Opinion of the Scientific Panel on Biological Hazards on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals[1]

Question EFSA-Q-2007-039

Adopted on 8 March 2007

PANEL MEMBERS

Olivier Andreoletti, Herbert Budka, Sava Buncic, Pierre Colin, John D Collins, Aline De Koeijer, John Griffin, Arie Havelaar, James Hope, Günter Klein, Hilde Kruse, Simone Magnino, Antonio Martínez López, James McLauchlin, Christophe Nguyen-The, Karsten Noeckler, Birgit Noerrung, Miguel Prieto Maradona, Terence Roberts, Ivar Vågsholm, Emmanuel Vanopdenbosch.

ACKNOWLEDGMENT

Umberto Agrimi, Olivier Andreoletti (Chairman & Rapporteur), Jean-Noël Arsac, Thierry Baron, Herbert Budka, John D Collins, John Griffin, Martin Groschup, Jim Hope, Danny Matthews, Marion Simmons, Mick Stack, Kath Webster**.**

[1] For citation Purposes: Opinion of the Scientific Panel on Biological Hazards on a request from the European Commission on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals, *The EFSA Journal* (2007), 466, 1-10

SUMMARY

The European Food Safety Authority (EFSA) and its Scientific Panel on Biological Hazards were asked by the European Commission (EC) to provide an assessment on (1) the existence of new available data which could provide evidence of any epidemiological or molecular link between classical and/or atypical scrapie and Transmissible Spongiform Encephalopathies (TSEs) in humans and on (2) the performance of the current discriminatory analytical methods used for further examination of TSE positive cases in small ruminants and their ability to differentiate Bovine Spongiform Encephalopathy (BSE) from known atypical and/or classical scrapie strains.

The experts of the Scientific Panel on Biological Hazards examined the previous opinions of the Scientific Steering Committee (SSC) and of EFSA as well as new scientific information published. The experts of the BIOHAZ panel concluded that there is no evidence for an epidemiological or molecular link between classical and/or atypical scrapie and TSEs in humans. The BSE agent is the only TSE agent identified as zoonotic. However, in view of their diversity. it is currently not possible to exclude transmissibility to humans of other animal TSE agents. Furthermore, the current discriminatory tests as described in the EC legislation to be used for discrimination between scrapie and BSE appear, up to now, to be reliable for the differentiation of BSE from classical and atypical scrapie. However, at the current stage of scientific knowledge, neither their diagnostic sensitivity nor their specificity can be assumed to be perfect.

KEY WORDS: Bovine Spongiform Encephalopathy, BSE, sheep, scrapie, atypical scrapie, public health, Regulation (EC) No 999/2001.

1. BACKGROUND

Regulation (EC) No 999/2001 of the European Parliament and of the Council lays down rules for the prevention, control and eradication of transmissible spongiform encephalopathies in ovine and caprine animals.

One of the strategic goals of the TSE roadmap prepared by the European Commission (EC, 2005) is a review of the TSE eradication measures in small ruminants taking into account the new diagnostic tools available but ensuring the level of consumer protection. In the framework of this TSE roadmap, the Commission started in the beginning of 2006 a discussion with the Member States (MS) on a proposal to review the eradication measures to apply in TSE affected flocks where BSE has been excluded. However, recently, the French Food Standards Agency (AFSSA, 2007) issued an opinion on the measures to be applied in flocks where scrapie, classical or atypical, has been detected. In that opinion AFSSA considers that the performance of the discriminatory methods used to differentiate BSE from scrapie could be limited and that the risk of transmission of classical and atypical scrapie to humans can not be excluded.

The Commission proposal to review the eradication measures to apply in TSE affected flocks where BSE has been excluded lies exactly on the basis of these two main basic concepts: (1) the fact that there's no proven epidemiological or molecular links between scrapie and TSEs in humans and (2) the ability to differentiate, by a molecular test, BSE from known atypical and classical scrapie strains. As those two main essential concepts are questioned in the AFSSA opinion it is considered necessary to request an assessment from EFSA on that subject.

2. TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is therefore invited to provide an assessment on:

1. the existence of new available data which could provide evidence of any epidemiological or molecular link between classical and/or atypical scrapie and TSEs in humans,
2. the performance of the current discriminatory analytical methods used for further examination of TSE positive cases and their ability to differentiate BSE from known atypical and classical scrapie strains.

3. ASSESSMENT

3.1. Pre-amble

The questions refer to the proposal of the European Commission to review the eradication measures to apply in TSE-affected flocks where BSE has been excluded. This is important to note since this limits the response to flocks in which cases of classical and/or atypical scrapie have been identified. Any positive case of TSE in small ruminants is termed as “TSE in small ruminants” which encompasses classical scrapie, atypical scrapie including Nor98 in sheep and goats as well as BSE in these species if found.

Practical definitions of TSE in small ruminants are summarized in the EFSA opinion on the classification of atypical and classical scrapie and BSE in small ruminants (EFSA, 2005) in the Table "Criteria for the categorisation of TSEs in small ruminants" (in annex 1 of the said EFSA opinion).

3.2. Small ruminants TSE agents and human

3.2.1. TSE agent diversity in ruminants

Since the Institute for Animal Health, Neuropathogenesis Unit (NPU, Edinburgh) described the biodiversity of scrapie field strains in the mid-1960's using inbred mice, a large panel of different TSE field agents has been described. BSE-derived natural cases were described in cattle, felines, humans, captive large ruminants and more recently in a goat. The introduction of active surveillance programmes in the European Union has led to the recognition of isolates that do not conform to previous phenotypes of BSE in cattle and classical scrapie in sheep. These are currently termed for operational reasons as "atypical" BSE or scrapie, but probably reflect part of a wider spectrum of isolates not previously recognised, and in the case of atypical scrapie affecting genotypes highly resistant to clinical scrapie (Biacabe *et al.*, 2004; Casalone *et al.*, 2004; Benestad *et al.*, 2003; Gavier-Widen *et al.*, 2004; Buschmann *et al.*, 2004).

Substantial progress has been made in recent years in the molecular characterisation of TSEs in ruminants and in humans, notably following studies aiming to differentiate BSE from other TSEs, and in investigating the link between BSE and the Variant Creutzfeldt-Jakob disease (vCJD). Such investigations have led some authors, when comparing atypical forms of TSEs in ruminants to classical scrapie and to BSE, to notice some molecular similarities between atypical TSEs in ruminants and some human forms of prion diseases (sporadic or genetic CJD). Such findings have been reported in both atypical forms of BSE (Casalone *et al.*, 2004; Biacabe *et al.*, 2007) and in Nor98 or atypical scrapie in small ruminants (Everest *et al.*, 2006; Klingeborn, 2006; Saunders *et al.*, 2006; Arsac *et al.*, 2007). However, these data only suggest some similarities in the molecular mechanisms of these prion diseases, which can lead to shared phenotypic features, but do not demonstrate any causal link. Any relationship between two prion diseases in two different species would require a detailed comparison of the biological properties (Bruce and Fraser, 1991; Fraser *et al.*, 1992; Bruce *et al.*, 1994; Bruce *et al.*, 1997).

3.2.2. Species and transmission barriers

When prions are passaged to a new species, the incubation period is typically prolonged and the transmission rate can be lowered. Further intra-species passages then reduce and stabilize the incubation period. In some cases there is no evidence of this interspecies transmission during the lifespan of the recipient.

While this species barrier depends mostly upon the PrP gene, it is also influenced by the prion strain, which at time may give the appearance of totally overcoming the barrier. BSE appears to behave in this way, appearing more promiscuous than other prion strains as implied by isolation in a number of species other than cattle, even in natural conditions. The lower prevalence of BSE in the other species could however suggest that there remained a real barrier to transmission, even if the few data available from primates suggest a low transmission barrier (Lasmezas, 2005). Host susceptibility to a prion has been shown to be altered on passage through an intermediate species, but the outcome in terms of pathogenicity and host range cannot be

predicted. Preliminary evidence from passage of BSE to sheep, and onward transmission to further sheep, suggests that the biological properties of BSE do not change, as determined by incubation period in sheep (no shortening on sub-passage), incubation and lesion profile in wild type mice, and molecular characteristics (Community Reference Laboratory (CRL) for TSEs, unpublished data). A recent experimental study of BSE transmitted from cattle to sheep has, however, established a higher virulence of BSE-after its passage in sheep in a bovine transgenic mouse model (Espinosa *et al.*, 2007).

The more general term of "transmission barrier" has been proposed to account for the strain dependence of the species barrier (reviewed in Hill *et al.*, 2003).

3.2.3. Human species barrier permeability

The risk of human infection after exposure to an animal TSE agent is usually considered to be low because of the species barrier phenomenon. Susceptibility to animal derived TSE has only been confirmed with the BSE agent so far.

Species barriers are considered to be efficient in limiting the propagation of the TSE agent between different species. However, our understanding of the nature and effectiveness of the human species towards ruminant derived TSE agents is still poor.

The assumed lack of association between TSEs in humans and those in small ruminants is mainly based upon epidemiological studies failing to establish a link between geographical distribution and prevalence of animal TSEs (except BSE) and human TSEs (Brown, 1987; van Duijn *et al.*, 1998), sporadic Creutzfeldt-Jakob disease (s-CJD) in particular being found in countries where no ruminant-derived TSEs have been diagnosed (New-Zealand and Australia). A case control study has also failed to establish links with consumption of or exposure to materials derived from small ruminants (van Duijn *et al.*, 1998).

It is important to recognise that the lack of association may be biased by a number of factors:

(i) The lack of a data on the historical real prevalence and distribution of small ruminant TSEs, at a time where only passive surveillance was performed;

ii) the lack of understanding of the true biodiversity of TSEs in small ruminants in terms of both classical and atypical agents;

(iii) the lack of understanding of the diversity of TSEs in humans due to the limited molecular and bioassay characterisation of human TSEs also in relation to the number and spectrum of neurodegenerative diseases of humans;

(iv) the predicted phenotype of disease that might arise should an animal derived TSE transmit to humans.

The recognition of vCJD in human was only enabled by the very typical and particular features of this form of TSE (such as age at onset and geographic location, and phenotypic features such as the peculiar neuropathologic features and the molecular pattern aspect of PrPres). Together, these features facilitated the detection of the emergence of a new pathology. Since vCJD cases are still greatly outnumbered by cases of other human TSEs, even in the UK, and thus contribute to the overall prevalence of human TSEs in only limited fashion, epidemiology on its own could have failed to identify this particular disease.

The human species barrier can be investigated experimentally by inoculation to primates that are believed to represent the closest species to human. However it does not allow to take into account the human gene PRNP polymorphisms (in particular the M/V 129), that have been identified to play a major role on relative susceptibility towards prion disease. In addition, genes other than the PrP gene may also be influential in determining overall susceptibility to TSEs. Moreover, the cost and duration of such experiments do not make it possible to consider large scale studies.

In two experiments that were conducted with (i) scrapie adapted in hamster in squirrel monkey (*Saimiri sciureus*) (oral challenge) (Gibbs *et al.*, 1980) and (ii) natural sheep scrapie isolate in cynomologus monkey (*Macaca fascicularis*) and marmoset monkey (*Callithrix jacchus*) (intra-cerebral challenge) a transmission was demonstrated (Gibbs & Gajdusek, 1972; Baker, 1988). A recent study demonstrated the transmission by the intra-cerebral route in a macaque of one of the two atypical forms of BSE recently identified in cattle (BASE or L-type) (Casalone *et al.*, 2004), with a shorter incubation period than that previously described with typical BSE in this primate model (Lescoutra-Etchegaray *et al.*, Prion 2006).

Currently several project are under way to estimate the extent of the human species barrier to animal-derived TSEs, using transgenic models as surrogates for humans. The outcome of such experiments is to date unpredictable, and results could be difficult to interpret (Gombojav *et al.*, 2003).

Finally, route of exposure, dose, and cumulative exposures are also postulated to influence the abilities of TSE agent to cross species barriers (Jacquemot *et al.*, 2005).

In conclusion, no scientific data currently enable us to consider any TSE agent other than BSE as a zoonotic agent. However, there are significant scientific uncertainties associated with the question whether TSE agents in their whole spectrum may cross the human transmission barrier under natural conditions.

3.3. TSE discriminatory test

Any positive case of TSE in small ruminants is termed as “TSE in small ruminants” which encompasses classical scrapie, atypical scrapie including Nor98 and BSE in sheep and goats. Following the confirmation of BSE in a goat, EC (DG Sanco) proposed new measures to the MS and which came into force in January 2005 by the adoption of EC regulation 36/2005. They require a three step mandatory strategy for differential testing between scrapie and BSE for all confirmed positive scrapie cases in both sheep and goats. Steps include the discriminatory rapid testing (by defined Western blot protocols) of index cases detected in flocks, and further molecular testing and possibly when necessary mouse bio-assay (“strain typing”) of any isolates which look BSE-like on initial blot. All such cases are required to be submitted to testing by an approved discriminatory western immunoblot specific for the protease-resistant prion protein (PrP^{res}), initially at the National Reference Laboratory (NRL) or other approved laboratory.

Evidence of PrP^{res} with a low apparent molecular mass of the unglycosylated band, close to that observed in cattle with typical BSE, or a poorer staining with monoclonal antibody P4 (or equivalent N-terminal antibody according to the discriminatory blot method adopted) when compared with 6H4 (or equivalent core-directed primary antibody), or a poor PrP^{res} signal on the blots examined with core antibodies, should trigger submission of further material to the CRL for extra consideration. Given the current uncertainty about the interpretation of molecular results,

full agreement of the results of ring trial tests (one WB, ELISA plus IHC) is required for the definitive identification of a suspect case of BSE in small ruminants, and the case should be further characterised by mouse bio-assay.

In its opinion adopted in January 2007 (EFSA, 2007) the BIOHAZ Panel indicated that:

- *The scrapie/BSE discriminatory tests are robust judging by their performance against a small number of samples in a blinded ring trial organised by the EU TSE Community Reference Laboratory (Stack et al., in preparation);*
- *application as part of small ruminant surveillance was continuing to improve the accuracy of these prevalence estimates. However, balanced against this optimistic scenario, the BIOHAZ panel accepted that the sensitivity and specificity of the discriminatory tests had, for logistical reasons, not been experimentally evaluated and potential confounding factors, such as concomitant infection of the same animal with scrapie and BSE, remained to be investigated.*

4. CONCLUSIONS

The experts of the BIOHAZ panel conclude that:

1. There is no evidence for an epidemiological or molecular link between classical and/or atypical scrapie and TSEs in humans.
2. The BSE agent is the only TSE agent identified as zoonotic. However, in view of their diversity it is currently not possible to exclude transmissibility to humans of other animal TSE agents.
3. Current discriminatory tests as described in the EC legislation to be used for discrimination between scrapie and BSE appear, up to now, to be reliable for the differentiation of BSE from classical and atypical scrapie. However, at the current stage of scientific knowledge, neither their diagnostic sensitivity nor their specificity can be assumed to be perfect.

5. DOCUMENTS PROVIDED TO EFSA

Letter (plus annex) with the ref. D(2006) SANCO.E.2/JOV/mtd – D (2007) 520045 from the European Commission, Health & Consumer Protection Directorate-General, requesting an assessment from the European Food Safety Authority (EFSA) on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals.

Opinion from the French Food Standards Agency (AFSSA) of 15 January 2007 (AFSSA, Saisine 2006 SA-0343 and 2006 SA-0195 plus annex) on the measures to be applied in flocks where scrapie, classical or atypical, has been detected. English translations provided by the AFSSA.

6. REFERENCES

AFSSA (2007) Opinion of the French Food Safety Agency (AFSSA) on changes the control measures for sheep and goat herds in which a case of classical or atypical scrapie has been detected.

Arsac, J. N., Andreoletti, O., Bilheude, J. M,, Lacroux, C., Benestad, S. L. and Baron, T. (2007) Similar biochemical signatures and prion protein genotypes in atypical scrapie and Nor98 cases, France and Norway. *Emerg. Infect. Dis*.,13 (1), 58-65

Baker, H.F., Ridley, R.M., Wells, G.A., Ironside, J.W. (1988) Prion protein immunohistochemical staining in the brains of monkeys with transmissible spongiform encephalopathy. *Neuropathol. Appl. Neurobiol.*, 24 (6),476-86

Benestad, S. L., Sarradin, P., Thu, B., Schonheit, J., Tranulis, M. A. and Bratberg, B. (2003) Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. *Vet Rec 153* **(7)**, 202-8

Biacabe, A. G., Laplanche, J. L., Ryder, S. & Baron, T. (2004) Distinct molecular phenotypes in bovine prion diseases. *EMBO Rep*, **5**, 110-115

Biacabe, A.G, Jacobs, J.G, Bencsik, A., Langeveld. J.P.M. and Baron, T. (2007) H-type bovine spongiform encephalopathy: complex molecular features and similarities with human prion diseases. *Prion*. 1 (1), *In press*.

Brown, P., Cathala, F., Raubertas, R. F., Gajdusek, D. C. & Castaigne, P. (1987) The epidemiology of Creutzfeldt-Jakob disease: conclusion of a 15-year investigation in France and review of the world literature. *Neurology*, **37**, 895-904.

Bruce, M. E. and Fraser, H. (1991) "Scrapie strain variation and its implications." *Curr. Top. Microbiol. Immunol.*, **172**, 125-38

Bruce, M., Chree, A., McConnell, I., Foster, J., Pearson, G. and Fraser, H. (1994) Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **343**(1306), 405-11

Bruce, M. E., Will, R. G., Ironside, J. W., McConnell, I., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., Cousens, S., Fraser, H. and Bostock, C. J. (1997) Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* **389** (6650), 498-501

Buschmann, A., Biacabe, A. G., Ziegler, U., Bencsik, A., Madec, J. Y., Erhardt, G., Luhken, G., Baron, T. and Groschup, M. H. (2004) Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. *J Virol Methods* **117** (1), 27-36

Casalone, C., Zanusso, G., Acutis, P., Ferrari, S., Capucci, L., Tagliavini, F., Monaco, S. and Caramelli, M. (2004) Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. *Proc. Natl. Acad. Sci. USA*, **101**(9), 3065-70

EC (2005) Communication from the European Commission of 15 July 2005 on the future of TSE measures: "The TSE roadmap".

EFSA (2005) Opinion of the Scientific Panel on Biological Hazards on the request from the European Commission on classification of atypical Transmissible Spongiform Encephalopathy (TSE) cases in Small Ruminants. *The EFSA Journal*, **276**, 1-30.

EFSA (2007) Opinion of the Scientific Panel on Biological Hazards on a request from the European Commission on the quantitative risk assessment on the residual BSE risk in sheep meat and meat products. *The EFSA Journal*, **442**, 1-10.

Espinosa, J. C., Andreoletti, O., Castilla, J., Herva, M. E., Morales, M., Alamillo, E., San-Segundo, F.D., Lacroux, C., Lugan, S., Salguero, F. J., Langeveld, J. and Torres, J. M. (2007) "Sheep-passaged bovine spongiform encephalopathy agent exhibits altered pathobiological properties in bovine-PrP transgenic mice." *J. Virol.* **81**(2), 835-43

Everest, S.J., Thorne, L., Barnicle, D.A., Edwards, J.C., Elliott, H., Jackman, R., and Hope, J. (2006) Atypical prion protein in sheep brain collected during the British scrapie-surveillance programme. *J. Gen. Virol*. **87**, 471-7.

Fraser, H., Bruce, M. E., Chree, A., McConnell, I. and Wells, G. A. (1992) Transmission of bovine spongiform encephalopathy and scrapie to mice. *J. Gen. Virol.* **73** (Pt 8), 1891-7

Gavier-Widen, D., Noremark, M., Benestad, S., Simmons, M., Renstrom, L., Bratberg, B., Elvander, M. and af Segerstad, C. H. (2004) Recognition of the Nor98 variant of scrapie in the Swedish sheep population. *J Vet Diagn Invest* **16**(6), 562-7

Gibbs, C. J. Jr. and Gajdusek, D. C. (1972) Transmission of scrapie to the cynomolgus monkey (Macaca fascicularis). *Nature* **236**(5341), 73-4

Gibbs, C. J. Jr., Amyx, H. L., Bacote, A., Masters, C. L. and Gajdusek, D. C. (1980) Oral transmission of kuru, Creutzfeldt-Jakob disease, and scrapie to nonhuman primates. *J Infect Dis* **142**(2), 205-8

Gombojav, A., Shimauchi, I., Horiuchi, M., Ishiguro, N., Shinagawa, M., Kitamoto, T., Miyoshi, I., Mohri, S. and Takata, M. (2003) Susceptibility of transgenic mice expressing chimeric sheep, bovine and human PrP genes to sheep scrapie. *J. Vet. Med. Sci.* **65**(3), 341-7

Hill, A. F., Collinqe, J. (2003) Subclinical prion infection. *Trends. Microbiol*., **11**(12), 578-84

Jacquemot, C., Cuche, C., Dormont, D. and Lazarini, F. (2005) High incidence of scrapie induced by repeated injections of subinfectious prion doses. *J Virol* **79**(14), 8904-8

Klingeborn, M., Wik, L., Simonsson, M., Renstrom, L. H., Ottinger, T. and Linne, T. (2006) Characterization of proteinase K-resistant N- and C-terminally truncated PrP in Nor98 atypical scrapie. *J Gen Virol* **87**(Pt 6), 1751-60

Lasmezas, C. I., Comoy, E., Hawkins, S., Herzog, C., Mouthon, F., Konold, T., Auvre, F., Correia, E., Lescoutra-Etchegaray, N., Sales, N., Wells, G., Brown, P.and Deslys, J. P. (2005) Risk of oral infection with bovine spongiform encephalopathy agent in primates. *Lancet* **365**(9461): 781-3

Lescoutra-Etchegaray, N., Comoy, E., Jacquin, S., Brown, P., Lasmezas, C.and Deslys, J. P. (2006) Standardized blood samples of Creutzfeldt-Jakob-infected primates for the evaluation of future prion diagnostic tests. Prion 2006 conference. http://www.neuro-prion.org/pdf_docs/conferences/prion2006/abstract_book.pdf

Saunders, G.C., Cawthraw, S., Mountjoy, S.J., Hope, J., and Windl, O. (2006) PrP genotypes of atypical scrapie cases in Great Britain. *J. Gen. Virol*. **87**, 3141-9.

Van Duijn, C. M., Delasnerie-Lauprêtre, N., Masullo, C., Zerr, I., de Silva, R., Wientjens, D. P. W. M., Brandel, J.-P., Weber, T., Bonavita, V., Zeidler, M., Alpérovitch, A., Poser, S., Granieri, E., Hofman, A., and Will, R. G. (1998) Case-control study of risk factors of Creutzfeldt-Jakob disease in Europe during 1993-95. *Lancet* **351**, 1081-1085