie is not greater than that of the general sheep population (tested at the slaughterhouse and during quartering).

Moreover, a case-control study was conducted by the same teams to identify the risk factors for atypical scrapie⁴, and none of the factors identified are indicative of a contagious disease.

These two studies suggest that the atypical scrapie agent would be scarcely transmissible or non-transmissible in a farming environment. These conclusions could call into question the relevance of animal health control measures implemented in livestock affected by atypical scrapie in comparison with measures already taken for the general population of small ruminants (screening by spot check during quartering and at the slaughterhouse, and monitoring of clinical cases).

Accordingly, with respect to the results from these two studies and on the basis of current scientific knowledge, on 4 February 2009 AFSSA sent a request to the Scientific Panel on TSSE so that it could update its previous recommendations for animal health control measures to be applied to a herd of small ruminants whose index case is a case of atypical scrapie.

Method of Assessment

The Scientific Panel on TSSE delivered the following opinion after discussions at the 30 April, 28 May and 30 June 2009 meetings.

The Panel has:

- reconsidered all available data on atypical scrapie in small ruminants (genetic susceptibility, pathogenesis, sensitivity relative to screening tests, intra-herd prevalence);

³ "Tremblantes ovines atypique et classique. Etude de la prévalence des cas secondaires dans les foyers français détectés entre 2002 et 2007" [Classical and atypical sheep scrapie. Study of the prevalence of secondary cases in French outbreaks detected between 2002 and 2007]. A. Fediaevsky (AFSSA – INRA), P. Gasqui (INRA) D. Calavas (AFSSA), C. Ducrot (INRA)

⁴ "Enquête cas-témoins sur les facteurs de risque de la tremblante atypique chez les ovins" [Case-control survey of the risk factors for atypical scrapie in sheep], A. Fediaevsky, E. Morignat, C. Ducrot, D. Calavas, AFSSA Lyon, INRA Theix.

- examined the two studies which led to the request and their potential impact on animal health control measures for small ruminants.

Regulatory context

The current animal health control measures for small ruminants are defined by the 27 January 2003 Order, modified by the 17 August 2005 Order (waiver protocol for goats), and the 7 November 2008 Order (measures regarding the use of milk of small ruminants from herds affected with TSE).

As of this time, the distinction between atypical scrapie and classical scrapie has been taken into account:

- in the NS DGAL/SDSPA/N2007-8074 guidance note of 21 March 2007, which introduces – provisional – animal health control measures distinguishing between classical scrapie and atypical scrapie.
- in the orders concerning the use of milk from affected herds.

We should note that the provisional measures anticipated by the 21 March 2007 guidance note differ from those of the 27 January 2003 Ministerial Order providing for animal health control measures on the following points: limiting the genotyping to animals sent to the slaughterhouse, suspension of preventive slaughter measures, (see tables in the Annex). Moreover, the guidance note specifies that the measures apply “while awaiting a change in specific regulations for atypical scrapie”.

Discussion.

I. Review of recently published data on atypical scrapie

a) Pathogenesis

No recently published study has provided any new facts about the distribution of the atypical scrapie pathogen in the peripheral tissues or body fluids of affected animals.

b) Genetic determinism and atypical scrapie in small ruminants

The conclusions regarding the greater susceptibility to the disease in sheep which have AFRQ and AHQ alleles, and in goats which have the AHQ allele of the PRP [Prion Protein] gene have been confirmed by new publications (Moreno *et al.* 2007 – Colussi *et al.* 2008).

Consistent with these data, research conducted on 1041 sheep at the VLA Weybridge [Veterinary Laboratory Agency] in the UK, from a flock made from a group of animals imported from New Zealand (a country whose animals are reputedly unaffected by TSE) has made it possible to identify 3 cases of atypical scrapie to date, all in animals having at least one AFRQ allele (Simmons *et al.* 2009).

c) Detection of cases of atypical scrapie and rapid testing

In its 20 July 2006⁵ and 15 January 2007⁶ Opinions, the Panel expressed its reservations about the estimates of prevalence of atypical scrapie based on data collected by active monitoring programmes.

⁵AFSSA Opinion regarding evaluation of the risk associated with the potential presence of BSE [bovine spongiform encephalopathy] in ovine species, dated 20 July 2006.

In fact, the nature of the samples examined (obex), as well as the fact that the tests used for detecting atypical scrapie produced unequal results, seemed likely to diminish the ability of the system to identify infected animals.

Studies published since then confirm:

- the existence of pronounced inter-individual variations, in terms of the distribution of PrPSc in the encephalon, in affected animals. The obex, and in some cases the cerebellum sometimes prove to be unsuitable for detecting affected animals (Seuberlich *et al.* 2007 – Colussi *et al.* 2007) ;
- the existence of conflicting results among the rapid tests used for initial TSE screening in small ruminants in the case of atypical scrapie (Seuberlich *et al.* 2009).

Finally, the actual ability of the tests (based on the detection of PrPSc) to detect animals incubating the disease (infective carriers) has not been determined. Some of the biochemical characteristics of abnormal PrP associated with atypical scrapie (increased sensitivity to a treatment by proteinase K) could account for the discrepancy between the measures of infectivity level (assessed by bioassay, for example, in ovine PrP-transgenic mice) and the detection of PrPSc by rapid test (Le Dur *et al.* 2005).

d) Detection of cases of atypical scrapie and population demographics

In its 15 January 2007 Opinion, the Panel had stressed the fact that cases of atypical scrapie are, on average, observed in older animals as compared with the cases of classical scrapie (Gavier-Widén *et al.*, 2004).

The published data confirm these observations (Seuberlich *et al.* 2007 and Simmons *et al.* 2009).

Given our current state of knowledge, two hypotheses could explain this phenomenon:

- 1) the pathological process starts early as in cases of classical scrapie, but:
 - either the speed of progression of atypical scrapie is slower;
 - or detection capabilities for cases of atypical scrapie in the field are inferior to those for cases of classical scrapie.
- 2) the pathological process starts later than in cases of classical scrapie.

In both hypotheses, in a population surveillance programme, the demography of the population tested is likely to have a direct influence on the ability to detect infected animals.

II. Review of work conducted by AFSSA Lyon and INRA Theix

Two studies conducted by AFSSA Lyon together with INRA Theix were submitted to the Panel.

The first deals with the examination of risk factors associated with the presence of a case of atypical scrapie in infected sheep flocks identified by the active monitoring programme in place). The second concerns the analysis of surveillance data (rapid tests) collected as part of the animal health control measures applied to flocks of sheep affected with scrapie and from active monitoring at the slaughterhouse and rendering plant.

⁶ AFSSA Opinion dated 15 January 2007 regarding changes in animal health control measures in sheep and goat flocks in which a case of classical or atypical scrapie has been detected.

a) Case-control survey of risk factors for atypical scrapie

This study was conducted in flocks of sheep affected with atypical scrapie (n=95) and matched case-control flocks (n=225). Using a questionnaire, it sought to identify breeding factors or practices associated with an increased risk of occurrence of the disease. From this study it was not possible to attribute responsibility to the risk factors traditionally associated with classical scrapie: introduction of animals and contacts with other flocks.

The authors concluded that the results obtained are consistent with the hypotheses of either a 'spontaneous' disease or one of infectious origin, which was very mildly contagious.

These results reinforce those of the study conducted by Hopp *et al.* (2006) in Norway, which the Panel had considered in its 15 January 2007 Opinion.

b) Study of the prevalence of secondary cases in French outbreaks detected between 2002 and 2007

During APDIs (*Arrêté Préfectoral portant Déclaration d'Infection* [infection declared by prefectural order]) declared in flocks of sheep affected with scrapie (classical or atypical), the animal health control measures applied according to the 27 January 2003 Order allowed for:

- either the total slaughter of all flocks placed under APDI;
- or the implementation of genetic selection measures with elimination of all ovine carriers of the VRQ allele within two months and gradual elimination (over a period which may last 2 years) of animals which are not carriers of an ARR resistance allele;
- as well as reinforced surveillance measures for the flocks placed under APMS (*Arrêté Préfectoral de Mise sous Surveillance* [prefectural monitoring order]) following detection of the index case, including a screening of all the animals eliminated from the farm according to the procedures stipulated in the active epidemio-surveillance programme (rapid test on obex for animals older than age 18 months).

More recently (guidance note of 21 March 2007), another series of measures was proposed to farmers whose flocks were affected by atypical scrapie. The note suggested putting them under surveillance (while awaiting a specific regulation for atypical scrapie) without any specific elimination of animals, combined with restrictions in the transfer of the animals to unaffected farms.

The data analysed in the study conducted by INRA Theix and AFSSA Lyon was provided by all of these programmes, combining preventive slaughter (flocks under APDI) and reinforced surveillance (flocks under follow-up APMS and flocks concerned by the aforementioned guidance note).

Analysis results indicate that the detected prevalence of secondary cases in the outbreaks of atypical scrapie subject to preventive slaughter and the flocks subject to reinforced surveillance is not higher than that of the general population. In addition, the simulations done in this study indicate that the distribution of the number of outbreaks observed with one or more than one secondary case is consistent with a probability that the animals of these flocks will be affected by the disease equal to the prevalence observed in general populations undergoing active monitoring. This supports the hypothesis of very low or even no inter-individual contagion with this disease. It should be kept in mind that the probability of detecting cases of atypical scrapie increases with the age of the animals. Nevertheless, the prevalence of

secondary cases in the outbreaks of atypical scrapie under reinforced surveillance is still not significantly different from that of the general population when considering only the oldest animals (AFSSA-INRA report⁷). Lastly, the analysis of epidemiological data from monitoring in 11 European countries allows us to reach the same conclusions (Fediaevsky *et al.* article submitted for publication⁸).

Conclusions and recommendations

The recently published data and the studies conducted jointly by AFSSA Lyon and INRA Theix in flocks affected with atypical scrapie support the hypothesis of low (or no) contagiousness in atypical scrapie.

In a situation in which,

- the number of flocks affected by atypical scrapie identified each year should remain low (decrease in annual number of tests);
- the type of sampling and tests applied would not be optimised

The application of a reinforced surveillance programme, identical to the animal health control measures for classical scrapie (carrying out of tests for animals leaving the flock, slaughtered or quartered), to flocks affected with atypical scrapie will not provide more information about inter-individual transmission of the agent of atypical scrapie within these flocks.

In addition to these conclusions shared by the group of members of the Panel:

Since a majority of experts regard the prevalence of these secondary cases in the flocks affected by atypical scrapie as being no different from that measured in the general population, the maintenance of animal health control measures for flocks affected with atypical scrapie is not justified. In other words, the probability of detecting a case of atypical scrapie in a flock in which a first case has been detected is no higher than the probability of detecting a case in the general population. For the same reasons, these experts consider that it is unnecessary to restrict exchanges of animals within these flocks.

A minority of experts believe that the data available to date do not allow inter-individual transmission of atypical scrapie to be excluded. Consequently, for flocks affected by atypical scrapie, these experts consider it necessary to maintain:

- some restrictions in movements of animals, particularly exchanges of breeding stock;
- a reinforced surveillance programme for affected flocks, targeting old animals, the carrying-out of efficient rapid tests for detecting atypical scrapie in cerebellum and obex samples, and genotyping of the PrP gene at the 4 codons of interest.

They propose maintaining these measures until new physiopathological data have been obtained that provide additional information about the infectious nature of peripheral tissues and biological fluids in atypical scrapie cases.

⁷ "Tremblantes ovines atypique et classique. Etude de la prévalence des cas secondaires dans les foyers français détectés entre 2002 et 2007" [Classical and atypical sheep scrapie. Study of the prevalence of secondary cases in French outbreaks detected between 2002 and 2007]. A. Fediaevsky (AFSSA – INRA), P. Gasqui (INRA) D. Calavas (AFSSA), C. Ducrot (INRA)

⁸ A.Fediaevsky, C.Maurella, M.Nöremark, F.Ingravalle, S.Thorgeirsdottir, L.Orge, R. Poizat, M. Hautaniemi, B. Liam, D.Calavas, G. Ru, P. Hopp, The prevalence of atypical scrapie in sheep from positive flocks is not higher than in the general sheep population in 11 European countries. Article submitted for publication.

Main Bibliography

Moreno CR, Moazami-Goudarzi K, Laurent P, Cazeau G, Andreoletti O, Chadi S, Elsen JM, Calavas D. Which PrP haplotypes in a French sheep population are the most susceptible to atypical scrapie? *Arch Virol.* 2007;152(6):1229-32. Epub 2007 Apr 12.

Colussi S, Vaccari G, Maurella C, Bona C, Lorenzetti R, Troiano P, Casalnuovo F, Di Sarno A, Maniaci MG, Zuccon F, Nonno R, Casalone C, Mazza M, Ru G, Caramelli M, Agrimi U, Acutis PL Histidine at codon 154 of the prion protein gene is a risk factor for Nor98 scrapie in goats. *J Gen Virol.* 2008 Dec;89(Pt 12):3173-6.

Seuberlich T, Botteron C, Benestad SL, Brünisholz H, Wyss R, Kihm U, Schwermer H, Friess M, Nicolier A, Heim D, Zurbriggen A Atypical scrapie in a Swiss goat and implications for transmissible spongiform encephalopathy surveillance.. *J Vet Diagn Invest.* 2007 Jan;19(1):2-8

Seuberlich T, Doherr MG, Botteron C, Nicolier A, Schwermer H, Brünisholz H, Heim D, Zurbriggen A Field performance of two rapid screening tests in active monitoring of transmissible spongiform encephalopathies in small ruminants *J Vet Diagn Invest.* 2009 Jan;19(1):97-101

Nentwig A, Oevermann A, Heim D, Botteron C, Zellweger K, Drögemüller C, Zurbriggen A, Seuberlich T. Diversity in neuroanatomical distribution of abnormal prion protein in atypical scrapie. *PLoS Pathog.* 2007 Jun;3(6):e82

Simmons HA, Simmons MM, Spencer YI, Chaplin MJ, Povey G, Davis A, Ortiz-Pelaez A, Hunter N, Matthews D, Wrathall AE Atypical scrapie in sheep from a UK research flock which is free from classical scrapie. *BMC Vet Res.* 2009 Feb 10;5:8.

Le Dur A, Béringue V, Andréoletti O, Reine F, Lai TL, Baron T, Bratberg B, Vilotte JL, Sarradin P, Benestad SL, Laude H A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. *Proc Natl Acad Sci U S A.* 2005 Nov 1;102(44):16031-6.

Hopp P, Omer MK, Heier BT. A case-control study of scrapie Nor98 in Norwegian sheep flocks. *J Gen Virol.* 2006 Dec;89(Pt 12):3729-36.

Gavier-Widén D, Nöremark M, Benestad S, Simmons M, Renström L, Bratberg B, Elvander M, af Segerstad CH. Recognition of the Nor98 variant of scrapie in the Swedish sheep population. *J Vet Diagn Invest.* 2004 Nov;16(6):562-7.

Key words: animal health, atypical scrapie, TSE

ANNEX

Main APDI animal health control measures
Farms with confirmed cases of sheep affected with atypical scrapie (sedentary sheep)

	Mandatory Animal health control measure 27 January 2003	Provisional measures 21 March 2007 guidance note	European Regulation (EC) No. 999/2001
Cleaning and disinfecting of the farm	Yes	Yes	Cleaning/disinfecting for goats only (Annex VII, Chapter A, Point 3.1c)
Genotyping	Genotyping all animals on the farm at 3 codons	Genotyping (3 codons) only animals sent for slaughter but suspended for the rest of the animals on the farm.	Cases with atypical scrapie must undergo genotyping at 4 codons + A representative sample of the national ovine population (G%). No genotyping of all the animals in a flock in which a case has been confirmed.
Preventive slaughter	Limited to animals of genotypes susceptible to classical scrapie.	Suspended	By waiver, none
Restriction of movements	Prohibition of release or introduction of animals with susceptible genotypes until application of preventive slaughter.	Prohibition of release or introduction of animals with susceptible and highly susceptible genotypes.	No exchanges among Member States or non-EU countries
Consumption (slaughterhouse)	Before preventive slaughter: lambs less than 2 months of age with extended list of SRM [Specified Risk Materials] (head+intestines). Lambs from 2 to 6 months of age for which one parent is of genotype ARR/ARR After preventive slaughter: animals from flocks with no restrictions	Animals with resistant genotypes + Lambs less than 2 months of age with extended list of SRM [Specified Risk Materials] (head+intestines). Lambs from 2 to 6 months of age for which one parent is of genotype ARR/ARR	Authorised (see tests) but no exchanges among Member States. Surveillance of animals from flocks affected with atypical scrapie (routine test at the slaughterhouse on OV>18m)
Milk	Milk consumption authorised	Milk consumption authorised	Milk consumption authorised
Tests	Testing of all susceptible animals having undergone preventive slaughter of more than 12 months of age, or of a sample if there are more than 120.	Tests of susceptible animals, provisionally isolated from batches intended for slaughter	During surveillance : All animals more than 18 months of age sent to the slaughterhouse. All animals more than 18 months of age found dead or destroyed at the farm. No exchanges between Member States
Surveillance period	3 years after the application of animal health control measures.	Not specified "Pending regulatory changes specific to atypical scrapie..."	2 years

**Main APDI animal health control measures
Farms with confirmed cases of goats affected with atypical scrapie (sedentary goats)**

	Mandatory animal health control measure 27 January 2003	Provisional measures 21 March 2007 guidance note	European Regulation (EC) No. 999/2001
Cleaning and disinfecting of the farm	Yes	Yes	Yes
Preventive slaughter	All animals in the flock	Suspended	By waiver, none
Restriction of movements	Introduction to or release of goats from the farm is prohibited until the end of the APDI except when bound directly for a research study facility	Introduction to or release of goats from the farm is prohibited until the end of the APDI except when bound directly for a research study facility	No exchanges among Member States or third countries
Consumption (slaughterhouse)	After preventive slaughter: animal repopulation without restrictions	Goats may not be sent to the slaughterhouse	Authorised (see tests) But no exchanges among Member States
Milk	Milk consumption authorised (farm with atypical scrapie)	Milk consumption authorised (farm with atypical scrapie)	Milk consumption authorised
Tests	Testing of animals (all or from a sample) more than 12 months of age and which have been slaughtered as a preventive measure	Animals which are not temporarily kept	During surveillance: All animals more than 18 months of age sent to the slaughterhouse. All animals more than 18 months of age found dead or destroyed at the farm. No exchanges between Member States
Surveillance period	3 years after the application of animal health control measures	Not specified <i>"Pending regulatory changes specific to atypical scrapie..."</i>	2 years