EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C1 - Follow-up and dissemination of scientific opinions

OPINION ON

SAFE SOURCING OF SMALL RUMINANT MATERIALS

(SAFE SOURCING OF SMALL RUMINANT MATERIALS SHOULD BSE IN SMALL RUMINANTS BECOME PROBABLE: GENOTYPE, BREEDING, RAPID TSE TESTING, FLOCKS CERTIFICATION AND SPECIFIED RISK MATERIALS)

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 4-5 APRIL 2002

OPINION ON SAFE SOURCING OF SMALL RUMINANT MATERIALS

(SAFE SOURCING OF SMALL RUMINANT MATERIALS SHOULD BSE IN SMALL RUMINANTS BECOME PROBABLE: GENOTYPE, BREEDING, RAPID TSE TESTING, FLOCKS CERTIFICATION AND SPECIFIED RISK MATERIALS)

BACKGROUND AND TERMS OF REFERENCE

In its opinion of 19 October 2001 the Scientific Steering Committee (SSC) considered that, should BSE in small ruminants become probable, its previous opinions on safe sourcing of materials by exclusion of certain Specified Risk Materials would no longer be adequate, but that a more comprehensive approach, involving for example also testing and genotyping of animals, would be needed. However, as available data did not allow to comprehensively judge on genotyping as a possible tool for consumer protection, the SSC recommended that the concept of genotyping as a tool for consumer protection be urgently verified.

The SSC further announced the preparation of an opinion on certification of flocks as part of a policy to guarantee safe sourcing at the long term. The SSC finally announced the preparation of an opinion on the criteria which should be used in a widespread breeding programme for resistance to Scrapie/BSE in small ruminants, should such programme be decided upon as an overall long term strategy for the reduction / elimination of Scrapie/BSE prevalence in small ruminants.

In addition, Commission services invited the SSC to address in an opinion, provisions on culling of TSE affected flocks. They also asked the SSC whether, in the light of the scientific elements contained in 5 recent opinions adopted in 2001 and 2002 by the French Food Safety Agency and of possible other new scientific data and evidence, it maintains its opinions of March and October 2001 implying that the probability that BSE is present in small ruminants has not increased since its previous opinions and that there is no reason to amend at this stage the list of Specified Risk Materials to be currently removed from the food chain.

The current opinion deals with the above issues.

The SSC invited the TSE/BSE *ad hoc* Group to prepare a detailed scientific report, to be used as input for preparing an opinion on the above questions. The TSE/BSE *ad hoc* Group finalised its report on 7 February 2002. It is annexed to this opinion.

The present opinion is an exploitation of the opinions and documents already adopted by the SSC and of new data in order to develop a scientific approach to support risk reduction strategies should the presence of BSE in small ruminants become probable. It is based on a detailed report (attached) prepared by a special working group and by the TSE/BSE *ad hoc* Group.

1. Distribution of infectivity in experimentally infected BSE-susceptible animals.

Unlike the situation in experimentally-infected cattle, the distribution of infectivity in experimentally infected sheep tissues at different time intervals from exposure by the oral route to a large dose of the BSE agent indicate a widespread involvement of lymphoid tissues early in the incubation period. In fact, already after one month from exposure to the BSE agent, susceptible sheep show an estimated significant load of BSE infectivity, in the intestine, lymph nodes, tonsils, stomach and spleen. After 36 months of exposure the estimated total BSE infectivity load in the animal body is much higher and the distribution of infectivity very different. As compared to the central nervous system tissues, the PrP^{Sc} load in the intestine of BSE-infected small ruminants is relatively higher at the beginning of the incubation period and of the same order of magnitude toward the end of the incubation.

The tissues/organs of BSE-infected susceptible small ruminants that, according to current knowledge, contain, or may contain BSE-infectivity are as follows:

- The head;
- The spinal cord and associated dorsal root ganglia;
- The spleen;
- Peripheral nervous tissues;
- Other lymphoid tissues (e.g., tonsils) and lymph nodes (e.g., prescapular lymph nodes and supra mammary lymph nodes, …);
- The liver;
- The pancreas;
- The placenta;
- The alimentary tract from oesophagus to rectum**,** not only the intestine itself, the forestomachs and the abomasum, but also closely related lymph nodes, the mesenteric lymph nodes and other lymph nodes, e.g. the mediastinal lymph nodes, as well as the innervation.

2. Scrapie and BSE- resistant and susceptible small ruminant genotypes

Results to date indicate that the relationship between genotype and susceptibility is similar for scrapie and BSE. In sheep susceptibility to these two TSEs is linked to PrP genotype, with codons 136, 154 and 171 being of major importance. Most affected sheep are homozygous for glutamine (Q) at codon 171 and succumb to BSE disease. Available findings summarized in Section 1 indicate that, after an exposure to a high dose of BSE-infectivity, detectable infection may be widespread in the lympho-reticular system after a few months from exposure. Furthermore, in natural scrapie of Romney sheep (to which pathogenetically experimental BSE in sheep bears a close semblance), PrP^{Sc} can be detected from two months of age in Peyer's patches and mesenteric lymph node in the VRQ/VRQ genotype. This being the case, there is no basis on which to recommend an age cut-off for the presence of BSE-infectivity in small ruminant tissues listed in Section 1 for *susceptible genotypes*.

Available evidence indicates that sheeps which are homozygous for the arginine (R) allele at codon 171 are the most resistant to development of the disease upon challenge with BSE-infected material. Although infection of this genotype cannot be completely excluded, the likelihood of it becoming infected with BSE subsequent to exposure is very small. Moreover, the development of the disease in resistant sheep, if it occurred, is likely to be very slow and not to result in significant infectivity levels in young animals. Therefore, based on available but relatively limited data indicating no evidence of scrapie nor BSE infectivity in resistant animals of any age, it is a reasonable worst case assumption that the tissues from sheep which are homozygous for the arginine (R) allele at codon 171, including those listed in Section 1, do not pose any significant BSE-risk below 18 months of age[1].

Semi-resistant sheep which are heterozygous with one arginine (R) at condon 171 show an intermediate degree of resistance to BSE-infection and a distinct pathogenesis, as indicated by a different pattern amount and distribution of infectivity in tissues and a much longer incubation period compared to susceptible genotypes. In fact, for any given level of exposure to the BSE agent, the probability of finding clinical BSE or infectivity in tissues is lower in the ovines of a semi-resistant genotype than in the susceptible ovines. Moreover, during the pre-clinical phase, PrP^{Sc} does not appear to be detectable in the digestive autonomomic nervous system of heterozygotic

---

[1] This threshold also corresponds approximately with the lower 10% percentile of the incubation period distribution in susceptible sheep in which scrapie has been observed under field circumstances.

C:\Documents and Settings\BCerrato\My Documents\DOc des mails\531.doc   3

ARR/ARQ or ARR/VRQ ovines. For these reasons, the sheep tissues from heterozygous ARR/ARQ or ARR/VRQ ovines, including those listed in Section 1, can be considered free from BSE infectivity only below 6 months of age.

The above suggested age-thresholds should be revised once more information on the genotype of TSE-positive animals has become available.

As available information on BSE and genotype in goats is very limited, it is reasonable to assume, for the time being that all goats are susceptible to TSE by the oral route under certain conditions. Further research on goat PrP genetics is required.

3. Rapid tests to identify BSE-affected small ruminants

The currently available rapid post-mortem bovine BSE tests, if confirmed to be applicable to sheep central nervous system tissues, would certainly be useful to identify affected small ruminants. However, they would not offer the same degree of consumer protection as for bovines, particularly because of the pattern of pathogenesis in BSE-susceptible small ruminants which results in the presence of infectivity in peripheral tissues very early in the incubation period.

Tests applicable on tissues that show infectivity in the early stages of incubation such as the lymphoid tissues are still being developed and will probably not be available for routine applications soon. Such tests would only permit an early identification of the susceptible small ruminants that pose a BSE risk, if sensitive enough to detect low levels of BSE-infectivity. On the other hand, only tests applied to CNS at the end of the incubation period are likely to be useful to detect BSE-affected semi-resistant sheep due to the fact that detectable infectivity may be absent in certain lymphoid tissues of these genotypes.

4. Breeding for TSE resistance in small ruminants.

As available data indicate that the relation between sheep genotype and susceptibility to a TSE is very similar for scrapie and BSE, breeding for scrapie resistant sheep is expected to result also in BSE-resistant sheep.

Breeding for resistant PrP genotypes is now being carried out in a number of countries, including, for example, the UK, the Netherlands and France. Worries have been expressed about the potential long-term effects of such nation-wide and generalised programmes. Their discussion as well as an elaboration on the practicalities of breeding programmes fall beyond the scope of this opinion, but it may be expected that breeding

for ARR/ARR genotypes in some breeds of sheep would be a multi-step process involving (a) ram genotyping scheme to increase frequency of ARR allele in healthy flocks, (b) monitoring for scrapie on farms taking part in the programme and (c) dealing with scrapie affected flocks.

The SSC considers that such a programme should in a first instance be targeted at risk population or risk areas and would require:

- The availability of an acceptable method of identifying individual sheep (*for example*, electronic chips or boluses);

- For each important breed, an approximate knowledge of the frequency of ARR/ARR sheep to give an estimate of how quickly the breed would be able to move towards use of ARR/ARR rams only.

- An agreed procedure on scrapie monitoring taking into account that very young animals or animals of heterozygous genotype may not show easily identifiable PrP^{Sc} in peripheral tissues.

- A programme of genotype monitoring of scrapie cases as recommended in the SSC's opinion of 30 November 2001 on *Requirements for statistically authoritative BSE/TSE surveys*, in order to have warning of the potential emergence of new scrapie strains able to cause disease more easily in the heterozygous genotypes (at the moment judged to be of intermediate susceptibility). Also the monitoring of the PrP^{Sc} profile will be needed in conjunction with strain typing. [*]

- With respect to the occurrence of possible adverse effects, an effective monitoring of breed characteristics in scrapie resistant genotypes to obtain reliable information on any undesirable changes (e.g. in birth weight, growth rates, strength and resistance to particular other diseases).

- Careful monitoring for completeness of protection against infection within the flock.

Very little is known about genotype frequencies in goats in general and goats should be investigated in their own right. Goats have the equivalent of the sheep ARQ allele and arginine at codon 171 has not been described to date. In order to reach agreement about main feature of goat-breeding programmes, further information on goat PrP genetics is required.

Strain-typing is discussed in more detail in a opinion of 4-5 April 2002 of the SSC suggesting a *Strategy a to investigate the possible presence of BSE in sheep*

5. Flock certification

Animals from a certified "Scrapie/BSE-free" (or preferably**:** "Scrapie/BSE-negligible risk") flock would represent no risk. However, infectivity can be present for years in animals and flocks that were apparently TSE-free in terms of clinical manifestation before coming under observation. The implementation of a comprehensive programme leading to the possible certification of flocks would therefore in most cases and for most countries require many years. An approach of less stringent "provisional certification", is a possible alternative in the short term if, where necessary, it is applied in combination with other criteria such as testing and genotyping. The report of the TSE/BSE *ad hoc* Group attached to the present provides details on possible approaches to flock certification, which apply to both sheep and goats.

Note: In countries with a geographical BSE risk level (GBR) above 1, the closure of a flock is essential to obtain the provisional certification as this guarantees that no new TSE (scrapie, BSE, …) infectivity can be introduced into the flock, including from other small ruminant (including: goat) flocks or bovine herds.

6. Culling strategies

Because of the transmissibility of the infection within a flock and between flocks by direct or indirect contacts, the elimination of the index case only will not eliminate the enhanced risk in a flock (of sheep and/or goats) where a clinical or sub-clinical TSE case has been confirmed. Therefore, a culling strategy should ideally be applied which covers the entire flock where the index case was found and, in the case BSE was confirmed, the flocks that were in contact with the original flock via other small ruminants[2] or via grazing areas. Such culling would, however, have little or very little risk reducing effect for sheep of the ARR/ARR genotype[3] or if the risk of transmission to other flocks was negligible. The assessment whether the risk for transmission to other flocks was negligible would require that the animals introduced into a flock are identifiable and their history traceable and that they are genotyped. The risk would, for example, be negligible in the case of contacts with or imports from flocks certified to represent a negligible risk, if the contact only concerned the use of breeding rams (as compared to pregnant ewes) or if the imported animals tested negative with a validated

---

[2]   Including via the offspring of the case

in vivo test (once available). The application of one of the certification scenarios as described in the Report of the TSE/BSE *ad hoc* Group could be considered as an alternative for the slaughter of contact flocks at risk.

Much of the above described approach will have to depend on the availability of detailed records and identification, and it may not only be impossible to trace sheep that have moved out of a flock or cohort historically, but the identification of cohort and offspring may also be impossible. If no tracing of animals exported from a BSE infected flock is possible other approaches (e.g. ad hoc epidemiological investigations) could be helpful to identify the potentially -exposed flocks.

The Report of the TSE/BSE *ad hoc* Group provides some examples where such may be the case and careful weighting of the advantages and disadvantages of various culling options is thus required.

7. Safe sourcing of small ruminant materials

Should the presence of BSE in small ruminants become probable, safety of sourcing of small ruminants materials (i.e. minimisation of the risk of inadvertently slaughtering BSE-infected animals) could be achieved by combining different approaches including removal of tissues known to pose a risk of infectivity as from a given age, testing for BSE, genotyping and breeding for BSE-resistance, flock certification and individual animal and flock tracing. The report of the TSE/BSE ad hoc Group provides an example of a structured approach that requires all the relevant information be made rapidly available at the slaughterhouse. To achieve a maximum risk reduction, sourcing should, in principle, take place from animals in certified flocks.

Moreover, the possible risk of materials sourced from small ruminants potentially infected with BSE is likely to change with the geographical origin of the animals, depending on factors such as possible local unsafe feeding practices, possible episodic imports of BSE-affected animals and different reliability of the existing surveillance system. The SSC opinion on the methodology to assess the geographical BSE risk of small ruminants is not expected to be rapidly available given the complexity of the model to be developed and the data limitations on sheep. It is, nevertheless, expected that the programme of active rapid test-based monitoring of Scrapie/BSE in sheep in

---

3   Rapid TSE testing at slaughter of the spleen or brain of ARR/ARR animals above the age of 18 months from flocks with TSE would gradually provide conclusive evidence / reduce to negligible the risk that this genotype is a carrier of detectable infectivity levels.

2002 and the application of SSC's forthcoming protocol to investigate the presence of BSE in sheep, could rapidly provide essential information in the future.

8.   On the need to revise previous SSC-opinions on BSE in small ruminants

- With respect to the question whether there is currently a higher probability that BSE is present in small ruminants, the SSC confirms its opinion of 18-19 October 2001, that "there is (nevertheless) at present no evidence that BSE is present in small ruminants under field conditions".

- Whether or not casings obtained from sheep intestines can be considered as representing a negligible risk will depend upon the reduction resulting from the casing production process, the potential presence of infectivity in the parts of the intestine used for casing production and the age of the animal. Relevant data and information to assess the risk possibly posed by casings, are currently being collected by a number of research bodies and a final conclusion should await the outcome of this exercise[4].

- Regarding the safety of milk, the SSC considers that its Statement of 30 March 2001 remains valid, namely that the evidence available to date does not point to milk or colostrum representing a possible risk but that for precautionary reasons the milk, colostrum or milk products from suspect BSE cases should not be offered for consumption. The recommendations for research made in the above SSC statement and in several of the previously adopted scientific opinions remain valid.

---

[4]   The European Natural Sausage Casings Association (ENSCA) recently commissioned two studies related to the safety of small ruminant casings. One is as risk assessment of the use of sheep Casings, carried out by Det Norske Veritas (DNV) Consulting. The objective of the proposed study is to assess the potential exposure of the human population to the BSE agent through the consumption of sheep sausage casings on the assumption that BSE is present in sheep. Draft results are expected for end April 2002. In addition, the Department of Veterinary Medicine of the University of Utrecht has been asked to provide additional data for the risk analysis. These aim to determine the presence and decrease in possible infectious material during the cleaning process of sheep intestines and to assess the relative amount of possible infectious material in a typical leg of lamb as compared to a defined length of sheep casings. (Philip Comer, pers.comm., 24.03.02).

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C1 - Follow-up and dissemination of scientific opinions

REPORT ON SAFE SOURCING OF SMALL RUMINANT MATERIALS

(SAFE SOURCING OF SMALL RUMINANT MATERIALS SHOULD BSE IN SMALL RUMINANTS BECOME PROBABLE: GENOTYPE, BREEDING, RAPID TSE TESTING, FLOCKS CERTIFICATION AND SPECIFIED RISK MATERIALS)

FINALISED BY THE TSE/BSE *AD HOC* GROUP
AT ITS MEETING OF ON 21 MARCH 2002.

TABLE OF CONTENTS:

PREAMBLE:

The Scientific Steering Committee received between September and December 2001, various requests for scientific advice on issues related to BSE in small ruminants. The requests relate to (1) specified risk materials, (2) certification of flocks as representing a negligible TSE risk, and (3) genotype and TSE susceptibility.

Different specific reports have been prepared by 3 separate rapporteurs. These have been integrated into the current single report addressing the overall issue of *Safe sourcing of small ruminant materials should BSE in small ruminants become probable: genotype, breeding, rapid TSE testing, flock certification and Specified Risk Materials.*

This opinion and report complete, where appropriate, the opinions on Specified Risk Materials (SRM), genotyping, breeding for resistant flocks and flock certification adopted so far by the Scientific Steering Committee.

I.   BACKGROUND AND TERMS OF REFERENCE

a. In its opinion of 18-19 October 2001 the Scientific Steering Committee (SSC) considered that, should BSE in small ruminants become probable, its previous opinions on **Specified Risk Materials in small ruminants** would no longer be adequate.

b. In the same opinion the SSC considered that available data would not allow to comprehensively judge on **genotyping** as a possible tool as part of consumer protection strategy, e.g., in combination with rapid tests or removal of specified risk materials. The SSC recommended that the concept of genotyping as a tool for consumer protection be urgently verified.

c. The SSC further recommended that the general and EU-wide introduction of **breeding programmes** as an overall long term strategy for the reduction / elimination of TSE prevalence in populations of small ruminant should be evaluated in detail in the light of the existing uncertainties on the policy of breeding and genotyping of sheep. Meanwhile, the SSC announced the preparation of an opinion on the criteria which should be used in a widespread breeding programme for resistance to TSEs in small ruminants, should such programme be decided upon.

d. The SSC also regarded the implementation of a policy of **certification of flocks** as essential to guarantee safe sourcing at the long term. It announced the preparation of an opinion on the subject that would make provision for the application of expected future developments in the field of rapid tests and genotyping.

In addition, Commission services invited the SSC to address in its opinion provisions on culling of TSE affected flocks. They also asked the SSC whether, in the light of the 5 recent opinions of the French Food Safety Agency (AFSSA) related to the risk of BSE in small ruminants (dates of adoption: 14 February 2001, and 18 July 2001, two on 8 November 2001, 18 February 2002) and of possible other new scientific data and evidence, it maintains its opinions of March and October 2001 implying that there is currently no higher probability that BSE is present in small ruminants and that there is no reason to amend at this stage the list of Specified Risk Materials to be currently removed from the food chain.

The current report mainly deals with the above issues.

Keywords: TSE, BSE, flock certification, records, surveillance, testing, genotyping, specified risk materials, sheep, goats, small ruminants.

II. DEFINITIONS

For the purpose of this report:

- a *flock* is defined as a group of small ruminants kept under the same conditions

- a *certified TSE-negligible risk flock* is defined as a flock of small ruminants which gives sufficient guarantees with regard to those factors which could introduce the TSE agent into a flock in which no TSE has been observed so far or into a flock after total elimination of all TSE infected and possibly exposed animals. (Note: As the zero risk level does not exist, it is preferable to use the wording "TSE negligible risk" flocks rather then "TSE-free" flocks.)

- "*closed flock*": Flock for which sufficient guarantees can be given that the TSE agent can no longer be introduced (i.e., *via* live animals, feed, horizontal, vertical or environmental routes).

- "BSE in sheep being probable" implies[5] that there would be strong indications (including on the basis of circumstantial evidence from any domestic flock) or evidence of BSE actually being present in domestic flock. It includes thus the scenario that the actual presence of BSE in small ruminants would have become likely but not necessarily confirmed.

  Note: If it is assumed that exposure of small ruminants was from BSE though feed, then two separate issues need to be considered to assess the current risk: first regarding the infected feed itself and secondly the likelihood of any introduced 'new' strain being sustained in the small ruminant population with the same efficiency as established strains.

III. GENOTYPE AND SUSCEPTIBILTY OF SHEEP TO TSEs.

III.1. STATE OF KNOWLEDGE

Several summary overviews on the relationship between sheep genotype and susceptibility to TSEs have been prepared recently. To be mentioned are:

a) The Report of April 1999 of the SEAC[6] Subgroup on Research and Surveillance for TSEs in sheep.

b) The SSC opinion of 22-23 July 1999 on the policy of breeding and genotyping of sheep, i.e. The issue of whether sheep should be bred to be resistant to scrapie.

c) The WHO Consultation on Public Health and Animal Transmissible Spongiform Encephalopathies: Epidemiology, Risk and Research Requirements with the participation of Office International des Epizooties Geneva, Switzerland 1-3 December 1999. (Published as: WHO (2000) WHO/CDS/CSR/APH/2000.2

d) The SSC's Pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions. Adopted by the Scientific Steering Committee at its meeting of 8-9 February 2001.

e) The Opinion of the French Food Safety Agency (AFSSA[7]) of 8 November 2001 on Health monitoring measures concerning scrapie in sheep and goats.

---

[5] Definition based on the SSC's pre-emptive risk assessment of 8-9 February 2001,

[6] SEAC: Spongiform Encephalopathy Advisory Committee (United kingdom)

Annex 1 provides an overview of the genotype-related information contained in the above documents b), c) and d). In **Annex 2**, the genotype-related passages in the AFSSA opinion (above document e) are quoted[8]. In addition, the SSC opinion and corresponding report on *TSE Infectivity distribution in ruminant tissues (state of knowledge, December 2001)* (EC, 2002) provides an overview of the knowledge on relations between TSE infectivity and distribution in ruminant tissues. Finally, recent research on natural scrapie by Heggebo *et al* (2002) on the distribution and accumulation of PrP in gut-associated and peripheral lymphoid tissue of scrapie-affected Suffolk sheep, provides further corroborative information on the relationship between genotype and TSE susceptibility included in the above reports and papers.

As far as the relations between genotype and susceptibility of sheep to TSEs are concerned, Annexes 1, 2 and 3, EC (2002) and Heggebo *et al* (2002) can be summarised as follows **:**

III.1.1. As for **scrapie in sheep**, the state of knowledge can be summarised as follows:

- Susceptibility to TSEs depends on the genotype of the animal and also in sheep the incidence of TSEs is linked to PrP genotype, with codons 136, 154 and 171 being of major importance. Most scrapie-affected sheep are homozygous for glutamine (Q) at codon 171 and succumb to disease with ARQ/ARQ, VRQ/VRQ, VRQ/ARQ genotypes. [9] However, it should be noted that, for a given genotypic configuration, different breeds may show different susceptibility levels to a TSE.

- Occasionally scrapie occurs in ARQ/ARR and VRQ/ARR sheep. However, for a given level of exposure to a source of infection, the likelihood of becoming scrapie-infected is lower than with sheep that are homozygous for glutamine (Q) at codon 171. Also, during the preclinical phase, in heterozygote ARR/VRQ or ARR/ARQ ovines, PrP^{Sc} does not seem to be easily detectable in lymph tissue or in the digestive autonomous nervous system.

- Sheep of ARR/ARR genotype are considered to be the most resistant to scrapie. ARR/ARR animals so far have not shown to carry detectable infectivity or PrP^{Sc}, with the exception of one PrP^{Sc} positive case out of the genotyped scrapie cases[10]. Therefore although this cannot be 100% excluded, the likelihood of this genotype to become infected with scrapie seems to be very small.

- The available information is not enough to provide an answer to the question of what is the risk that flocks of scrapie resistant sheep would carry the scrapie agent without showing clinical signs and at the same time being able to transmit the agent horizontally, vertically or via rendering. This hypothesis can therefore not be excluded. Such a situation, if shown to exist in sheep (and as yet there is no

---

[7]   AFSSA: Agence Française de Sécurité Sanitaire des Aliments

[8]   Translated into English

[9]   V stands for valine, A for alanine, R for arginine, H for histidine and Q for glutamine

[10]   Ikeda *et al* (1995) describe a Western blot positive PrP^{sc} animal of ARR/ARR genotype. This animal is the one mentioned as the only reported ARR/ARR scrapie case. There are, however, no photos of the blot., There is also no mention of infectivity in the paper. The Working Group considers that detection of PrP^{Ss} by an unseen Western blot cannot be equated with detection of infectivity.

field proof of this), could lead to the maintenance of a low level of infection in a flock without any clinical signs.

For animals which remain healthy throughout a natural life-span, those genotypes of sheep which occasionally have scrapie cases, ARQ/ARR Suffolks and VRQ/ARR Cheviots for example, could be considered as potential carriers of hidden infection, whether or not they can pass it on to other animals. ARR/ARR animals so far have not been shown to carry detectable infectivity *or PrPres* at all, with the exception of the one equivocal case mentioned above.

It can be assumed that in the case of hidden infection / silent carriers, in an apparently resistant population would have very much lower titres of infection in tissues than would be found in susceptible sheep. This could also be reflected in the current efficacy of tests for TSE on peripheral lymphoid tissues or brain, as the concentration of PrP^{Sc} will be much lower (if any is present) in these animals and most probably under the detection limit of the present tests.

- The fact that sheep that are homozygous for glutamine (Q) at codon 171 are susceptible to scrapie, that ARR homozygous animals are resistant and that animals that heterozygous with one arginine (R) at codon 171 show an intermediate state of susceptibility, seems to be universal and not affected by the breed as such.

  Note: The output from TSE surveillance in sheep recommended in the SSC opinion of 29-30 November 2001 on TSE surveillance, should permit to derive, per country, the percentage of adult slaughtered sheep that are homozygous or heterozygous for glutamine or for arginine at codon 171.

- Available date (EC, 2002) suggest that unlike the situation in cattle experimentally infected by the oral route with a relatively large exposure dose of BSE agent, the results in sheep indicate a potentially widespread involvement of lymphoid tissues early in the incubation period at least in ARQ/ARQ scrapie/BSE susceptible sheep. This is corroborated by Heggebo *et al* (2002) who studied the distribution and accumulation of PrP in gut-associated and peripheral lymphoid tissue in eight animals (20-24 months of age) from (natural) scrapie-affected Suffolk sheep.

  The four clinically affected PrP(ARQ/ARQ) sheep had widespread accumulations of disease-associated PrP in the CNS, lymphoreticular system and peripheral ganglia. In the two PrP(ARQ/ARQ) sheep that did not show clinical signs of scrapie, only limited vacuolation and PrP accumulation were detected in the brain, but the results from the lymphoreticular system and peripheral nervous system were comparable with the clinically affected animals. The remaining PrP(ARR/ARR) and PrP(ARR/ARQ) sheep did not show proteinase K-resistant PrP accumulations in the lymphoid tissues examined and immunohistochemistry did not reveal the presence of disease-associated PrP. In lymphoid tissues of the PrP(ARQ/ARQ) sheep, the dominant localization of disease-associated PrP was in lymphoid nodules.

- From their ongoing research, J.Grosclaude, H.Rezaei and J.M. Elsen, (pers.comm, 31.10.01) conclude that current physicochemical data on the PrP protein support the hypothesis of ARR/ARR homozygous animals being fully resistant against TSE and the absence of carriership in animals with this genotype.

III.1.2. As for **BSE in sheep**, research data are available for only a few tens of animals:

- Results to date indicate that the relation between sheep genotype and susceptibility to a TSE is similar for scrapie and BSE: the ARR genotypes are apparently resistant to development of clinical disease on challenge with BSE and animals carrying the glutamine (Q) allele at codon 171 are potentially susceptible to BSE and to scrapie. The influence of the genotype at codon 136 and 154 is not yet known for BSE but is being tested by direct challenge studies at IAH, UK.

- New research data summarised in EC (2002) are consistent with the previously expressed view that BSE in sheep after oral exposure is pathogenetically closely similar to scrapie, particularly with respect to the tissue distribution of infectivity and/or PrP^{Sc}.

- Regarding the age, preliminary findings from a single experiment which examines the oral transmission of BSE to QQ$_{171}$ sheep indicate that, after what may considered a high exposure, detectable infection may be widespread in LRS by 10 months after exposure and may be present as soon as 4 months. Furthermore, natural scrapie of Romney sheep (to which pathogenetically experimental BSE in sheep bears a close semblance), PrP^{Sc} can be detected from two months of age in Peyer's patches and mesenteric lymph node in the VRQ/VRQ genotype (Andreoletti *et al.*, 2000). *This being the case LRS from BSE susceptible sheep must be considered a hazard at all ages of sheep* and there is thus no basis on which to recommend a [generally applicable] age cut-off for the presence of TSE infectivity in small ruminant tissues, at least for susceptible genotypes. This clearly includes the lymphoid tissue of the entire alimentary tract from oesophagus to rectum. The central nervous system may become involved as early as 10 months after infection; it is not possible from the available data to suggest whether the differences between the response in this respect in Romney and Suffolk sheep is significant in the context of populations of sheep. Nevertheless, it is encouraging that experimentally exposed sheep, semi-resistant to BSE infection have no evidence of PrP^{Sc} in tissues up to two years after a relatively high exposure dose. Moreover, with a range of much lower exposures in field situations that might be anticipated in endemic BSE in sheep and possibly different susceptible PrP genotypes in sheep, there may well be proportionally longer incubation periods and correspondingly later involvement of the CNS.

III.1.3. As for **TSE and genotype in goats** available information is very limited. In the absence of a more complete knowledge of the susceptibilities of different genotypes of goats to the BSE or scrapie agent it is therefore reasonable to assume for the time being that perhaps all goats may be susceptible to TSE by the oral route under certain conditions. Further research on goat PrP genetics is required[11].

---

[11] Nora Hunter (pers.comm, 2002) and colleagues have recently published data on challenge of goats with scrapie and with BSE and have described an association of codon 142 genotype in goats with incubation period. Although the data is limited, the current tendency to assume that goats will all be susceptible and with no resistance may be a reason for concern. There is no evidence either way. It may be safer to assume goats are all susceptible but further research is needed on this point.

III.2. FURTHER COMMENTS

From what precedes it may be concluded that homozygocity for the arginine (R) allele at codon 171 on its own is not a sufficient criterion to fully guarantee total absence of TSE risk. However, the probability of TSE infectivity being present is significantly reduced and, if present, the infectivity levels at a given period in time after exposure to the TSE agent, are likely to be much lower as compared to animals that are homozygous for glutamine (Q) at codon 171. Animals that are heterozygous with one arginine (R) at codon 171 show an intermediate state of susceptibility.

In practice this implies:

a) For flock certification and breeding programmes: that the TSE incidence and TSE-infective load in a flock or population will decrease with the introduction of the arginine (R) allele at codon 171.

b) For consumer protection: that animals that are homozygous for arginine at codon 171 represent a significantly lower risk but it cannot [yet] be concluded that they offer 100% protection.

The SSC opinion of 30 November 2001 on *Requirements for a statistically authoritative TSE survey* recommends to include in such surveys a genotyping programme to (a) adequately map scrapie susceptible sheep genotypes per country, and (b) confidently identify scrapie resistant sheep genotypes per country. Such programme would possibly make available within 1-2 years, a significant sample of genotyped test positive animals per country. As part of rapid testing, genotype information will indeed compliment the results. Animals testing positive for a TSE should be of a susceptible or semi-resistant genotype. If they are of a semi-resistant genotype, they should be significantly older than positive susceptible animals. Genotypes considered to be resistant should in principle not be found positive. A genotyping sub-programme would thus gradually provide sufficient data to conclude on issues such as absence of TSE infectivity in tissues of ARR/ARR animals regardless of the breed, possible presence of infectivity in lymphoid tissues of older resistant (ARR/ARR) or semi-resistant (**R/***] genotypes (indicating a possible status of silent carrier-ship in these animals), etc. The evidence will be even firmer if TSE-validated tests would be applicable on peripheral lymphoid tissues and spleen, possibly permitting detection of the presence of PrP^{res} indicating silent carriers.

Apart from the flock certification frame, genotype information will also be useful to:

- identify, before or at slaughter, animals that possibly pose a risk;

- identify, in a flock affected by TSE or epidemiologically related flocks, the animals that could possibly be exempt from the eradication strategy.

IV. BREEDING

a. On the basis of the same information as presented in Chapter III, breeding for resistant PrP genotypes is now being carried out in a number of countries, including in, for example, the UK, the Netherlands and France.

Worries have, however, been expressed about the potential long-term effects of such nation-wide and generalised programmes. If only ARR/ARR sheep were considered for commerce one really has virtually no information on how breeding for this allele might affect sheep either in respect of management (hardiness, resistance to harsh weather conditions, etc) or susceptibility to other diseases. Scrapie resistant animals could, for example, be less healthy in other respects or could be more susceptible to other diseases or have a lower weight at slaughter, etc. In addition, it has been suggested that the development of an entire national flock of ARR/ARR genotype (the most resistant known) could result in inadvertent selection of rare scrapie strains able to cause disease in this genotype.

In countries where the prevalence of TSE in sheep is considered to be significant, breeding for TSE-resistance can nevertheless be useful because it is sure that the VRQ allele codes for TSE susceptibility and is therefore an undesirable trait. Such programme should then in first instance be targeted at risk populations or areas.

The implementation of genetic programmes is extended over several years which provides opportunities to adjust or rectify the programme should it appear that unexpected and unwanted characteristics are introduced. The important condition is then, of course, that the (national) breeding programme co-operates with a close monitoring of its effects.

Although there is no evidence for any of the above-suggested hypothetical adverse effects, other options for disease control should remain the object of further investigations.

b. Few countries have experience with nation-wide programmes of breeding for TSE resistance in sheep. An example which can be mentioned is the UK National Scrapie Plan (NSP) which has three stages: (a) ram genotyping scheme to increase frequency of ARR allele in healthy flocks (b) monitoring for scrapie on farms taking part in the NSP (c) dealing with scrapie affected flocks.

Breeding for ARR/ARR genotypes in some breeds of sheep would be a multi-step process as in some breeds there are no ARR/ARR rams available and the frequency of ARR allele is low. In order to deal with this difficulty, there are two categories of membership of the NSP. A first category including farmers breeding using rams of the most scrapie resistant ARR homozygous genotype, and a second category with farmers breeding using only rams of the ARR homozygous and/or heterozygous genotype.

c. In the light of the above, the criteria for a breeding programme could therefore include:

-   An acceptable method of identifying individual sheep: e.g. electronic chips or boluses.

-   For each important breed: an approximation of the frequency of ARR/ARR sheep to give an estimate of how quickly the breed would be able to move towards use of ARR/ARR rams only. This could also apply generally within one country. There would have to be an agreement on how many animals per

breed or per country would be needed to be genotyped in order to say with any confidence what the ARR frequency is.

- An agreed procedure on scrapie monitoring given that very young animals or animals of heterozygous genotype may not show easily identifiable PrP^{Sc} in peripheral tissues.

- Genotype monitoring of all scrapie cases (or as many as possible) in order to have warning of the potential emergence of new scrapie strains able to cause disease more easily in the heterozygous genotypes (at the moment judged to be of intermediate susceptibility).

Very little is known about genotype frequencies in goats in general. Goats have the equivalent of the sheep ARQ allele and arginine at codon 171 has not been described to date although perhaps not enough animals have been studied. In order to reach agreement about a goat-breeding programme, further information on goat PrP genetics is required.

With respect to the occurrence of possible adverse effects, the following can be mentioned:

- Difficulties in some breeds or countries if ARR/ARR genotypes not available: e.g. in Iceland this genotype is rarely (if ever) found and this may be true in other cases. There should be a willingness to breed for other suitably identified genotypes in these cases.

- The last point also is relevant to goats. If a general rule is made that in goats also, ARQ/ARQ animals should be eliminated, this may wipe out goats entirely from Europe. We know already that codons other than 136, 154 and 171 are of importance in control of incubation period of experimental TSEs in goats e.g. codon 142 which has no importance in sheep scrapie. This information strongly suggests that goats should be investigated in their own right.

- New scrapie strains. It will be necessary to monitor the genotype of scrapie cases to see if genotype targeting of scrapie is changing or if a new strain is emerging. Also the monitoring of the PrP^{Sc} profile will be needed in conjunction with strain typing. This aspect is discussed in more detail in a forthcoming report of the TSE/BSE *ad hoc* Group presenting a *Protocol to investigate the possible presence of BSE in sheep*.

- Breed characteristics. It will be necessary to monitor breed characteristics in scrapie resistant genotypes to obtain reliable information on any changes in birth weight, growth rates, strength, resistance to other diseases etc.

V.   RAPID TSE TESTING

The use of rapid tests has the potential to form the basis for the establishment of the TSE status of an animal and flock.

- As the classical confirmatory tests such as histopathology, immunohistochemistry, Scrapie Associated Fibril (SAF) examination or classical Western Blot test are not suitable for large scale testing, in the framework of the certification of herds and large scale surveys, rapid tests should be used and the development of new rapid *in vivo* tests on peripheral lymphoid tissues, and ideally on blood should be an absolute priority.

- In terms of consumer protection, the use of rapid TSE tests on small ruminants will only result in a significant risk reduction if they can be applied on tissues that show infectivity in the early stages of incubation, for example lymphoid tissues.

  Currently, 3 "rapid TSE tests" are considered to be reliable detectors of BSE PrP^{Sc} in cattle brain material in the final stages of disease incubation. Initial validation results show that these tests are capable of detecting PrP^{Sc} also in sheep brains.

  The applicability of these tests on other tissue material, e.g., lymph nodes or spleen, can be accepted on theoretical grounds and limited data provided by the test companies suggest that these products may also work for such purposes. This would need to be validated because the infectivity levels in these tissues are lower than those in brain material and because the chemical and physical characteristics of the material itself are different.

- The application of the currently available tests to brain material from a large representative sample of slaughtered animals would rapidly provide a quantitative estimate of both the field incidence of TSEs in small ruminants and the age distribution of field TSE cases. This would, secondarily, provide a most valuable tool for assessing the real risk of exposure of consumers to BSE by consuming possibly infected small ruminants (if any) if subsequent study of TSE positive brains identified BSE[12].

  These rapid tests should also offer data to confirm the absence of PrP^{Sc} in fully resistant genotypes.

  It is however not expected that the *currently available* tests used on CNS material or after their validation for use on lymphoid or other tissues will provide a satisfactory level of direct and immediate consumer protection, for the following reasons:

  a) their current limits of detection cannot exclude the presence of infectivity levels that still would constitute a risk.

  b) testing of CNS tissues will not offer direct consumer protection because of the TSE pathogenesis pattern in TSE-susceptible small ruminants which causes infectivity to be present in peripheral tissues early in the incubation period. Indirectly they will, however, significantly increase the level of consumer protection, because they will contribute to improved culling policies and eradication programmes and to a better knowledge of the geographical and within-flock distribution of TSE.

---

[12] It should however be stressed that a positive test result would only indicate the presence of a TSE in the animal and not necessarily of BSE.

c) Tests that can be applied on, for example, lymphoid or peripheral tissues may be of limited use when applied on animals of a semi-resistant genotype because of the apparently limited involvement of peripheral tissues in the TSE pathogenesis of TSE in such animals.

- The possible application of immunological tests allowing early detection of PrP^{res} in peripheral tissues would offer a more accurate picture of the prevalence of infected animals.

VI.   CERTIFICATION OF FLOCKS

Annex 3 provides a detailed background on the issue of flock certification as possible part of TSE risk reduction strategies. From the annex it can be derived that:

- As a rough estimate, for approx.10% of the scrapie cases, the age at onset is above 5 years;
- TSE infectivity can be present for several years in an animal and in a flock before clinical cases appear;
- The probability of TSE infection developing into clinical disease would also be determined by the genotype composition of a flock,
- There is no evidence for silent carriership in resistant genotypes, although it cannot be excluded yet and,
- There are indications for horizontal / peri-natal transmission in sheep,

It follows that, *ideally*, a flock could be declared as being of negligible TSE risk only if the prevalence of TSE can be ruled out in a significantly large number of animals of the generation following the "closure" of a flock, that were kept alive for a number of years that corresponds to the upper limits of the incubation period. The data on age of onset show that 90% of the clinical TSE cases in sheep appear at an age below 5 years[13]. So, full certification on the basis of records alone of a flock would probably not be possible within 7 – 10 years and provided a sufficiently large number of animals[14] have been kept alive to exclude the prevalence of TSE infectivity in the flock.

Such full certification is probably not an approach that is readily applicable under real field conditions. The Working Group considers that a practical alternative, which is compatible with field reality and with current knowledge on TSEs in sheep, would be to option for the concept of a "provisional certificate of negligible TSE risk", attributed on the basis of less stringent time criteria but to be applied in combination with other criteria such as testing and genotyping.

---

[13]   For semi-resistant animals, this age would be higher and, moreover, the likelihood to find a TSE case would be lower.

[14]   The flock size corresponding to "a sufficiently large number of animals" depends on many factors, as indicated above: genotype composition, age at slaughter, etc. For flocks entirely composed of "susceptible" genotype(s), the likelihood to find any clinical disease in an infected environment is higher. For flocks entirely composed of "resistant" genotype(s), the likelihood is high to never find any clinical disease and the flock size would therefore need to be very large. However, although the absence of detection of clinical disease in such "resistant" flock during a period of 5 years would not automatically imply total absence of infectivity, one can nevertheless accept that the levels of infectivity, if present, would be very low.

Depending upon the available information, time horizons between 0 and 5 years could be envisaged, as illustrated in the scenarios presented in **Annex 3**. They suggest a possible sequential and evolving scheme of certification which recognises the current ability to test for PrP^{Sc} in post-mortem material, the validated and sensitive *in vivo* tests that are likely to become available in a foreseeable future and the advantage that can be taken from genotype information.

VII. DISTRIBUTION OF TSE INFECTIVITY IN SMALL RUMINANT TISSUES

From **Annexes 1 and 2** on TSE susceptibility and genotype and from EC (2002) on infectivity distribution in ruminant tissues, the following can be derived:

VII.1. Infectivity in tissues and genotype of an animal with respect to TSE-resistance.

a) Results to date indicate that the relationship between sheep genotype and susceptibility to a TSE and tissue distribution of infectivity and/or PrP^{Sc} is similar for scrapie and BSE.

b) For a given level of exposure to a source of TSE infectivity, and compared to animals of a TSE-susceptible genotype, the probability of finding infectivity or clinical TSE is significantly lower for animals of a semi-resistant genotype (ARQ/ARR and VRQ/ARR animals, for example) and very low (if existing at all) for animals of resistant genotypes (ARR/ARR). Also, during the preclinical phase, in heterozygotic ARR/VRQ or ARR/ARQ ovines, PrP^{Sc} does not seem to be detectable in lymph tissue or in the digestive autonomous nervous system.

VII.2. Infectivity distribution in tissues in TSE-susceptible animals

a) Unlike the situation in cattle experimentally infected by the oral route with a relatively large exposure dose of BSE agent, the results in sheep indicate a potentially widespread involvement of lymphoid tissues early in the incubation period at least in ARQ/ARQ scrapie/BSE susceptible sheep.

b) The SSC opinion of 10-11 January 2002 (E.C., 2002) summarises the knowledge available in December 2001 on the distribution of TSE infectivity in the tissues of ruminants. For the purpose of the current report, and with regard to BSE, the following can be mentioned

   - The opinion confirms (i) the possible presence of infectivity in central nervous tissues, peripheral nervous tissues, lymphoid tissues including the ones associated with the intestine and a number of other tissues such as liver, placenta, etc., and (ii) the absence of detectable TSE infectivity in skeletal muscle in both sheep with scrapie and with experimental BSE.

   - In experimental BSE in sheep after oral exposure disease specific PrP is described in the stomachs (abomasum and forestomachs) of susceptible sheep (Jeffrey *et al* 2001) and this is complimented by the finding of infectivity in these tissues relatively late in the incubation period (Bellworthy, personal communication in E.C., 2002). This contrasts with the earlier report (Foster *et al* 2001) in which assays of infectivity on stomachs of BSE-dosed terminally affected susceptible sheep were negative.

- No infectivity has been detected in **milk** of sheep with scrapie or BSE, but as far as BSE is concerned, the experimental data are very limited. It is understood that further research with regard to BSE and milk safety is ongoing (FSA, December 2001, pers.comm.).

  Notes:

  - The TSE/BSE *ad hoc* Group took note of the risk assessment of milk products under the hypothesis that BSE would be present in small ruminants presented in AFSSA (2002). This assessment also includes a theoretical analysis of the exposure if consumers to milk products and cheese produced from milk from animals a flock with BSE. The Group considers that some of the assumptions made may need to be justified further, for example the assumptions on the estimation of possible theoretical maximum levels of infectivity in milk on the basis of infectivity titres found in blood buffy coat.

  - Regarding the one single case reported on in 1992 of a 38-year-old pregnant woman with sporadic CJD whose colostrum was found to be infected when injected i/c into mice (Tamai *et al*, 1992) and mentioned in AFSSA (2002), the TSE/BSE *ad hoc* Group signals a Statement 30 March 2001 of the SSC on the safety of milk which relates to the importance to be attributed to Tamai et al (1992).

  The TSE/BSE *ad hoc* Group considers that the Statement of the SSC of 30 March 2001 on the safety of milk remains valid, namely that the evidence available to date does not point at milk or colostrum representing a possible risk but that for precautionary reasons the milk, colostrum or milk products from suspect BSE cases should not be offered for consumption. The recommendations for research made in the above SSC statement and in several of the previously adopted scientific opinions remain valid.

- Infectivity has been detected inconsistently in the **placenta** of sheep with preclinical and clinical scrapie. (Race et al, 1998) There has been no study as yet of placental infectivity on BSE-exposed sheep.

- Infectivity has been detected in the **liver** of sheep with clinical scrapie and recently (S.J.Bellworthy, pers.comm, 2002), low levels of infectivity have been found during the incubation period of TSE-susceptible sheep experimentally infected with BSE.

- As in scrapie (EC, 2002), there is a widespread alimentary tract involvement during the incubation of experimental BSE in TSE-susceptible sheep (Bellworthy, pers.comm., 2002; unpublished results from the UK Veterinary Laboratories Agency), not only covering tissues and organs such as the intestine itself, the forestomachs and the abomasum, but also closely related lymph nodes, the mesenteric lymph nodes and other lymph nodes, e.g. the bronchial/mediastinal lymph nodes.

The SSC opinion of 11-11 January 2002 on tissue infectivity distribution (E.C., 2002) also considers that, should the presence of BSE in small ruminants become probable, the whole head should be considered to pose a possible risk.

c) In its opinion of 26-27 October 2000 on the Implications of Houston *et al* (2000) on the Transmission of BSE by **blood** transfusion in sheep,[15] the Scientific Steering Committee considers that, pending confirmation, the data in this experiment show that the exchange by transfusion of (400 ml of) whole blood taken during the incubation period of a sheep infected with the BSE agent can transmit BSE to a healthy sheep. This ovine model adds to data obtained in mouse and hamster models of scrapie and human TSE. The SSC considered that the data are preliminary and still lack results from the controls and do not confirm the identity of the strain (scrapie or BSE) in both the donor and recipient animals. They can therefore only be considered a tentative evidence of the transmissibility of the BSE agent through blood but neither justify nor add arguments for the introduction of new methods or approaches to the assessment of blood safety.

Given the current uncertainty concerning the infectivity of blood, and should BSE become probable in sheep, as a precaution different measures should be applied concerning the meat and milk of sheep depending on their genotype.

d) Heggebo *et al* (2002) found large accumulations of (scrapie-)disease associated PrP in the lymphoid nodules of the alimentary tract at the late pre-clinical and clinical stage of infection in natural scrapie-affected animals of Suffolk sheep. (The affected animals were of the ARQ/ARQ genotype. No disease-associated PrP was found in the ARR/ARR and ARR/ARQ animals of the flock.)

Lacroux *et al* (2001) and AFSSA (2001c) elaborate on the possible presence of infectivity in the intestine after the process of cleaning and preparation for use as casings. It may be concluded that this process significantly reduces the amount of cells (e.g. lymphoid cells of Peyer patches, autonomous nerve plexus cells) that possibly may contain infectivity should the animal be incubating, but that this reduction is not total.

e) **Summary**

Taking into account the scientific evidence and uncertainties listed above, the information contained in the SSC opinion of 10-11 January 2002 on TSE infectivity distribution in ruminant tissues (EC, 2002), the information contained in Lacroux *et al* (2001) and AFSSA (2001c) and Jeffrey *et al* (2002)[16] and Heggebo *et al* (2002) the following can be concluded regarding tissues potentially posing a risk in small ruminants (as very limited data are available for goats, the list for sheep is considered to be a reasonable approximation also for goats):

- The entire head;
- The spinal cord and associated DRG;

---

[15] **Houston, F., Foster, J.D., Chong, A., Hunter, N., Bostock, C.J., 2000.** Transmission of BSE by blood transfusion in sheep. Research letter. The Lancet, **356:** 999-1000; 955-956; 1013.

[16] See annex 1, section II.d

- The spleen;
- The entire alimentary tract from oesophagus to rectum of BSE exposed sheep of a susceptible genotype. This would also include closely related lymph nodes, the mesenteric lymph nodes and other lymph nodes, e.g. the mediastinal lymph nodes (which can also be taken when the oesophagus and lungs are removed from the carcass) as well as the innervation.

  Note: The Working Group supports the opinion of 8 November 2001 of the French Food Safety Agency (AFSSA, 2001c), considering that cleaning and processing of the intestine will reduce its infectivity level, but that there is evidence that such cleaning under practical circumstances is not thorough enough to remove all cell- and tissue material that could contain infectivity.

- Other lymphoid tissues (e.g., tonsils) and lymph nodes (e.g., prescapular lymph nodes, supra mammary lymph nodes, …);
- The liver; the pancreas;

Whether or not these tissues effectively pose a risk in the food, feed or animal by-product recycling chains will depend upon a number of factors such as whether or not the presence of BSE in domestic sheep flocks is probable, the origin of an individual animal and the TSE status of its flock of origin, its age and genotype and whether it was TSE-tested or not. This is further clarified in the next sections.

VII.3. Infectivity distribution in tissues and age.

a) Infection under natural conditions most likely takes place at young ages (e.g. peri-natal).

b) Regarding the age, preliminary findings on TSE susceptible sheep indicate that, after what may be considered a high exposure, detectable BSE infection may be widespread in LRS at 10 months after exposure and may be present as soon as 4 months in homozygous ARQ/ARQ animals (but such early presence of infectivity was not observed in heterozygous animals carrying an arginine ??(3) allele at codon 171). Furthermore, in natural scrapie of Romney sheep (to which pathogenetically experimental BSE in sheep bears a close semblance), PrP^{Sc} can be detected from two months of age in Peyer's patches and mesenteric lymph node in a susceptible genotype.

c) The central nervous system may become involved as early as 10 months after infection.

Nevertheless, it is encouraging that experimentally exposed sheep, semi-resistant to BSE infection have no evidence of PrP^{Sc} in tissues up to two years after a relatively high exposure dose of 5 grams. Moreover, with a range of much lower exposures in field situations that might be anticipated in endemic BSE in sheep and possibly different susceptible PrP genotypes in sheep, there may well be proportionally longer incubation periods and correspondingly later involvement of the CNS.

Note: The determination of the exact age of small ruminants may pose practical problems. Ferguson *et al* (2002) state: "*Without an individual sheep-tracing system it is impossible to identify individual animals definitively. Thus, verifying an animal's age and flock of origin is extremely difficult. Although age-based controls might be, in part, based on markers of age (as the current SRM restrictions relate to sheep and goats aged over 12 months or that have a permanent incisor erupted through the gum), tooth-based ageing of lambs may be less reliable but might be based on the eruption of molar 1 at 3 months, molar 2 at 9 months or molar 3 at 18 months.*"[17] **Approximately 6 months** would in practice correspond with an animal that has not yet 2 molars erupted.

VII.4. Relative TSE infectious loads associated with tissues in sheep.

Recently, a report and a publication became available that provide information which merits to be exploited in the context of a quantitative assessment of the possible TSE exposure risk associated with tissues in sheep:

- Ferguson *et al* (2002) and its supplement, propose, on the basis of recent and still ongoing research, an *estimation* of an incubation-stage dependent profile of ovine BSE tissue infectivity titres. The incubation stages are at 1, 3, 6, 18 and 36 months. 36 months corresponds, in the proposed profile, with the time of onset of clinical disease. The infectivity titres are given as $\log_{10}$ ID_{50}/g mice i/c values.

  Note: (From Ferguson and Donnelly, 2002): "Very limited data on the level of PrP^{Sc} infectivity in different body tissues for BSE in sheep. All available data have been used, but current assays have limited accuracy, and data from only two time points in the incubation period of experimental BSE infections in sheep have currently been collected (6 months post inoculation, and at clinical onset). Hence data derived from scrapie pathogenesis studies have been used to allow extrapolation of infectivity estimates across the entire BSE incubation period. Quantitative infectivity titre data for many tissues is simply not available for either BSE or scrapie; in such cases we have used semi-quantitative data from PrP^{Sc} detection studies to derived assumed values of infectivity with reference to data from tissues where both titre and detection data are available."

- DNV (2001) develops a risk assessment of exposure to BSE infectivity in UK sheep. On the basis of the SSC's opinion on (E.C., 2000b) on oral exposure of humans to the BSE agent: infective dose and species barrier it suggests that the infectivity density for humans of brain of clinical diseased (sheep) animal has a *median* value of 0.25 human oral ID_{50} per gram and a 95 percentile range of 0.002 to 50 human oral ID_{50} per gram The DNV report also provides data on the weights of sheep tissues and carcasses.

A combined exploitation of both publications **would** permit to tentatively *estimate* the reduction of risk obtained by the removal of certain tissues at various stages of incubation. **Table 1** presents the *estimated* approximate ovine BSE *infectivity loads (expressed as total mouse i/c ID_{50})* values at 5 time points during incubation.). No correction for variation with age of tissues has been introduced. Such correction

---

[17] These are approximate ages, as there exist a breed-specific variability. For example, certain goat breeds have their teeth eruptions at later ages. This biological variability should be taken into account when developing practical measures.

would not significantly affect the comparative value of the estimates presented. It is currently very difficult if not impossible to convert these comparative risk (reduction) levels into absolute values of human exposure risk: a number of essential input data are indeed still unknown (e.g, the species barrier and infectious dose, …) or debated (e.g./, the efficiency of i/c as compared to oral transmission; see also: Taylor *et al*, 2001).

Given the current limited knowledge on TSEs, the values in **Table 1** can only be very approximative. It appears nevertheless that, as compared to the CNS tissues, the potential PrP^{Sc} load in the intestine of small ruminants is higher in the beginning of the incubation period and of the same order of magnitude to wards the end of the incubation.

Table 1: **Estimated approximate ovine BSE infectivity loads, expressed as total mouse i/c ID$_{50}$ (and as a fraction of the total infectivity load recovered in all tissues listed at 36 months) at 5 time points during incubation. (Table integrating Ferguson *et al* (2002) and DNV (2001))[+].**

Tissue	Weight* (grams)	Estimated approximate BSE infectivity load (expressed as total mouce i/c ID$_{50}$) Months after infection (% into incubation period)				
		1 m [2.78 %]	3 m [8.33 %]	6 m [16.67 %]	18 m [50.00 %]	36 m [100 %]
Brain	100	°1×10^1 (1.1×10^{-7})	°1.0×10^2 (1.1×10^{-6})	°3.2×10^2 (3.6×10^{-6})	3.2×10^4 (3.6×10^{-4})	3.2×10^7 (0.36)
Spinal cord	40	°0.4×10^1 (4.6×10^{-8})	°1.3×10^2 (1.5×10^{-6})	1.3×10^3 (1.5×10^{-5})	4.0×10^4 (4.6×10^{-4})	1.3×10^7 (0.15)
Peripheral nervous system	20	°6.4×10^1 (7.3×10^{-7})	°6.4×10^2 (7.3×10^{-6})	6.4×10^3 (7.3×10^{-5})	6.4×10^4 (7.3×10^{-4})	6.4×10^4 (7.3×10^{-4})
Lymph nodes, tonsils,	40	°1.3×10^3 (1.5×10^{-5})	4.0×10^3 (4.6×10^{-5})	4.0×10^4 (4.6×10^{-4})	1.3×10^6 (1.5×10^{-2})	1.3×10^6 (1.5×10^{-2})
Spleen	100	3.2×10^2 (3.6×10^{-6})	3.2×10^3 (3.6×10^{-5})	3.2×10^6 (3.6×10^{-2})	3.2×10^6 (3.6×10^{-2})	3.2×10^6 (3.6×10^{-2})
Intestines[§]	1200	°1.2×10^4 (1.4×10^{-4})	°3.8×10^4 (4.4×10^{-4})	3.8×10^5 (4.4×10^{-3})	3.8×10^7 (0.44)	3.8×10^7 (0.44)
Liver	610	°6.1×10^2 (7.0×10^{-6})	°6.1×10^3 (7.0×10^{-5})	6.1×10^4 (7.0×10^{-4})	1.9×10^5 (2.2×10^{-3})	1.9×10^5 (2.2×10^{-3})
Stomachs (rumen)	1000	°1.0×10^3 (1.1×10^{-5})	°1.0×10^3 (1.1×10^{-5})	°3.2×10^3 (3.6×10^{-5})	3.2×10^4 (3.6×10^{-4})	1.0×10^5 (1.1×10^{-3})
Thymus	300	°9.5×10^1 (1.1×10^{-6})	°3.0×10^2 (3.4×10^{-6})	9.5×10^2 (1.1×10^{-5})	9.5×10^3 (1.1×10^{-4})	3.0×10^4 ($3.'\times10^{-4}$)
Other: heart, lung, kidney, etc.	750	°7.5×10^1 (8.6×10^{-7})	°7.5×10^2 (8.6×10^{-6})	°2.4×10^3 (2.7×10^{-5})	7.5×10^3 (8.6×10^{-5})	7.5×10^3 (8.6×10^{-5})
Totals	**4160**	**1.5×10^4 (1.8×10^{-4})**	**5.4×10^4 (6.2×10^{-4})**	**3.7×10^6 (4.2×10^{-2})**	**4.3×10^7 (4.9×10^{-1})**	**8.7×10^7 (1)**

Notes: + The presented estimated loads assume that the animal is BSE-susceptible. Table 1 is a compilation of information from various sources and experiments and it is there fore impossible to specify the animal breeds or mice lines on which the data were obtained. For full list of references: see Ferguson et al, 2002.

* Tissue weights: approximate for adult animals; no corrections for age have been introduced.

\# The total weight of the peripheral nerves is estimated to correspond to ½ of the spinal cord mass.

° conservatively assumed values

§ The weight of the intestine of adult sheep, manure stripped at the abattoir, before further cleaning and processing is, according to Leatherhead Food RA (Leatherhead FRA, 1997), 1200g. Weights of sheep small intestine have been provided by other authorities:

- UK hoggets, manure-stripped intestine less the ileum and small part of the jejunum, mean weight of a single intestine from a set of ten intestines - 1600g (range: 1200 - 2000 grams) (P.Comer, pers.obs., 26.03.02.)

- UK spring lambs, manure-stripped intestine less the ileum and small part of the jejunum, mean weight of a single intestine from a set of ten intestines - 590g (Bradley, 2002; referring to R Harder, pers.comm).

- Dutch sheep intestine of unspecified age and anatomical region and unspecified as to whether manure-stripped or not: 960 – 1120g (B.Berends, referring to P.Koolmeees, pers.obs.).

The range of weights (variation) is attributed to the age of the sheep, the time of year (that also influences the mean age of lambs for slaughter) and the diet.

After processing to produce a natural casing the weight of the intestine is reduced by between 77% - 89%. The resultant casing weight (excluding the ileum and small part of the jejunum) varies in the range from 60g – 370g. For example, the mean weight of one casing from a set of ten casings from UK Spring lambs (see above) is 136g.

One sheep intestine has a length of 18-34 metres. When processed to produce a natural casing approx. 0.5 m are needed to envelop 5 sausages.

The natural casing at the point in time where it is ready to be filled to produce links of sausages, is histologically almost entirely collagen that contributes to its strength, versatility and usefulness as a sausage envelope. However, it is likely to consistently retain autonomic nervous tissue from Meissner's plexus and variable/inconsistent, but generally small amounts of mucosa and lamina propria and residues from some Peyer's patches, particularly those that penetrate the muscularis mucosae, which otherwise forms the inner boundary of the casing.

The residual infectivity in natural casings prepared from sheep intestines will depend upon the extent of removal of infectious components of the original intestine. The amount of overall reduction of infectivity resulting from the casing production process will depend upon whether infectivity is homogeneously distributed within the parts of the intestine used for casing production, the timing of exposure to infection in relation to the timing of slaughter, the age of the sheep, its *PrP* genotype and the strain of agent.

What is of interest here is the titre of infectivity present in the intestine before processing, its anatomical and histological distribution within the intestine and the residual titre of infectivity in the natural casing prepared from an infected intestine including if the residual infectivity is homogeneously or variably distributed throughout its length. It is also important to know if a human infectious oral dose could be consumed from a single robust meal of sausages. Critical data to answer these questions are absent. However, immunohistochemical and other appropriate methods to detect PrP^{Sc} have been used on sheep intestines and casings from animals with scrapie. If detection of PrP^{Sc} is equivalent to detection of infectivity and if this can be quantified, some additional insight might be provided into the residual risks from TSE infectivity in natural sheep casings in the event that natural BSE occurs in sheep. Such interpretations should be cautiously used until the necessary supporting data are produced.

The estimated infectivity reduction resulting from the casing production process varies according to the source from 100 (e.g., DNV, 2001) to 10 (e.g., Ferguson and Donnelly, 2002). A key unknown in this context is to what extent infectivity is homogenously distributed within the intestine and the initial starting titre for natural BSE in sheep. If one does assume homogenous distribution then the relevant net infectivity reduction would be 85-90 %. Aggregation of infectivity could of course make the figure much higher in specific parts of the intestine and much lower in other parts. All this depends on what precise tissues the infectivity studies were actually testing in the case when 'intestine' is listed. (Ferguson, pers.comm., 26.03.02). On the basis of currently available data (AFSSA, 2001c; Bradley, 2002) it is not possible at this time to use the anatomical description of the three parts of the small intestine (duodenum, jejunum and ileum) as a specific criterion to judge on the (level of) residual infectivity in any particular small section within these three anatomical parts. However, it is likely that of these three parts the ileum would present the greatest risk if only for the reason that the largest continuous Peyer's patch occurs there and there is clear evidence from other TSE (BSE in cattle and scrapie in sheep) that infectivity is consistenty present there and from an early age.

VIII. **SOURCING OF SMALL RUMINANT MATERIALS SHOULD BSE IN SMALL RUMINANTS BECOME PROBABLE.**

Safe sourcing of small ruminant materials, should the presence of BSE in such population(s) become probable, needs to combine several strategies, including testing, genotyping, removal of tissues known to pose a risk of containing infectivity as from a given age and individual animal and flock tracing. These strategies will need to be implemented where the probability of BSE infectivity being present in an animal offered for slaughter is considered to be non-negligible. The latter depends on the overall TSE incidence and the size of the fraction of it probably being BSE.

a. Sourcing from geographically safer areas as a possible tool for risk reduction.

As for BSE, the possible risk of materials sourced from small ruminants potentially infected with BSE, will vary with the geographical origin of the animals which in its turn will determine risk factors such as possible unsafe feeding practices, possible imports of animals with a high BSE risk, reliability of the existing surveillance system, etc. The SSC has been invited to prepare an opinion on the geographical BSE risk of small ruminants. The opinion is, however, not expected to be rapidly available given the complexity of the model to be developed and the data limitations on sheep. It is nevertheless expected that the programme of active rapid test-based monitoring of TSEs in sheep that [is about to] started in early 2002 and the application of the SSC's forthcoming protocol to investigate the presence of BSE in sheep, could provide essential informations in the future.

b. The use of rapid tests

In terms of consumer protection, the use of rapid TSE tests on small ruminants will only result in a significant risk reduction if they can be applied on tissues that show infectivity in the early stages of incubation, for example lymphoid tissues, and if the detection limits are sufficiently low.

The currently available rapid bovine TSE tests, if confirmed to be applicable to sheep central nervous system tissues, will not offer full consumer protection because of the TSE pathogenesis pattern in TSE-susceptible small ruminants which results in infectivity in peripheral tissues early in the incubation period.

The development of tests applicable to lymphoid tissues is still ongoing and will probably not be available for routine applications in the near future. Once available they permit to early identify the animals that pose a TSE risk provided they are senstitive enough to detect low levels of infectivity/PrP^{Sc}. Conversely, in semi-resistant animals, detectable infectivity may never be present in certain lymphoid tissues so that only tests applied to CNS at the end of the incubation period would detect the case.

c. Genotype and age as a sourcing criteria

Animals of a susceptible genotype or animals for which the genotype is unknown, are potentially TSE-susceptible. The currently available rapid TSE tests do not permit to confidently guarantee the absence of infectivity levels that could pose a risk to humans. Research data show that there is no [generally applicable] age cut-off below which infectivity levels in certain or all tissues would be below levels that pose a risk. for the small ruminant SRMs were BSE to be confirmed in small ruminants, at least those of susceptible genotypes. Therefore, the entire animal at all ages and its (derived) products (except wool and hooves and similar) poses a potential BSE risk.

For animals of a resistant genotype (ARR/ARR): the tissues listed in section VII.2. pose a risk for animals above *(suggested, expert judgement:)* approximately **18** months. Rapid testing will provide additional confirmation that infectivity levels are below detection level.

For semi-resistant animals: the tissues listed in section VII.2. pose a risk for animals above *(suggested, expert judgement:)* approximately **6** months. Rapid testing of CNS tissues will not provide information useful to reliably judge the safety of a carcass. Tests on lymphoid tissues, once available, are expected to provide additional confirmation that infectivity levels in other tissues are below detection level.

The above suggested age thresholds have been chosen because the (limited) available scientific evidence indicates that:

-   For resistant genotypes: the probability of nevertheless becoming TSE infected is very low, and the disease pathogenesis would then most likely be very slow and not result in significant infectivity levels in young animals. (The threshold of 18 months also approximately corresponds with the lower quintile of the incubation period distribution: most animals are older, if they develop a clinical TSE.)

-   for semi-resistant animals: as compared to susceptible genotypes, the probability is lower of becoming TSE infected, and the disease pathogenesis seems then to be significantly slower.

The suggested age thresholds could be revised in the light of the outcome of genotyping programmes which could show, for example, that infectivity is never found in animals of a semi-resistant or resistant genotypes below a given age.

d. Flock certification

Animals from a certified TSE-free or TSE-negligible risk flock would represent no risk. However, infectivity can be present for years in animals and flocks that were apparently TSE-free in terms of manifestation of the disease before coming under observation. The implementation of a comprehensive programme leading to the possible certification of flocks as being "TSE-free" or (preferable) representing and negligible TSE risk, would therefore in most cases and for most countries require many years. An approach of less stringent "provisional

certification", is a possible alternative which could be implemented in 4-5 years, if, where necessary, it is applied in combination with other criteria such as testing, genotyping and removal of tissues potentially representing a risk. **Annex 3** provides details on flock certification. The framework presented hereafter shows how the concept of certified flocks may fit in an overall strategy of risk management.

e. An example of possible framework for the future.

Ideally, a structured sourcing based on testing, SRM removal, and utilisation of carcasses, according to age, could be applied. Such approach would require individual animal tracing and identification systems, flock certification systems, validated tests that can be applied earlier in the incubation period on non-CNS tissues, routine facilities for genotyping and a system that would make all this information rapidly available at the slaughterhouses. **Table 2** provides an *example* of a structured approach.

Table 2:   **Sourcing of small ruminant materials** *should BSE in small ruminants become probable***.**

			Certified as "TSE-negligible risk flock"?						Full certificate		
			No			Provisional					
			Genotype			Genotype			Genotype		
Result rapid TSE test			-/?	+	++	-/?	+	++	-/?	+	++
	No testing	Age (months) < 6	HH	SRM	LL	SRM	LL	LL	LL		
		6-18	HH	SRM	LL	SRM	SRM	LL			
		> 18	HH	HH	SRM	SRM	SRM	LL			
	Negative (CNS)	< 6	HH	SRM	LL	SRM	LL	LL			
		6-18	HH	SRM	LL	SRM	SRM	LL			
		> 18	HH	SRM	SRM	SRM	SRM	LL			
	Negative (LN)	< 6	SRM	SRM	LL	SRM	LL	LL			
		6-18	SRM	SRM	LL	SRM	SRM	LL			
		> 18	SRM	SRM	SRM	SRM	SRM	LL			

Comments and footnotes to **Table 2**:

++   resistant genotype;   +: semi-resistant genotype;   -: susceptible genotype;   ?: genotype unknown

HH   Highest risk (no safety guarantee); no safe consumption; the whole animals is an SRM wit the possible exception of hooves and wool

SRM   Risk present (limited level of safety), but controllable by SRM removal (see list in VII.2)
LL   Lowest risk (negligible risk / safe); no removal of SRMs needed.

IX. **ASSESSMENT OF THE NEED TO REVISE PREVIOUS SSC OPINIONS.**

In addition to the issues addressed in the previous chapters, the SSC was asked whether, in the light of the 4 recent opinions of the French Food Safety Agency (AFSSA, 2001a, 2001b, 2001c, 2001d) and of possible other new scientific data and evidence, it maintains its opinions of March and October 2001 implying that there is currently no higher probability that BSE is present in small ruminants and that there is no reason to amend at this stage the list of Specified Risk Materials to be currently removed from the food chain.

With respect to the question whether or not there is currently higher probability that BSE is present in small ruminants, the Working Group subscribes the SSC opinion of 18-19 October 2001, that "*there is [nevertheless] at present no evidence that BSE is present in small ruminants under field conditions*". The present report is therefore to be considered as a conversion and further exploitation of the opinions and documents already adopted by the SSC, into a set of scientific platforms ~~bases~~ to support risk reduction strategies should the presence of BSE in small ruminant populations become probable.

Regarding the question whether or not there are reasons to amend at this stage the list of Specified Risk Materials (SRMs) to be currently removed from the food chain, the Working Group considers that from the data and evidence provided in Lacroux *et al* (2001), AFSSA (2001c), DNV (2001), Ferguson *et al* (2002) and Heggebo *et al* (2002) it appears that, as compared to the CNS tissues which are currently included in the list of SRMs, the potential PrP^{Sc} load in the intestine of small ruminants is higher in the beginning of the incubation period and of the same order of magnitude to wards the end of the incubation.

These data also show as from approx. half the incubation period, a potentially significant infectious load in the lymph nodes and tonsils (spleen excluded) in TSE-susceptible animals. For at least 95% of the field cases of a TSE in small ruminants, the incubation period exceeds 12 months.

IX **REFERENCES:**

AFFSA (Agence Française de Sécurité Sanitaire des Aliments), 2001a. Avis sur l'actualisation de la liste des matériaux à risque spécifié chez les ovins et les caprins**.** Scientific Opinion Adopted by AFFSA on 14 February 2001. [Text translated into English.]

AFFSA (Agence Française de Sécurité Sanitaire des Aliments), 2001b. Avis concernant un projet d'arrêté modifiant l'arrêté du 17 mars 1992 relatif aux conditions auxquelles doivent satisfaire les abattoirs d'animaux de boucherie pour la production et la mise sur le marché de viandes fraîches et déterminant les conditions de l'inspection sanitaire de ces établissements. Scientific Opinion Adopted by AFFSA on 18 July 2001. [Text translated into English.]

AFFSA (Agence Française de Sécurité Sanitaire des Aliments), 2001c. Avis concernant l'évaluation des effets de la technique du délimonage appliquée aux intestins d'ovins au regard du risque d'encéphalopathies spongiformes subaiguës transmissibles. Scientific Opinion Adopted by AFFSA on 8 November 2001. [Text translated into English.]

AFFSA (Agence Française de Sécurité Sanitaire des Aliments), 2001d. Avis concernant les mesures de police sanitaire relatives à la tremblante des ovisn et caprins. Scientific Opinion Adopted by AFFSA on 8 November 2001. [Text translated into English.]

AFFSA (Agence Française de Sécurité Sanitaire des Aliments), 2002. Avis sur l'analyse des risques liés aux encéphalopathies spongiformes transmissibles dans les filières petits ruminants, les forces et faiblesses du dispositif actuel et les possibilités d'évolution. Scientific Opinion Adopted on 18 February 2002 by AFFSA and made public on 5 March 2002.

AFFSA (Agence Française de Sécurité Sanitaire des Aliments), 2002. Avis du Comité sur l'analyse des risques liés aux encéphalopathies spongiformes transmssibles dans les filières petits ruminants, les forces et faiblesses du dsipositif actuel et les possibilités d'évolution. Annex to AFSSA (2002), adopted by the Comité d'Experts Spécialisé sur les ESST on 31 December 2001 and made public on 5 March 2002. [Text partly translated into English.]

Andreoletti O. *et al*. (2000) 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.

Belt, P. B. G. M., Muileman, I. H., Schreuder, B. E. C., Bos-de Ruijter, J., Gielkens, A. L. J. and Smits, M. A. (1995). Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. Journal of General Virology **76**: 509-517.

Belt, P., Schreuder, B., Muileman, I., Bossers, A. and Smits, M. (1995) PrP genotype and modulation of disease onset in natural scrapie. Prion Diseases, Gottingen, Germany.

Bossers, A., Schreuder, B. E. C., Muileman, I. H., Belt, P. B. G. M. and Smits, M. A. (1996). PrP genotype contributes to determining survival times of sheep with natural scrapie. Journal of General Virology **77**: 2669-2673.

Bradley, R., 2002. The safety of sheep intestine and natural casings derived therefrom in regard tro risks from animal TSE and BSE in particular. Preliminary report submitted on 9 March 2002 to the TSE/BSE ad hoc Group via the SSC secretariat. 17 pages.

Chandler, R. L. (1963). Experimental scrapie in the mouse. Research in Veterinary Science **4**: 276-285.

Clouscard, C., Beaudry, P., Elsen, J. M., Milan, D., Dussaucy, M., Bounneau, C., Schelcher, F., Chatelain, J., Launay, J. M. and Laplanche, J. L. (1995). Different allelic effects of the codons 136 and 171 of the prion protein gene in sheep with natural scrapie. Journal of General Virology **76**: 2097-2101.

Coghlan A (2001) New evidence for vCJD risk from lamb. New Scientist.

Collis, S. C. and Kimberlin, R. H. (1985). Long-term persistence of scrapie infection in mouse spleens in the absence of clinical-disease. Fems Microbiology Letters **29**: 111-114.

Dickinson, A. G., Fraser, H. and Outram, G. W. (1975). Scrapie incubation time can exceed natural lifespan. Nature **256**: 732-733.

Dickinson, A. G., Stamp, J. T. and Renwick, C. C. , Rennie, J.C., (1968). Some factors controlling the incidence of scrapie in Cheviot sheep injected with a Cheviot-passaged scrapie agent. J.Comp.Pathol., **78**: 313-321.

DNV Consulting (Det Norske Veritas) (2001). Risk of Exposure to BSE Infectivity in UK Sheep. Report prepared for the UK Food Standards Agency and made available to the SSC in October 2001 on a confidential basis**.**

E.C. (European Commission) (1999a). Listing of Specified Risk Materials: a scheme for assessing relative risks to man. opinion adopted by the Scientific Steering Committee at its meeting of 9 December 1997 (re-edited, 22-23 January 1998).

E.C. (European Commission) (1999b). Opinion on the possible vertical transmission of Bovine Spongiform Encephalopathy (BSE) Adopted by the Scientific Steering Committee at its meeting of 18-19 March 1999.

E.C. (European Commission) (1999c). The policy of breeding and genotyping of sheep, i.e. The issue of whether sheep should be bred to be resistant to scrapie. Scientific Opinion Adopted by the Scientific Steering Committee at its meeting of 22-23 July 1999.

E.C. (European Commission) (2000a). Report and Opinion on The criteria for diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical diagnosis of TSE agent strains. Adopted by the Scientific Steering Committee at its meeting of 13-14 April 2000**.**

E.C. (European Commission) (2000b). Oral exposure of humans to the BSE agent: infective dose and species barrier. Opinion and report adopted by the Scientific Steering Committee at its meeting of 13-14 April 2000 Following a public consultation via Internet between 6 and 27 March 2000.

E.C. (European Commission) (2000c). Opinion on specified risk materials of small ruminants. (Follow-up to the SSC opinion of 24-25 September 1998 on *the Risk of Infection of Sheep and Goats with BSE Agent*). adopted by the Scientific Steering Committee at its meeting of 13-14 April 2000.

E.C. (European Commission) (2000d). Opinion on The Implications of the Houston *et al* paper in *The Lancet* of 16 September 2000 on the Transmission of BSE by blood transfusion in sheep. (The Lancet, Vol. 356, pp 999-1000; 955-956; 1013). Adopted by the Scientific Steering Committee at its meeting of 26-27 October 2000.

E.C. (European Commission) (2001a). Opinion pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions. Adopted by the Scientific Steering Committee at its meeting of 8-9 February 2001.

E.C. (European Commission) (2001b). Statement of the SSC on the safety of milk. Adopted on 30 March 2001.

E.C. (European Commission) (2002). TSE infectivity distribution in ruminant tissues (state of knowledge, December 2001). Adopted by the Scientific Steering Committee at its meeting of 10-11 January 2002.

Elsen, J. M., Barillet, F., Khang, J. V. T., Schelcher, F., Amigues, Y., Laplanche, J. L., Poivey, J. P. and Eychenne, F. (1997). Genetics of susceptibility to scrapie in sheep: Current research and prospect. Productions Animales **10**: 133-140.

Elsen, J.M., Amigues, Y., Schelcher, F., Ducrocq, V., Andereoletti, O., Eychenne, F., Tien Khang, J. V., Poivey, J.-P., Lantier, F. and Laplanche, J.-L. (1999). Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Archives of Virology **144**: 431-445.

Ferguson, N.M., Ghani, A.C., Donnelly, C.A., Hagenaars, T.J., Anderson, R.M., 2002. Estimating the human health risk from possible BSE infection of the British sheep flock . In: letters to Nature. *Nature* AOP, Published online: 9 January 2002; DOI: 10.1038/nature709.

Ferguson, N.M., Ghani, A.C., Donnelly, C.A., Hagenaars, T.J., Anderson, R.M., 2002. Estimating the human health risk from possible BSE infection of the British sheep flock. (Supplementary information) In: letters to Nature. *Nature* AOP, Published online: 9 January 2002; DOI: 10.1038/nature709.

Ferguson, N.M., Donnelly, C.A., 2002. Note of 12 February 2002 on the estimated BSE risk-reduction associated with current SRM controls for sheep.

Foster, J., Goldmann, W., Parnham, D., Chong, A.and Hunter, N. (2001). Partial dissociation of PrP sc deposition and vacuolation in the brains of scrapie and BSE experimentally infected goats. Journal of General Virology, 82, 267-273.

Foster, J.D., Parnham, D.W., Chong, A., Goldman, W., Hunter, N. (2001) Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats. The Veterinary Record, **148**: 165-171.

Foster, J.D., Parnham, D.W., Hunter, N., Bruce, M. (2001). Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission (2001). *Journal of General Virology* **82**, 2319-2326.

Goldmann, W., Hunter, N., Benson, G., Foster, J. D. and Hope, J. (1991). Different scrapie-associated fibril proteins (PrP) are encoded by lines of sheep selected for different alleles of the Sip gene. Journal of General Virology **72**: 2411-2417.

Goldmann, W., Hunter, N., Smith, G., Foster, J. and Hope, J. (1994). PrP genotype and agent effects in scrapie - change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. Journal of General Virology **75**: 989-995.

Hadlow, W.J.; Kennedy, R.C. and Race, R.E. (1982) Natural infection of Suffolk sheep with scrapie virus. Journal of Infectious diseases 146, 657 - 664.

Hadlow, W.J.; Race, R.E. ; Kennedy, R.C.; Eklund, C.M. (1979) Natural infection of sheep with scrapie virus IN: Slow transmissible Diseases of the Nervous system. Vol 2. eds S.B. Prusiner and W.J. Hadlow pp 3-12 Academic Press New York.

Heggebo, R., Press, C.M., Gunnes, G., Gonzales, L., Jeffrey, M., (2002). Distribution and accumulation of PrP in gut-associated and peripgeral lymphoid tissue of scrapie-affected Suffolk sheep. J.Ge,.Virol., **83 (2):** 479-489.

Hoinville, L., McLean, A.R., Hoek, A., Gravenor, M.B., Wilesmith, J., 1999. Scrapie occurrence in Great Britain. The Veterinary record, **145**: 405-406.

Houston, F., Foster, J.D., Chong, A., Hunter, N., Bostock, C.J., 2000. Transmission of BSE by blood transfusion in sheep. Research letter. The Lancet, **356:** 999-1000; 955-956; 1013.

Hunter, N. (2000). Transmissible Spongiform Encephalopathies. In: Breeding for disease resistance in farm animals. RFE Axford CABI Publishing, New York. Pp 325-339.

Hunter, N., Goldmann, W., Foster, J., Cairns, D. and Smith, G. (1997). Natural scrapie and PrP genotype: case-control studies in British sheep. The Veterinary Record **141**: 137-140.

Hunter, N., Goldmann, W., Smith, G. and Hope, J. (1994). The association of a codon 136 PrP gene variant with the occurrence of natural scrapie. Archives of Virology **137**: 171-177.

Hunter, N., Moore, L., Hosie, B., Dingwall, W. and Greig, A. (1997). Natural scrapie in a flock of Suffolk sheep in Scotland is associated with PrP genotype. Veterinary Record **140**: 59-63.

Hunter, N.,Foster, J., Dickinson, A.G., Hope, J. (1989). Linkage of the gene for the scrapie-associated fibril protein (PrP) to the Sip gene in Cheviot sheep. The Veterinary Record, **124**: 364-366.

Hunter, N.,Foster, J., Goldmann, W., Stear, M., Hope, J. and Bostock, C. (1996). Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. Archives of Virology **141**: 809-824.

Ikeda, T., Horiuchi, M., Ishiguro, N., Muramatsu , Y., Kai-Uwe, G. and Shinagawa, M. (1995). Amino acid polymorphisms of PrP with reference to onset of scrapie in Suffolk and Corriedale sheep in Japan. Journal of General Virology **76**: 2577-2581.

Jeffrey, M., Ryder, S., Martin, S., Hawkins, S.A.C., Terry, L., Berthelin-Baker, C. and Bellworthy, S.J. (2001). Oral inoculation of sheep with the agent of BSE. 1. Onset and distribution of disease specific PrP accumulation in brain and viscera. Journal of Comparative Pathology **124**, Iss 4, pp. 280-289.

Jeffrey, M.; Ryder, S.; Martin, S.; Hawkins, S.A.C.; Terry, L.; Berthelin-Baker, C.; Bellworthy, S.J. (2001) Oral inoculation of sheep with the agent of Bovine Spongiform Encephalopathy. 1. Onset and Distribution of Disease-specific PrP Accumulation in Brain and Viscera. Journal Comparative Pathology 124, 280-289.

Junghans, F., Teufel, B., Buschmann, A., Steg, G., Groschup M.H.(1998) Genotyping of German sheep in respect to scrapie susceptibility. Veterinary Record**. 143:** 340-341.

Lacroux, C., Grandjean, C., Corbière, F., Aandréoletti, O., Schelcher, F., 2001. Recherche de PrP Sc par immunohistochimie dans la paroi d'intestins délimonés et non délimonés, issus d'ovins en phase clinique de tremblante. Protocole AFSSA/DGAL/INRA-ENVT (Version of 17 November 2001). Document N° UMR INRA-ENVT 959. 4pp + annexes.

Laplanche, J. L., Chatelain, J., Beaudry, P., Dussaucy, M., Bounneau, C. and Launay, J. M. (1993). French autochthonous scrapied sheep without the 136Val PrP polymorphism. Mammalian Genome **4**: 463-464.

McLean, A.R., Hoek, A., Hoinville, L.J., Gravenor, M.B., 1999. Scrapie transmission in Britain: a recipe for a mathematical modelm. Proc.R.Soc.Lond.B, **266**: 2531-2538.

O'Rourke, K., Melco, R. and Mickelson, J. (1996). Allelic frequencies of an ovine scrapie susceptibility gene. Animal Biotechnology **7**: 155-162.

Race, R. and Chesebro, B. (1998). Scrapie infectivity found in resistant species. Nature **392**: 770.

Race, R.,Jenny, A. and Sutton, D. (1998). Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: Implications for transmission and antemortem diagnosis. Journal of Infectious Diseases **178**: 949-953.

Roels, S., Vanopdenbosch, E., 2001. TSE/Scrapie bij schapen: uitbraak en verspreiding door verkoop. (TSE / scrapie in sheep: an outbreak spread by trade). Vlaams Diergeneeskundig Tijdschrift, **70**: 127-130.

Schmerr, M.J., Jenny, A.L., Bulgin, M.S., Miller, J.M., Hamir, A.N., Cutlip, R.C., Goodwin, K.R., (1999). Use of the capillary electrophoresis and fluorescent labelled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. J.Chromatogr.A, **853**: 207-214.

Schreuder, B. E. C., van Keulen, L. J. M., Vromans, M. E. W., Langeveld, J. P. M. and Smits, M. A. (1998). Tonsillar biopsy and PrP^{Sc} detection in the preclinical diagnosis of scrapie. Veterinary Record **142**: 564-568.

Sigurdarson, S., Ducrot,C., 1998. Icelandic scrapie redaication programme. European scrapie network.

Tama, Y., Kojima, H., Kitajima, R., Taguchi, f., Ahtani, Y., Kawagichi, T., Miura, S., Sato, M., 1992. Demonstration of the transmissible agent in tissue from a pregnant woman with Creutzfeldt-Jakob disease. The New England Journal of Medicine, **327** (9): 649.

Taylor, D.M., Fernie, K., Steele, P.J., Somerville, R.A., (2001). The relative efficiency of transmitting bovine spongiform encephalopathy to RIII mice by the oral route. The Veterinary Record,**141** :345-346.

Thorgeirsdottir, S., Siurdarson, S., Georgsson, G. and Palsdottir, A. (1998). Association between the genotpes of the PrP gene and scrapie in Icelandic sheep. Symposium on Prion and Lentiviral Diseases, Reykjavik, Iceland.

Tranulis, M. A., Osland, A., Bratberg, B. and Ulvund, M. J. (1999). Prion protein gene polymorphisms in sheep with natural scrapie and healthy controls in Norway. Journal of General Virology **80**:1073-1077

van Keulen, L. J. M., Schreuder, B. E. C., Vromans, M. E. W., Langeveld, J. P. M. and Smits, M. A. (1999). Scrapie-associated prion protein in the gastro-intestinal tract of sheep with natural scrapie. J.Comp.Pathol., **121**: 55-63.

van Keulen-LJM; Schreuder-BEC; Vromans-MEW; Langeveld-JPM; Smits-MA; van-Keulen-LJM; Groschup-MH (ed.); Kretzschmar-H (2000) Pathogenesis of natural scrapie in sheep.in Prion-diseases:-diagnosis-and-pathogenesis. Pp 57-71 Springer-Verlag Wien; Wien; Austria

Wells, G.A.H.; Hawkins, S.A.C.; Green, R.B.; Austin, A.R.; Dexter, I.; Spencer, Y.I.; Chaplin, M.J.; Stack, M.J.; Dawson, M. (1998) Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (an update). Veterinary Record 142, 103-106

Westaway, D., Zuliani, V., Cooper, C. M., Dacosta, M., Neuman, S., Jenny, A. L., Detwiler, L. and Prusiner, S. B. (1994). Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. Genes & Development **8**: 959-969.

WHO (2000) WHO/CDS/CSR/APH/2000.2 WHO Consultation on Public Health and Animal Transmissible Spongiform Encephalopathies: Epidemiology, Risk and Research Requirements with the participation of Office International des Epizooties Geneva, Swizterland 1-3 December 1999.

Woolhouse, M. E. J., Stringer, S. M., Matthews, L., Hunter, N. and Anderson, R. (1998). A mathematical model of the epidemiology and control of scrapie in a sheep flock. Proceedings of the Royal Society B **265**: 1205-1210.

X.   ACKNOWLEDGEMENTS:

Dr.J.M.Elsen,   Dr.M.Jeffrey,   Dr.T.Baron,   L.Detwiler,   Dr.L.Hoinville, Prof.Dr.M.Ulvund, Dr.M.Savey, Dr.R.Somerville, Prof.Dr.P.James, Dr.M.Groschup.

ANNEX 1: STATE OF THE ART OF THE AVAILABLE SCIENTIFIC INFORMATION ON THE RELATIONS BETWEEN GENOTYPE OF SHEEP (AND, IF POSSIBLE GOATS) AND RESISTANCE AGAINST TSE INFECTION, PARTICULARLY BSE

I.   **MANDATE**

The SSC is invited to prepare a state of the art of the available scientific information on the relations between genotype of sheep (and, if possible goats) and resistance against TSE infection, particularly BSE.

If and where appropriate, issues should be addressed such as [risk of] silent carriership, length of the possible incubation period, cumulating of possible infectivity, replication of the TSE agent if present, infectivity levels in early stages of infection [even if clinical signs would possibly only appear after the end of the economic life of the agent), use of sentinel animals, risk of selection of a single [new, very resistant, ...] strain as the result of a breeding-for-resistance programme, etc.

II.   **STATE OF THE ART**

II.1.   **REGARDING SCRAPIE IN SHEEP:**

a.   Studies of natural scrapie in sheep have confirmed the importance of three amino acid codons in the sheep PrP gene (136,154 and 171) (Belt *et al*, 1995; Clouscard *et al,* 1995; Hunter *et al*, 1996) originally shown to be associated with differing incubation periods following experimental challenge of sheep with different sources of scrapie and BSE (Goldmann et al. 1991; Goldmann et al. 1994). The three polymorphic amino acids are shown in **Table 1**. Any particular combination of three codons is known as an allele but not all theoretically possible alleles have been found.

There are breed differences in the frequencies of the occurrence of PrP alleles and in the exact disease-associated alleles, however some clear genetic rules have emerged. The PrP genotype (given by specifying both alleles) of a sheep is described listing in order codons 136, 154 and 171 for each allele in turn.

Using this scheme, the most resistant sheep PrP genotype is ARR/ARR. To simplify, the ARR/ARR animals (carrying, in homozygous state, Alanine, Arginine and Arginine information at PrP codons 136, 154 and 171 respectively) were shown on a large number of observations (including 1700 cases observed across Europe within the 973305 project) to be resistant to natural and experimental scrapie (the Japanese case, Ikeda (1995), looks more and more unique) and increasing evidences indicate they are not healthy carriers of infectivity (Schreuder et al 1998, van Keulen et al, 1996; Andreoletti et al, 2000). Sheep of ARR/ARR genotype are also resistant to experimental injection with both scrapie and BSE (Goldmann *et al*, 1994).

The simplest UK breeds in terms of PrP genetics are Cotswold, Hampshire Down, Soay, Suffolk, and Vendeen which all normally encode just two PrP alleles: ARQ and ARR, although ARH is sometimes also found in some flocks of these breeds. Taking Suffolks as the paradigm breed for this group, Suffolk sheep of genotypes which encode Q on both alleles at codon 171 are most susceptible to scrapie. For example, many ARQ/ARQ Suffolks develop scrapie (**Table 2a**), although many also remain

healthy (Westaway *et al*, 1994; Hunter *et al*, 1997). Occasionally scrapie occurs in ARQ/ARR sheep.

Most UK sheep breeds are much more complex in PrP genetics than the Suffolk-type group, especially those breeds which encode the VRQ allele. For example Cheviot, Swaledale and Shetland sheep have 4 PrP gene alleles: VRQ, ARQ, ARR, and AHQ. Sheep with the genotype VRQ/VRQ appear to be extremely susceptible to scrapie (**Table 2b**) (Hunter *et al*, 1994; Hunter et al. 1996). It is reported that in Cheviots, where all three major alleles (VRQ/ARQ /ARR) are found, that natural disease is restricted to the VRQ allele but disease can be also be detected in ARQ/ARQ sheep of this breed following experimental challenge with some scrapie strains. Most complicated of all, are Texels and Lleyn sheep which have 5 PrP alleles and 15 genotypes. In the latter breeds, scrapie usually occurs in sheep of VRQ/ARQ, VRQ/ARH and VRQ/VRQ but is found occasionally in 5 other genotypes.

Table 1: PrP gene alleles and polymorphic amino acid codons

Amino acid number	Codon	Single letter code	Allele*
136	valine	V	VRQ
	alanine	A	ARQ,AHQ, ARR
154	arginine	R	ARR
	histidine	H	AHQ
171	arginine	R	ARR
	glutamine	Q	VRQ, ARQ, AHQ

* most codons are found on only one allele, some on three alleles. Alleles are given with codon 136, 154 and 171 in order.

Table 2: Suffolk and Cheviot sheep PrP genotypes and natural scrapie

a) **Suffolk sheep:**

| PrP Genotype	Natural scrapie
ARQ/ARQ	High risk of scrapie
ARQ/ARR	Occasional occurrence *
ARR/ARR	Resistant

* approximately 1 % in heavily infected flocks

b) **Cheviot sheep:**

PrP Genotype	Natural scrapie
VRQ/VRQ	Very high risk of scrapie
VRQ/ARQ	Very high risk of scrapie
VRQ/ARR	Occasional occurrence
ARQ/ARQ	Occasional occurrence
ARQ/ARR	Resistant
ARR/ARR	Resistant

Many other studies of sheep throughout Europe (both EU and non-EU countries) and the USA confirm and extend these findings of both genetically simple breeds at one extreme and complex breeds at the other (Laplanche *et al*, 1993; Westaway *et al*, 1994; Belt *et al*, 1995; Belt *et al*, 1995; Clouscard *et al*, 1995; Ikeda *et al*, 1995; Bossers *et al*, 1996; Elsen *et al*, 1996; O'Rourke *et al*, 1996; Elsen *et al*, 1997; Junghans *et al*, 1998, Thorgeirsdottir *et al*, 1998; Elsen *et al*, 1999; Tranulis *et al*, 1999). Within the simple breeds, like Suffolks, breeding for resistance involves the selection of ARR/ARR rams, there is little other choice. For more complex breeds of sheep, like Cheviots, there is much more choice in the range of relatively resistant genotypes. However in the UK, commercial testing for PrP genotype has resulted in the premium status of ARR/ARR animals of any breed and selection only for this genotype.

b.   The following figure provides an example of the differential mortality resulting from scrapie, according to the genotype. The date were obtained for enzootic scrapie [J.M.Elsen, pers.comm., 2001].
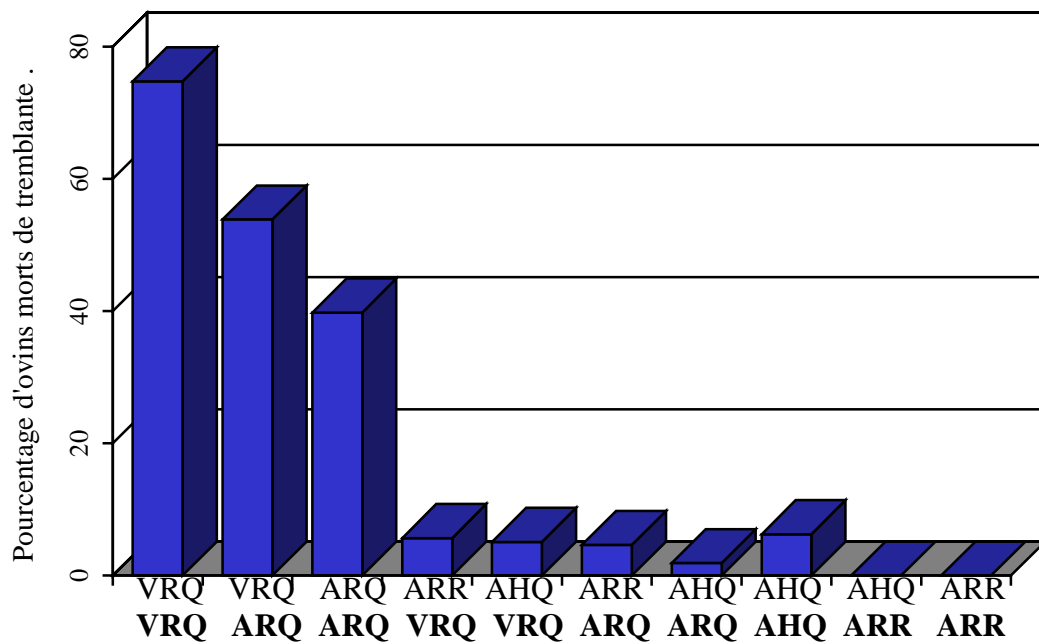
c.   Carrier animals are defined as those which have a latent infection and so have the ability to pass on the infection to other sheep. It is a possibility that sheep may harbour a latent scrapie infection and if so, that they could pass on infection to other sheep.. There is some limited experimental evidence for the existence of hidden infectivity. One of the earliest studies of scrapie in mice (Chandler, 1963), showed that incubation period was dose-dependent. In other words, incubation period lengthened as the amount of infectivity in the inoculum was reduced. At very low levels of scrapie infection, replication may take such a long time to build up, that disease does not develop within the animal's normal lifespan (Dickinson *et al*, 1975). Such a situation, if shown to exist in sheep (and as yet there is no proof of this) could lead to the

maintenance of a low level of infection in a flock without any signs of symptoms. Such sheep could be shedding scrapie infectivity for many years and be a source of infection for their flockmates. More recent evidence supporting such a phenomenon comes from another mouse model (scrapie strain 87V injected intraperitoneally (ip) as a 1% brain homogenate into IM mice) where there is long term persistence of high scrapie titres in the mouse spleen without either accompanying replication in the brain or development of scrapie symptoms. This could be a mechanism by which carrier status animals can be produced (Collis and Kimberlin, 1985). Cross species persistence may also occur, hamster scrapie injected into mice does not produce disease but the hamster scrapie remains in brain and spleen of the mice and can be recovered in a form still able to infect hamsters (Race and Chesebro 1998).

It is not known for certain whether resistant sheep are capable of replicating infectivity at a low level or whether such animals also have the ability to spread infection to other sheep. The former need not imply the latter. Resistant sheep may not show signs of clinical scrapie but could still harbour the infectious agent, posing a threat by maintaining the infectious agent and creating a potential situation in which a new strain, capable of causing clinical scrapie could be selected. Even if a new strain did not appear, computer modelling predicts that infection could remain hidden in the flock for many decades (Woolhouse *et al*, 1998) threatening the health of any susceptible animals introduced into the flock.

On the question which PrP genotypes of sheep may be most likely to harbour hidden infection, whether or not they can pass it on to other animals, it can be expected that it is almost certain that sheep of the most susceptible genotypes will carry infectivity within their bodies in the pre-clinical phase. However for animals that remain healthy throughout a natural lifespan, those genotypes of sheep that occasionally have scrapie cases could be considered as potential carriers of infection: ARQ/ARR Suffolks and VRQ/ARR Cheviots for example (**see Table 2**).

d.   Infectivity levels in tissues and age

Distribution of TSE infectivity through the carcase is primarily determined using mouse infectivity bioassays although distribution of disease specific PrP as determined by immunohistochemistry may also be used to indicate infected tissues. The sensitivity of the mouse infectivity bioassay is currently limited by the sheep/mouse species barrier and infectivity bioassay in sheep may be indicated where results are equivocal. Studies of the temporal distribution of scrapie infectivity in naturally infected Suffolk sheep (Hadlow et. al. 1979 and 1982) demonstrated infectivity in the lymphoreticular system and intestine from ten months post infection, spreading to give an initially low titre in the CNS at twenty-five months post infection. The degree of challenge to which each sheep was exposed was clearly random so although there was no detectable infectivity in the lambs killed at seven to eight months of age none of the dams and only two of the sires of these eight lambs developed clinical scrapie, whereas in the ten to fourteen month old kill group all the infected animals (8 of a group of 15) were progeny of dams that later were affected with scrapie. With this proviso, in comparing the clinically affected sheep with the ten to fourteen month old lambs the following conclusions could be drawn. In the CNS of

ten to fourteen month-old lambs, the level of infectivity is undetectable by mouse bioassay and is at least 1000 x below that in the clinical animals; the infectivity in lymph nodes, tonsil and spleen is approximately 10 x below that in the clinical animals; and the infectivity in the ileum and proximal colon is only slightly less (less than 1 log) than in the clinical animals. The Hadlow studies remain the main source of data on levels of scrapie infectivity in tissues other than the CNS in sheep. In more recent work looking at the sequence of spread of disease specific PrP using immunohistochemistry in scrapie infected sheep (van Keulen *et al*, 2000), the results were broadly similar with disease specific PrP in the intestine at five months post infection, subsequent spread through enteric nervous system to the spinal cord after ten months and widespread disease specific PrP by twenty one months post infection.

e.   **Conclusions:**

-    The incidence of TSEs in sheep is linked to PrP genotype, with codons 136, 154 and 171 being of major importance. Most scrapie-affected sheep are homozygous for glutamine (Q) at codon 171 and succumb to disease with ARQ/ARQ, VRQ/VRQ, VRQ/ARQ genotypes. The details of the genetic links with disease are complex, and are breed and probably country specific. However the common forms of scrapie do not represent a genetic disease as the susceptible PrP genotypes can be found easily in sheep from scrapie-free countries such as Australia and New Zealand. For an animal to develop scrapie, therefore, a susceptible PrP genotype and exposure to infection are both required.

-    Occasionally scrapie occurs in ARQ/ARR and VRQ/ARR sheep.

-    Sheep of ARR/ARR genotype are considered to be the most resistant sheep PrP genotype.

-    There is some limited experimental evidence for the existence of hidden infectivity but the available information is not enough to provide an answer to the question of what is the risk that flocks of scrapie resistant sheep would carry the scrapie agent without showing clinical signs but at the same time being able to transmit the agent horizontally, vertically or via rendering and this hypotehsis can therefore not be excluded. Such a situation, if shown to exist in sheep (and as yet there is no field proof of this) could lead to the maintenance of a low level of infection in a flock without any signs of symptoms. For animals which remain healthy throughout a natural lifespan, those genotypes of sheep which occasionally have scrapie cases could be considered as potential carriers of hidden infection, whether or not they can pass it on to other animals: ARQ/ARR Suffolks and VRQ/ARR Cheviots, for example. However it may be expected/assumed that in the case of hidden infection / silent carriership a resistant population would be expected to have very much lower titres of infection within them than would be found in susceptible sheep.

II.2. REGARDING BSE IN SHEEP:

a.   According to Goldmann *et al*, (1994), sheep of ARR/ARR genotype are resistant to experimental injection with both scrapie and BSE. Other research (Foster et al, 1993) showed that no infectivity nor PrP^Sc has so far been detected in any tissue of ARQ/ARR or ARR/ARR animals orally challenged with BSE up to 2̶ 3 years post challenge. On the assumption that PrP^Sc and infectivity are correlated this would suggest that if these animals are infected, the titre of infectivity in a substantial period (and up to 2̶ 3 years) following challenge is nil (or undetectable) in peripheral tissues of these genotypes compared with more susceptible genotypes. It will not be possible to determine certainly whether these peripheral tissues in challenged animals are detectably infected or not until the experiments have reached their conclusion in several years time. If infectivity were detected titrations would be requiredto assess titre for a quantitative risk analysis. Otherwise it may not be possible to conduct such a risk analysis unless no infectivity is detected and there are a statistically significant number of animals surviving to the termination of the study.

b.   Experimental BSE in sheep more closely resembles natural and experimental scrapie than BSE in cattle. Using immunohistochemical techniques Foster *et al* (2001) showed the distribution of disease specific PrP in sheep terminally affected with BSE and Jeffrey *et al* (2001) showed the progressive distribution of disease specific PrP from gut and peripheral lymphoid tissue through the lymphoreticular system, enteric nervous system and peripheral nerves to the CNS. In the former study Romney sheep of the three PrP genotypes ARQ/ARQ; ARQ/ARR and ARR/ARR were dosed at the age of six months with 5g of an inoculum prepared from the brains of cattle clinically affected with BSE and with an infectivity titre of $10^{3.97}$ log10 mouse i.c./i.p LD50/g of tissue as determined in RIII mice. The sheep were killed sequentially throughout the study and at post mortem examination a range of tissues were collected for histopathology, immunohistochemistry and RIII mouse infectivity bioassay. Interim updated (December 2001) results of studies by the VLA, UK of the tissue distribution of PrP^Sc (Jeffrey *et al*, 2001) and/or infectivity (mouse bioassay) in Romney (ARQ/ARQ) and Suffolk (ARQ/ARQ) sheep orally exposed to the BSE agent (5g affected brain homogenate) (S. Bellworthy, unpublished data) have established the earliest evidence of the presence of agent in tissues as follows:

Romneys (current data on incubation period range: 20-37 months):

- Retropharyngeal lymph nodes (LN)	4 months after exposure
- Peyer's patch	4 months after exposure
- Spleen	10 months after exposure
- Mesenteric LN	16 months after exposure
- Ileocaecal LN	16 months after exposure
- Mediastinal LN	16 months after exposure
- Tonsil	16 months after exposure
- Submandibular LN	16 months after exposure
- Distal ileum(excluding Peyer's patches)	16 months after exposure
- Mesenteric LN	16 months after exposure
- Prescapular LN	16 months after exposure
- Broncho-mediastinal LN's	16 months after exposure
- Brain and spinal cord	16 months after exposure
- Liver (low level of infectivity)	16 months after exposure

- Intestine	16 months after exposure	
- Vagus nerve	16 months after exposure	
- Forestomaches	22 months after exposure	
- Abomasum	22 months after exposure	
- Coeliaco-mesenteric ganglion (sympathetic)	22 months after exposure	

New Zealand Suffolk **(current data on incubation of initial clinical cases: 24 months)**

- CNS (including spinal cord)	}	10m
- Retropharyngeal LN	}	
- Submandibular LN	}	
- Prescapular LN	}	
- Spleen	}	
- Mesenteric LN	}	
- Peyer's patch	}	
- Ileo-caecal LN	}	
- Tonsil	}	
- Brain		16m

Preliminary qualitative infectivity bioassay results for this study, (Bellworthy, et al - in preparation), confirmed the presence of infectivity in ileal Peyer's patches at four months post infection, and in spleen at ten months post infection for the ARQ/ARQ genotype. Bioassay results for subsequent time points are still incomplete but initial results show a spread of infectivity through the lymphoreticular system, liver and spinal cord at sixteen months post infection. No infectivity has so far been detected in either of the other two genotypes (ARQ/ARR and ARR/ARR).

c. Similarly dosed ARQ/ARR (heterozygous for BSE/scrapie susceptibility) Romney sheep are currently approximately four years after dosing and remain healthy. Sequentially killed animals from this component of the study have not, as yet, shown PrP^{Sc} in any tissues suggesting, at least, that infectivity is extremely low in tissues, certainly up to two years after challenge.

These data suggest that unlike the situation in cattle experimentally infected by the oral route with a relatively large exposure dose of BSE agent, the results in sheep indicate a potentially widespread involvement of lymphoid tissues early in the incubation period at least in ARQ/ARQ scrapie/BSE susceptible sheep. New data are consistent with the previously expressed view that BSE in sheep after oral exposure is pathogenetically closely similar to scrapie, particularly with respect to the tissue distribution of infectivity and/or PrP^{Sc}.

d. **Conclusions:**

Research data available to date indicate that the relation between sheep genotype and susceptibility to a TSE is similar [identical?] for scrapie and BSE. Animals with a susceptible genotype, when experimentally infected with BSE, show the presence of the agent in certain tissues quiet soon after infection. This may be as early as 4 months post infection in the retropharyngeal lymph nodes and Peyer's patches. Animals with *PrP* genotypes that are more or less susceptible to scrapie and BSE may thus carry infectivity (or at least show PrP^{Sc} accumulation) early in their life. In resistant

genotypes on the contrary, no detectable infectivity has been found in any tissue at least until 2 years after experimental infection. (This research is still going on.)

II.3. REGARDING TSEs IN GOATS:

Regarding TSE and genotype in goats no recent information is available. Foster *et al* (1993) showed that BSE could be experimentally transmitted to Anglo-Nubian goats following either i/c (3 out of 3) or oral (2 out of3) challenge with brain material derived from cattle with BSE and Bruce (1994) further showed that the agent strain re-isolated from the brain of one i/c inoculated goat was indistinguishable from BSE. In regard to the orally BSE-challenged goats, 2 of 3 developed clinical and pathological signs of TSE. In the absence of complete knowledge of the different susceptibilities of different genotypes of goats to the BSE or scrapie agent it is reasonable to assume that perhaps all goats may be susceptible to TSE by the oral route under certain conditions. However, it has to be noted that the dimorphism in codon 142 of the caprine *PrP* gene appears to be associated with different incubation periods in goats, experimentally infected with the BSE agent and two types of scrapie agent including the C-type strain source CH 1641 (Goldman *et al,* 1996). No further information is available at the moment (Nora Hunter, pers.comm., 2002)

III. DISCUSSION:

a.   There is currently no evidence to suggest that resistant genotypes are silent carriers. It follows that animals of such genotypes in which it is impossible to detect PrP res by IHC or by WB are not expected to reflect a risk to other susceptible sheep within a flock. Genotyping can [could] thus be used as a tool for risk management.

However, the application of genotyping to risk management of BSE in small ruminants presents a particular problem in that the tissue infectivity data for ARR/ARR and ARR/ARQ genotypes experimentally exposed to BSE does not extend beyond about 3 years post challenge, although all remain healthy at this stage. If BSE follows the same pattern as scrapie then one could expect a ratio of about 1 ARR/ARQ BSE in sheep to every 50 ARQ/ARQ BSE's in sheep. There is therefore a risk that a small proportion of the exposed ARQ/ARR sheep may develop disease, though whether these will have a detectable peripheral pathogenesis phase is not known. Very little is known about BSE in the VRQ allele.

If fully susceptible sheep are to include those with the ARQ allele (which is clearly the case with the Suffolk) or VRQ alleles, then huge numbers of sheep would be regarded as commercially unviable. Indeed, if the overall sheep population is divided simply into susceptible (VRQ or ARQ alleles) and resistant (ARR/ARR) genotypes then there are some breeds of sheep which have very low numbers, perhaps in some cases none, of the most resistant ARR/ARR genotypes.

b.   Regarding the use of genetic information for BSE risk assessment, three hypotheses may be considered:

(i)   ARR/ARR animals are BSE resistant and not infectious

(ii) ARR/ARR animals are resistant but can transmit BSE to their mates

(iii) ARR./ARR animals are susceptible to BSE (but with a long incubation period) and carry infectivity.

If (i) is true, any action aiming at increasing ARR frequency in sheep populations would reduce the BSE risk. Breeds known to have a high frequency of this ARR allele have a limited risk to show TSE, including BSE. It may be recommended to select rapidly on a large scale for resistance to TSE, and flocks which were infected by BSE (or scrapie) should be replaced using ARR/ARR animals only (in a first step, these replacement animals should be at least ARR carriers). The success of such genetic plans will increase if: the PrP genotyping is cheap; the selection on PrP genotype is primarily organised in breeding flocks, which have an active role in the genetic evolution of the whole breed; the genetic variability of selected population is preserved (to make selection on the basis of *PrP* genotyping more acceptable by breeders); artificial reproduction (insemination, embryo transfer) are used to accelerate the genetic change; animals are surely identified, if possible using electronic devices; flocks are given BSE status/rank considering a risk scale to be established.

If (ii) is true, ARR/ARR animals bred in BSE environment are increasing the risk only if no susceptible animals are living in the same environment and showing infection. Sentinels are thus a solution for controlling the safety of the flock. On the other hand, non ARR/ARR animals are in any case more dangerous to humans (they carry higher amounts of infectivity, and considering the incubation period of TSE, this infectivity may be hidden for a while). On the whole, holdings where both susceptible and resistant sheep are bred are probably less at risk than holdings having solely susceptible or resistant sheep, and the proportion of resistant individuals within these mixed populations should be as high as possible.

Preliminary evidence shows that if (iii) is true, there is the possibility of some reduction in risk to the human population from the control of genetic structure of populations or flocks because of the lower level of infectivity in these animals. A reduction in clinical cases should occur.

c. Breeding for resistant PrP genotypes is now being carried out in the UK and elsewhere (including the Netherlands and France) bringing in with it worries about the potential long-term effects. If only ARR/ARR sheep were considered for commerce one really has virtually no information on how breeding for this allele might affect sheep either in respect of management (hardiness etc), susceptibility to other diseases on undesirable commercial traits. Scrapie resistant animals could, for example, be less healthy in other respects or could be more susceptible to other diseases. In addition, it has been suggested that the development of an entire national flock of ARR/ARR genotype (the most resistant known) could result in inadvertent selection of rare scrapie strains able to cause disease in this genotype. Although there is no evidence for any of these suggestions yet, other options for disease control are being investigated in a major epidemiological study of UK sheep flocks.

Annex 2: Genotype and susceptibility of sheep to TSEs – Selection of Extracts from the Opinion of 8 November 2001 of the French Food Safety Agency (AFSSA) on health monitoring measures concerning.

"(…)

The scientific grounds for genetic selection of ovines is the existence of genetic variability of susceptibility to TSSE (as in the mouse and in man). This variability has been well documented. Sheep lines selected during the scrapie incubation period were created in 1961 in the United Kingdom. In 1968 (Dickinson et al., 1968) a study of these lines demonstrated the existence of Mendelian genetic markers (Sip gene, for Scrapie Incubation Period with two sA (short) and pA (prolonged) alleles), which constitute the basis of our knowledge of the genetics of ovine susceptibility to this disease. Molecular genetics of scrapie took off in 1989 with the demonstration of the genetic link between this Sip gene and RFLP markers of the PrP gene (Hunter et al. 1989). It is largely explained by the polymorphism of the PrP gene which codes for an ubiquitous membrane glycoprotein called PrP. A total of 14 alleles of the PrP gene have been identified in sheep, corresponding to variations in different codons of this gene. Among the different alleles of the gene, the one that codes for the amino acids Alanine (A) in position 13 and Arginine (R), in positions 154 and 171 confers resistance to scrapie: this allele is codified ARR. As confirmed by thousands of observations made in all countries threatened by scrapie, sheep with two copies of the ARR type gene (homozygotic ARR/ARR sheep) are fully resistant to scrapie, regardless of the strain of the infective agent, while ARR heterozygotes are very rarely affected (Hunter 2000). For example, an epidemiological survey conducted at European level did not detect a single case of ARR/ARR in 1 587 cases of histopathology-confirmed scrapie, although this genotype was present in 15.5% of the population of 9141 controls (Annual Report No 3 on Project CT973305 - European Commission - DG VI). The only case of an ARR/ARR sheep affected by scrapie reported to date is that of a Japanese Suffolk (Ikeda et al. 1995). On the other hand, the allele which codes for Valine at 136, Arginine at 154 and Glutamine at 171 (the VRQ allele) is associated with high susceptibility to scrapie: in the European study, VRQ/VRQ animals represented 13.7% of scrapie cases and 0.6% of the controls.

Besides, the results of experiments in inoculating sheep with a BSE isolate do not suggest, as regards the influence of the ARR allele, a different genetic susceptibility spectrum for the BSE strain as opposed to the scrapie isolates. None of the ARR/ARR animals inoculated with BSE isolates contracted TSSE, nor did PrPres accumulate in detectable quantities (Goldman et al. 1994) (Jeffrey et al. 2001). However, the small number of ARR/ARR animals involved in these experiments means that there is still a margin of uncertainty as regards the notion of "absolute" resistance of this genotype to BSE.

(…)

Given the current state of knowledge, the asymptomatic presence of a TSSE strain in genetically resistant sheep has not been demonstrated. In cases of natural scrapie PrPres has not been detected in ARR/ARR ovines of any age and in particular in ovines which are older than those normally found in commercial herds. Experimental inoculation of these ARR/ARR ovines with strains of BSE or scrapie, either orally or intracerebrally, has not led to symptoms (Goldmann et al. 1994) (Jeffrey et al. 2001). Besides, the published results on the oral administration of the BSE agent to ARR/ARR ovines demonstrate the absence of PrPres tissue up to 24 months after inoculation

(Jeffrey et al. 2001). Research is currently being carried out on ARR/ARR ovines to confirm and extend these initial findings by evaluating the presence of infected tissue in the absence of PrPres in the case of maximised exposure via oral and intracerebral administration. As regards heterozygote animals which carry a copy of the ARR allele, accumulated observations to date in natural scrapie (Andreoletti et al. 2000; van Keulen et al. 1999) and in experimental BSE (Jeffrey et al. 2001) show that ARR/VRQ and ARR/ARQ animals are not carriers of PrPres before the age of one year at least in all the organs studied: the central nervous system, lymph tissues and the autonomous digestive nervous system. During tests involving oral administration of the BSE agent to six ARR/VRQ, ARR/ARQ and ARR/AHQ sheep, only one animal (ARR/VRQ) developed TSSE after a very long incubation period (1945 days), while PrPres in the encephalon remained negative in Western-Blot and doubtful in immunohistochemical tests (Foster et al. 2001).

(…)

The key parameter is the TSSE strain: the presence in natural conditions of a BSE strain in ovines and caprines has not yet been demonstrated. Nevertheless, small ruminants may have been exposed until recently, in various degrees, to sources of feed contamination. In view of the small number of isolates tested in the United Kingdom and France one cannot exclude this hypothesis with a sufficient degree of confidence. In this context of hypothetical risk, the Committee considers it justified, to the extent that scientific and technological progress makes it possible, to adopt rules limiting consumer exposure to ovine or caprine sources of TSSE. This approach is also consistent with the logic of the earlier recommendations of the Interministerial Committee in regard to specified risk material (SRM) from small ruminants.

Pathological deposits of PrP detected by immunohistochemistry, which must be considered as probable markers of infectivity, have been detected in animals infected by the scrapie agent at a very early age (as from two months in VRQ/VRQ ovines) (Andreoletti et al. 2000); during the preclinical phase, in ovines with a susceptible genotype, PrPres has been detected in all lymph tissues (with the exception of the thymus), in the myenteric nerve plexus, and also in the spinal marrow, the encephalon and the eye. PrPres has also been detected in blood (Schmerr et al. 1999); however, during the preclinical phase, in heterozygotic ARR/VRQ or ARR/ARQ ovines, PrPres does not seem to be detectable in lymph tissue or in the digestive autonomous nervous system.

Sheep of susceptible or very susceptible genotypes therefore differ considerably as regards infectability; hence, under similar exposure conditions, the frequency of infection of homozygotic VRQ/VRQ Romanov sheep is approximately 10 to 100 times higher than that of ARR/VRQ heterozygotes and twice that of ARQ/ARQ breeds (Elsen et al. 1999); within a given herd exposure seems to be heterogeneous notably as a function of the birth cohorts,

(…)

Nevertheless, given the slowness of the accumulation kinetics of infectivity in nervous tissues, a negative test in a sample of central nervous tissue does not conclusively demonstrate the absence of infectivity in the peripheral tissues. Consequently, the use of rapid tests of validated and satisfactory sensitivity both in respect of the central nervous system and peripheral tissues, such as the spleen, would seem to be the only way of reducing the risk of consumer exposure.

(…)"

ANNEX 3:   CERTIFICATION OF FLOCKS

VI.1.   FACTORS AFFECTING THE TSE STATUS OF A SMALL RUMINANT FLOCK

VI.1.1. Flock history with regard to scrapie/TSE.

    a) Introduction of scrapie into a country or an area has often been associated <u>with</u> imports of foreign sheep, or the purchase of a certain ewe or ram. In a scrapie-infected flock previously devoid of clinical scrapie, the disease can become overt several years after the introduction of a ram carrying susceptibility alleles. More frequently however, movement of infected sheep is generally considered the most important way of spreading scrapie from flock to flock (Hourrigan et al. 1979, others). In spite of the rather recently gained knowledge of different susceptibility among sheep of different *PrP* genotypes, there is still a lack of knowledge of infectivity carrier states related to genotypes. When scrapie occurs in a new flock, it is often [impossible / very difficult] to trace back the origin of infectivity.

        The highest risk is associated with lambing. The risk is likely to be realised more quickly if the infected animals are pregnant and have been mated to a scrapie-susceptible ram. This is because there is evidence that the placenta of infected sheep can carry infectivity and that this is infectious for sheep and goats by the oral route (Pattison et al 1972, 1974).

    b) Due to the relatively long incubation period with late appearance of clinical signs of TSE there is the potential of horizontal and vertical transmission within a flock to have occurred before the appearance of an initial clinical case. Thus an occurrence of a clinical case of TSE could point to a risk of exposure of other animals still alive in the herd. The risk of hidden TSE, (i.e. pre-clinical cases) remaining in the herd can be considered to be negligible only if the whole flock, with the possible exception of the fully resistant to clinical scrapie genotypes ARR/ARR was destroyed by incineration and if subsequent exposure of the newly established herd to the TSE-agent through feed, horizontal, vertical transmission or other ways of transmission as described above can be excluded[18]. This possibility may be only theoretical as illustrated by the recent case of restocking of fenced areas in Iceland from scrapie-negligible risk flocks, where scrapie seems to have re-appeared in spite of thorough cleaning of farm buildings and topsoil around farm buildings (Sigurdarson, 2001, pers.comm. see also Section VI.1.5.).

        It is therefore concluded that since the establishment of the flock no TSE case must have been diagnosed in the flock. Also all animals showing signs of neurological disorders should be examined to exclude the possibility of new

---

[18]   With respect to certification of previous cases of TSE there may need for be some degree of interpretation. Depending on whether the case was homebred or purchased, and for the latter depending on how soon after purchase the animal was clinically affected, the statement on past TSE history does indicate the extent to which the herd has been at risk of exposure.

infection with TSE[19]. Where possible, when a TSE has been rued out, an alternative diagnosis should be provided. Whenever such disease did not respond to treatment, or the animal died, a *post-mortem* examination and testing for TSE by an approved method at an approved reference laboratory should have excluded TSE. However, it will not always be possible to arrive at a definite diagnosis for all cases of neurological disease.

VI.1.2. Age at onset of clinical disease.

According to Dr.Linda Detwiler (Pers.comm 13.11.01.), scrapie occurs most frequently in sheep of either sex between 2 and 5 years of age (Dickinson, 1976; Sigurdarson, 1991). Cases of the disease are rare before 18 months (Dickinson and Stamp, 1969; Sigurdarson, 1991; USDA, APHIS, Veterinary Services, unpublished statistics). However a few cases of natural scrapie have been reported in sheep as early as 10-12 months of age (Zlotnik and Katiyar, 1961; Joubert et al., 1972). One report from Iceland states that scrapie was diagnosed in a 7 month old animal (Sigurdarson, 1991).

Since it is thought that most animals are infected at birth or shortly thereafter, age at onset of clinical signs and incubation period would be roughly the same.

Several observers have noted that once scrapie becomes endemic in a flock, age at death will decrease over time. The initial cases will usually be in 4-5 year old sheep. The age of occurrence progressively declines where cases develop in 18-24 month old animals (Kimberlin, 1979; Foster and Dickinson, 1989; Sigurdarson, 1991; Detwiler, unpublished observations). Foster and Dickinson, (1989) stated that the most plausible reason for this result was an increase in exposure to the agent.

Parry (1983), reporting on Parry (1962) in Heredity publishes an histogram for scrapie age of occurrence in 1008 sheep. The histogram is comparable to data published by other authors on Suffolk sheep**.** Data are also available for the USA (Source: USDA: APHIS:VS] and for France (estimates). The available data have been summarised in **Table 1** hereafter. It needs to be stressed, however, that the age distributions do not allow the estimation of 5% or 10% percentiles for susceptible or semi-resistant genotypes.

A survey amongst a limited number of scientists with field experience on TSEs in small ruminants yielded the following additional data:

United Kingdom:

L.Hoinville (pers.comm, 4.10.01) reports on field data on age at onsets that are not yet published but will be submitted for publication. In these data it is not possible to distinguish age at exposure and incubation period but the youngest case in her study was 15 months and the oldest was 126 months. In this study age at onset varies with genotype and median age at onset for different genotypes were in flocks with VRQ scrapie cases VRQ/VRQ 32 months, ARQ/VRQ and ARQ/ARQ 40 months, ARR/VRQ 69 months. There is also one

---

[19] On clinical and *post-mortem* examinations, it is essential to detect affected animals as soon as possible. In the absence of significant experience of TSE it is perfectly possible, however, that farmers and even veterinarians will fail to recognize some of the signs of TSE and in such circumstances a *post-mortem* examination of animals that do not recover could provide additional reassurance that the disease is not TSE.

flock in which no VRQ cases occurred and in that flock ARQ/ARQ animals have a median age at onset of 31 months and AHQ/AHQ animals a median age at onset of 41 months.

Norway:

Age of clinical and verified scrapie cases has been between 2 and 8 years. Among cases with known age and genotype (n=39), 12 has been ca. 2 years, 13 between 2 and 4 years, and 14 above 4 years, overall mean 4.5 years. Mean age of VRQ/VRQ genotypes with scrapie has been 3.3 years, ARQ/VRQ 3.9 years (n.s.), ARQ/ARQ 4.2 years, ARQ/ARR 3.5 years, AHQ/AHQ 4.6 years, and AAHRQQ 5.5 years (Ulvund, pers.comm., 11.10.01).

Table 1: Summary of data on scrapie incubation period distribution

Age	Percent	Age	Percent
< 12 months	0	< 12 months	0
13- 24 months	2.5	13- 24 months	10.0
25-36 months	37.5	25-36 months	52.5
37-48 months	47.5	37-48 months	30.0
> 49 months	12.5	> 49 months	7.5
Total sample: 930 ewes		Total sample: 78 rams	

UK (Source: Parry, 1983) (Figures estimated from a published histogram):

Age	Percent
< 12 months	4.5
12 to 24 months	25.0
25 to 36 months	25.0
> 37 months	44.5
Total sample: 178 animals	

France (Source: T.Baron, pers.comm., 10.10.01)

Age	Percent
< 17 months	0
17 to 24 months	6.9
25 to 36 months	34.3
37 to 48 months	36.4
49 to 60 months	12.8
61 to 72 months	6.2
> 72 months	3.4
Total sample: 697 animals	

USA (Source: USDA:APHIS:VS)

From what precedes it can be concluded that, as a rough estimate, for approx.10% of the scrapie cases, the age at onset is above 5 years.

VI.1.3. Feeding history of the small ruminant flock.

a) Management and feeding.

Sheep management and possible feeding of concentrates containing MBM.

Small ruminant feeding largely corresponds with three general periods (birth till weaning, growth period and finally production period) with the three critical points for concentrate feeding at flushing, steaming and lactation. Management and feeding practices vary very much both within the EU and among third countries. Main differences are in the use of the animals, i.e. for meat, wool or dairy purposes.

Sheep being kept mainly for wool, especially the fine wool breeds, are most often extensively managed on pasture, and not intensely fed. Hence the risk from feed is expected to be smaller for such sheep.

Sheep kept for meat only, or for meat and wool, which statistically is the most common practice, are usually given concentrates at least during late pregnancy and early lactation (often from six weeks before lambing until a month after, depending on the type and quality of the pasture or roughage). Concentrates are also fed around mating time in most regimes and countries, i.e. for 3-4 weeks. Pregnant ewes may also be supplemented with concentrates before parturition where pregnancy toxaemia disease is anticipated. In countries where animals are kept indoors throughout the winter, daily feeding of smaller amounts of concentrates is not uncommon. In sheep flocks where mating of lambs is common, lambs are often given concentrates from an age of about 5 months.

There are also possibilities for very young lambs to get hold of, or start eating, small amounts of concentrates when their mothers are being fed. In most housing and feeding systems the lambs are able to get admission to the feeding troughs. As the selection of pedigree lambs or replacements is most often not done until the age of 3-5 months, many of these thus have eaten concentrates very early in life. In the Scandinavian countries, for instance, sheep are fed indoors for several weeks after lambing until turnout to pasture, and lambs often get access to concentrates.

There is a market for meat production, or fat lambs, and such lambs are often fed concentrates from an early age. In GB, and also in other countries, early fat lambs for the Easter trade are slaughtered. There is, however, also a market for yearlings, and older sheep. In connection with the production of meat for specific ethnic niche products, sheep of older ages are sometimes preferred.

Milk production in sheep is less common, but some countries have a highly developed sheep milk industry. In such flocks, feeding concentrates is rather common from an early age and during most of the year, and lambs are removed at an early stage. Furthermore the lactating sheep may also receive concentrates to boost milk supply.

Goat management and and possible feeding of concentrates containing MBM.

Goats are mainly kept for milk, but some are kept for their fibre. A very small amount of concentrates is often recommended to be offered to kids from about two weeks. The amount is increased gradually as the kids grow. The adult goats are fed protein enriched feeds, most often containing concentrates, throughout the whole lactation period, which may amount to 6 months a year. Under certain

management regimes, the goats are therefore highly at risk if infected MBM is fed.

Feed components other than MBM

According to the opinions of the SSC on the safety of tallow, gelatine and hydrolysed proteins, these products could constitute a certain risk if they are produced from BSE-contaminated bovine or ovine/caprine sources. A certain risk remains if uncertainty exists about the source of the original raw material, even if the conditions for the safe production stipulated by the SSC are respected. It is therefore important that the animals in a certified TSE-negligible risk flock are not fed these products. For the same reason, feed of unknown origin, such as waste food, should not be given into TSE-negligible risk certified herds.

b) Feeding and scrapie

The origin of scrapie historically is unknown but the disease is perpetuated by sheep to sheep transmission and probably indirectly. It is theoretically possible in more modern times that index cases of scrapie could arise from exposure to scrapie-infected MMBM though this has not been formally proven. Thus, a detailed knowledge of the feeding system (including possibilities of cross-contamination of concentrate diets with infected MMBM), importation practices and epidemiology may assist in determining the most likely strain of agent (BSE or scrapie) responsible for the occurrence of a TSE/prion disease. It remains, however, that the only definitive answer to the strain of agent responsible is by biological strain typing in mice.

Up to now, there has in fact been very little focus on the possibilities of introduction of scrapie through mechanisms similar to those which led to the spread of BSE in cattle, i.e. through scrapie-infected MBM fed to small ruminants. If so, there would be no species barrier for sheep to sheep or goat to goat transmission of scrapie via MBM. One might then expect occurrence of the disease among susceptible animals in a region where the infected MBM has been used. This could lead to an increased infection pressure within the exposed flocks via overt clinical cases (infective foetal membranes, secretions/excretions/faeces – or other unknown routes - vectors etc., resulting in both horizontal and possibly maternal transmission. The initial introduction of scrapie through infected MBM could thus lead to a smaller or larger epidemic type of disease occurrence, dependent on the prevailing genotypes of the actual sheep in the region.

c) Feeding and BSE in small ruminants, should it occur.

The protein source in concentrates for small ruminants has often been vegetarian (grain based) or fish meal, but mammalian MBM has also been used, at least until effective feed bans were in place.

Present evidence suggests that index cases of BSE in sheep if they occurred would likely to be due to initial feed exposure to BSE-infected MMBM in the past. MBM derived from BSE-infected small ruminants could be a higher risk than that

derived from BSE-infected cattle (assuming the titres of infectivity were reasonably equivalent) because there would be no species barrier. It is assumed, of course, that BSE infection from infected sheep after heat treatment through rendering, transmits the disease back to the same species by the oral route. This assumption has not yet been formerly proved and precedents exist where oral challenge is ineffective, but mainly across species barriers. This oral route would be the most likely to perpetuate the disease since there could be widespread exposure through feed. Thus risk management procedures applied to control feed infection would have a dual approach namely, preventing initial exposure and preventing propagation.

Probably due to a lack of focus on concentrates fed to small ruminants, information on the origin of protein used in concentrates has been difficult to obtain. Accidental cross contamination of small ruminant diets could have occurred in the UK at least until August 1996, by which time the degree of feed security was very high. Some countries operated a ruminant feed ban for all ruminant species from 1990. Some other countries with scrapie did not operate any ban on MMBM in sheep feed until at least 1994 and other than the UK and Portugal no country operated until 1 January 2001 a complete ban on MMBM for all food animal species. In Britain, mammalian MBM was included in some diets until July 1988, when the practice was prohibited.

Current risks would be dependent on the effective enforcement of MBM and ruminant MBM bans. Other factors would include the prevailing possibilities of cross contamination of small ruminant concentrate feed with MBM intended for cattle[20], poultry and pigs; specified sheep risk materials (SRM) bans which are not fully implemented in many countries up to now; rendering parameters and feed processing; and scrapie related culling.

VI.1.4. Flock management in respect to environmental, horizontal and vertical transmission

McLean *et al* (1999) analyse potential risk factors associated with the occurrence of scrapie in a number of UK farms. Hopp *et al* (2001) put into evidence, for Norway, the different risk factors for the occurrence of scrapie in farming, and report in more detail on the risk of transmission between flocks following the introduction of ewes or rams or through common grazing/pasture. AFSSA (2002) provides a detailed account of the conditions that could maintain TSE in small ruminant flock in the absence of feed exposure.

a) Common grazing and environmental transmission

---

[20] Analysis of data on notifications of BSE and scrapie in UK reveals that 200 of the 22,966 farms that reported BSE between 1993 and 1999 notified both scrapie cases in sheep and BSE cases in cattle in this same period. This is not significantly greater than the number expected (196) if these two diseases occurred independently on all farms keeping both cattle and sheep (Francois Courtin, unpublished observations). This suggests that sheep farms that have had BSE in cattle are at no greater risk of having a TSE in sheep than those farms that have not had BSE. There is presently no knowledge as to how long, or for how many sheep generations, infectivity may be silently carried in less susceptible or more resistant genotypes of sheep and goats.

Common grazing could constitute another risk factor, especially around the lambing period but also permanently because of the persistence of infectivity via the soil or local vectors (hay mites, nematodes, etc). The average age of onset of clinical scrapie in Suffolk sheep is 3 to 4 years, but it is unknown whether, in case of natural BSE in sheep, the incubation would be (much) shorter or longer. This implies that the start of spread and appearance of clinical signs after the movement of an infected lamb or sheep into a TSE negligible risk flock may vary from a few days to several years. Moreover, there could be circumstances in which the infection can spread "silently". One circumstance is when ewes are culled before they had time to develop the disease. By the time infection in a flock has been revealed by the occurrence of clinical disease, several other animals could already be infected and may have been used for breeding in the flock of origin or in other flocks. Because of long incubation periods, the absence of BSE in a flock can only be established over long periods of time. The degree of certainty, in the absence of *in vivo* tests to detect all infected fertile rams and all infected dams before their first lambing, will be in direct proportion to the time that the flock has been continuously and thoroughly monitored.

The evidence for the transmission of natural scrapie from an infected environment is circumstantial (Hoinville, 1996), but two points are worthy of note when considering this issue. Laboratory strains of high titre hamster scrapie agent retain infectivity after burial for three years, though over 99% of the infectivity was lost (Brown and Gajdusek, 1991). Secondly, scrapie eradication programmes in several countries have failed to eliminate the disease. In Iceland, where the greatest effort has been made, success is close to achievement. This has followed close attention to the removal of the hazard of possible environmental contamination. The measures included extremely thorough cleaning and disinfection of farm buildings (flaming, burning, disinfection, creosoting, oil-based painting), leaving farms devoid of sheep for up to three years and removal of the topsoil from around farm buildings and other contaminated areas (Sigurdarson, 1991, 2000). Restocking was from scrapie-negligible risk flocks in fenced areas of Iceland. Despite these extreme measures, 2 new cases of scrapie occurred recently (Sigurdarson, 2001, personal communication). More generally it had already been shown by Sigurdarson and Ducrot (1998) that, in spite of prolonged eradication programmes in Iceland (20 years) followed in a number of cases by flock reconstitution, scrapie reappeared in approx. 5% of the reconstituted flocks.

b) Transmission of infectivity around birth (horizontal transmission)

Transmission of disease from one animal to another can occur by direct or indirect contact (including transmission from dam to offspring *in utero*, during parturition or in the immediate post-parturient period). Horizontal transmission is a more important mechanism than maternal transmission in transmitting sheep scrapie to other animals (and of course to other species) because it is a method that enables exposure of unrelated animals to take place (Hoinville, 1996). Woolhouse (1998) has shown that horizontal transmission is likely to account for the majority of

cases occurring within affected flocks. Potential methods are *via* placenta (proven, see below), milk, faeces or nasal discharges (all unproven).

As a result, the so-called maternal transmission of natural sheep scrapie could be a form of horizontal transmission.

It is known that the sheep placenta can be infected (Pattison *et al* 1972, 1974, Onodera *et al* 1993, Race, Jenny and Sutton, 1998) and that it can transmit disease by experimental exposure by the oral route to other sheep and goats (Pattison *et al* 1972, 1974). However, since the precise mechanism of maternal transmission in sheep is unclear, it is not possible to be entirely sure that the full range of infected source tissues that result in maternal transmission is known. It is therefore not possible to guarantee that risk management strategies to protect against this hazard will be completely effective. This view is supported by the fact that in practice, scrapie in sheep is very difficult to eliminate.

A pregnant ewe's placenta can be infected over a year before clinical signs develop in the ewe. Sequential pregnancies may show consistent infectivity/PrP^{Sc} of the placenta perhaps related to the genotype of the placenta that in turn results partly from that of the ram. The infection could be transmitted from ewe to lamb, but there could also be a possibility of horizontal transmission of infection between related and unrelated sheep which may be exposed as adults or as lambs. The risk for horizontal spread of infection is the highest when sheep are kept together, for example at lambing time.

c) **Transmission by genetic material (vertical transmission)**

Vertical transmission: transmission of disease can occur from either parent either genetically or environmentally via germ plasm at fertilisation. Transmission of TSE in small ruminants is not known to be due to mutations in the *PrP* gene or any other gene.

Transmission by semen. Infectivity was not found by bioassay of ovine semen from a ram with scrapie, in injected lambs. However, this study was done before the effects of the *PrP* genotype on the incubation period of scrapie were known. It is helpful to know that bioassay of testis and seminal vesicle of sheep with scrapie did not reveal any detectable infectivity (Hadlow, Kennedy and Race, 1982).

Transmission by ova or embryos. Wrathall, 2000, interprets the results of two experimental studies (Foster *et al* 1992, 1994, 1996 and Foote *et al,* 1993) relating to the use of sheep embryos from infected sheep with scrapie as follows: "I concluded that, taken overall, both Foster's and Foote's results provide good evidence that scrapie is not transmitted by sheep embryos". Foster *et al,*(1999) showed that experimentally BSE did not transmit to goats via goat embryos. From what precedes it remains unclear whether scrapie can be experimentally transmitted by embryos.

d) **Other forms of transmission**

Via vectors The evidence for transmission of scrapie via vectors is limited and this form of transmission cannot be entirely ruled out. Particular vectors potentially incriminated are (a) hay mites (Rubenstein *et al*, 1998), (b) fly larvae (Post *et al*, 1999) and (c) nematodes (Fitzsimmons and Pattison, (1968).

However, Hourrigan *et al,* (1979) reported that one cage of mice were infected when inoculated with *Haemonchus contortus* from a sheep with scrapie transmitted disease. Very little experimental detail was given of this study and it remains an isolated report. Elsen *et al* (1999) found high levels of scrapie transmission in a flock of sheep infected with the nematode *Teladorsagia circumcincta* and postulated that damage to the gut may have enhanced entry of scrapie infectivity into the tissues.

Although protozoon parasites like *Toxoplasma gondii* and *Sarcocystis* sp from the gut can sometimes enter brain tissue of sheep and goats they have not been incriminated in the transmission of TSE in any species.

From these reports it can be concluded that if vectors play a role at all it is a minor one and merits less management attention than other methods of transmission. Clearly parasitic infections should, however, be controlled.

Iatrogenic exposure of scrapie has probably occurred twice. The first report determined that the vehicle was a louping ill vaccine prepared from sheep tissues and this infected a large number of sheep sheep (Gordon, 1946, Greig, 1950). The second was more recent and in this case a vaccine against *Mycoplasma agalactiae* prepared from sheep tissues was incriminated (Agrimi *et al* 1999, Capucchio, 1998) but not all outbreaks could be linked to the use of the vaccine. In this episode goats were predominantly affected.

VI.1.5. Genotype of the flock animals with regard to TSE susceptibility

According to current knowledge, genotypic resistance to TSE infection will not [yet] provide a 100% proof of freedom from the presence of infectivity (i.e.a potential carrier of infectivity). However, a flock that is entirely composed of resistant or semi-resistant genotype(s) is much less likely to have an occurrence of TSE.Also, if infection was present, infectivity levels in younger animals are likely to be lower compared to animals of a susceptible genotype.

VI.1.6. Culling strategies applied to eradicate or control TSE in a flocks.

Ideally, all animals exposed to the same source of infection as the index case should be culled. The report attached to the SSC Opinion of 8-9 February 2001 presenting a pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions, mentions, as an example, two options of culling scheme options [21].

However, in the light of the current report and of the SSC's opinion of 18-19 October 2001 on the safety of small ruminant products should BSE in small ruminants become

---

[21]   The risk assessment also mentions that the emphasis might be adjusted to removal of susceptible sheep on the one hand whilst building up resistant genotypes on the other.

probable / confirmed, only one of the options offered in February 2001 would still be scientifically defendable, namely:

> *"> Slaughter followed by destruction (incineration) of all animals from flocks with TSE to prevent spread, except the ARR/ARR genotyped animals.*
>
> *> Tests on tonsils or retro-pharyngeal lymph nodes and brain of all healthy animals slaughtered above 6 months of age **[for destruction]** to detect pre-clinical cases from all flocks with a TSE case.*
>
> *> Examination and destruction of all offspring of clinical and pre-clinical cases and of the birth cohorts (including in-contact flocks). Restrict the movements of the animals from the in-contact flocks until tested negative by a validated **ex** vivo test)."*

Indeed, for cattle, with feed and maternal transmission being the commonly recognised routes of transmission, the birth cohort[22] is seen to be an approximation to the part of a herd that could have been equally exposed to BSE infectivity as the indicator case. *However,* given the various ways of transmission of TSE in sheep the possible "source of infection" cannot be limited to feed or the dam (as for cattle) but should include also the environment (common grazing areas) and other individual animals (contact exposure, eating of placenta's, …).

A study carried out in Belgium by Roels and Vanopdenbosch (2001) showed that the risk of spread of TSE in sheep by trade is far from theoretical. They describe an outbreak of TSE/scrapie in a flock of Hampshire Down sheep in Belgium. After the diagnosis was confirmed in an animal that had been sold, screening was performed in 16 other farms that also had bought animals from that same farm. Half of these flocks proved to be positive. In the primary focus, 2 sheep were affected.

Conclusion:

Because of the transmissibility of the infection within a flock and between flocks by direct or indirect contacts, the elimination of only the index case will not eliminate the enhanced risk in a flock where a clinical or sub-clinical TSE case has been confirmed. A culling strategy which covers the entire flock as well as the animals possibly exported from the flock and their offspring, but with the exception of the resistant animals[23], will result in a much higher level of risk reduction.

An additional risk reduction would be achieved by culling the flocks that were in contact with the original flock via other small ruminants[24] or via grazing areas. The extent of the further risk reduction can currently not be assessed because of lack of data. The risk reducing effect of culling sheep if the risk of transmission to other flocks was negligible, would, however, be limited if not nil. The assessment whether the risk for transmission to other flocks was negligible requires that the animals introduced into a flock are identifiable and their history traceable and that they are

---

[22] A cattle birth cohort covers the animals falling into the same age group (date of birth +/-12 months) as the case and for which the place of birth and/or of rearing during their first months of live is related to the case.

[23] Rapid TSE testing at slaughter of the spleen or brain of ARR/ARR animals above the age of 18 months from flocks with TSE would gradually provide conclusive evidence / reduce to negligible the risk that this genotype is a carrier of detectable infectivity levels.

[24] Including via the offspring of the case

genotyped. The risk would for example be negligible in the case of contacts with or imports from flocks certified to represent a negligible risk, if the contact only concerned the use of breeding rams (as compared to pregnant ewes) or if the imported animals tested negative with a validated in vivo test (once available). The application of one of the certification scenarios as described in the Report of the TSE/BSE *ad hoc* Group could be considered as an alternative for the slaughter of contact flocks at risk.

Much of the above will have to depend on the availability of detailed records and identification, and it may not only be impossible to trace sheep that have moved out of a flock or cohort historically, but the identification of cohort and offspring may also be impossible. If no tracing of animals exported from a BSE infected flock is possible, an ~~accurate~~ epidemiological investigation could be helpful to identify the potentially exposed flocks. (However, field investigations regularly prove that animal exchanges are enormous, easy to keep secret and difficult to trace without detailed identification and comprehensive flock records, especially for sheep.)

Note: Culling strategies as suggested above would reduce the TSE infectious load in flocks and the environment. However, when designing a strategy to be applied in practice, one should be aware that some measures might eventually appear to have a risk increasing rather then a risk reducing effect. Examples where such may be the case and where careful weighting of the advantages and disadvantages of various culling options is required, are:

- TSE reporting and surveillance may be discouraged;

- Selection / breeding programmes may be negatively affected;

- Culling of entire flocks (with the exception of ARR/ARR animals) and their replacement by ARR/ARR animals or the use of ARR/ARR rams, may significantly accelerate the introduction of unwanted characteristics possibly accompanying the ARR/ARR genotype and therefore render more difficult timely adjustments in the breeding policy;

- For some breeds it may be difficult / impossible to find replacement stock if they do (almost) not have ARR/ARR genotypes.

- The management of the environment and the landscape and certain lifestyles in less favoured areas (e.g., transhumance) may be adversely affected.

Culling programmes should therefore be conceived within an overall risk reduction strategy including also controlled breeding for resistance, flock certification, genotyping, testing, etc.

VI.2. **ELEMENTS IDEALLY NEEDED FOR ESTABLISHING AND MAINTAINING THE STATUS OF A "SMALL RUMINANT FLOCK CERTIFIED AS OF TSE-NEGLIGIBLE RISK"**

The minimal length of period for which information on records, surveillance and appropriate management may be required in the absence of other criteria to decide about the TSE status of a flock, will depend on the period after which a TSE can be first observed after its initial introduction.

VI.2.1. Records

Without good records it is impossible to certify the past health status of a flock and to verify that the small ruminants originating from the flock have not died elsewhere.

a) The fact that no clinical cases occurred in the flock during a given period of minimal length should be supported by complete records of births, deaths and all movements of the individual animals for that entire period They should allow the fate of all animals that have resided in the flock during these years to be determined.

b) The records should indicate that there was a negligible risk that TSE cases were present and that no infectivity was introduced during a given period mainly by providing information that permits to conclude on:

- Absence of TSE disease; also all animals showing signs of neurological disorders should be examined to exclude the possibility of new infection with TSE;

- The absence of imports/purchases or movements of animals from risk flocks or areas;

- The absence of exposure to TSE infected MMBM, especially for dairy sheep and goats (including also a detailed knowledge of the feeding system, the possibilities of cross-contamination of concentrate diets with infected MMBM and importation practices);

- The exclusion of environmental routes of TSE transmission, especially in cases of common grazing;

- The exclusion of horizontal routes, especially *via* placenta.

c) The availability of reliable records may imply that for the entire period, each animal must have been identified and monitored beyond doubt. For animals which have entered the herd during the period, records must allow their tracing back to the natal herd, in order to allow an assessment of their status with regard to TSE. If no reliable records of disease history exists the assumption should be that TSE may be present and this needs to be excluded by appropriate post mortem tests. When early pre-clinical *in vivo* tests are available, all animals in the flock testing could as alternative condition for certification.

d) For newly established flocks, sufficient guarantees are required that the flock is constituted only from animals from a country of negligible to zero TSE-risk or from flocks of an equivalent status.

VI.2.2. Surveillance data

a) Veterinary surveillance of the flock should be of such level that it is guaranteed that all cases of neurological disorders, for which TSE cannot be excluded, are immediately recognised[25].

b) Rapid TSE testing of animals of a flock, in complement to clinical surveillance or if no or only incomplete or unreliable data are available, would significantly increase the trustworthiness of a certification because infectivity can be detected shortly before clinical signs for the currently available tests that operate on CNS tissue and relatively early in the incubation period for tests applicable to lymphoid tissues, should they become available. The latter type of tests could probably also accelerate the procedure because it would be necessary anymore that a large number of animals reach the stage of adulthood.

The extent of the testing scheme could however been influenced by other factors such as the reliability of the historical data and genotypes of the sheep:

- The least intensive testing scheme would theoretically be required under a scenario that reliable historical data are available showing no history of TSE, and all sheep are genotyped and are of the ARR/ARR genotype: the possibility to detect PrP^{res} in sheep of such of flock would probably be limited (if PrP^{res} is present at all in such sheep) to older animals and PrP^{res} localized in the intestinal tract or spleen. So, in this scenario only older sheep would need to be tested and only on some target organs (spleen lymph nodes, tonsils).

- The most intensive scheme, including regular testing of al sheep and if available using also *in vivo* test, should be applicable for flocks with only full susceptible animals or no known genotype in the absence of any reliable historical data.

VI.2.3. Management

The management of the (candidate) certified TSE-negligible risk flock must be such that contact with other flocks is strictly limited to (i) exchanges via artificial insemination[26], (ii) exchanges between certified flocks and (iii) introduction of ARR/ARR rams for breeding and reproduction. Direct or indirect (common grazing, placenta from TSE-infected sheep) contact with small ruminants from other flocks as well as contact with potentially infected materials should thus be avoided.

Unless reared in complete isolation and kept under separate (e.g., feeding, grazing, …) conditions or unless a total ban on the use of MMBM is in place, chickens and pigs should not be on the same premises as the "certified TSE-negligible risk flock" because of the possibility of accidental feeding of small ruminants with pig and poultry feed containing MMBM. This condition is theoretically fulfilled in the EU since 01/01/2001 because of the total MMBM ban since then.

---

[25] The constitution of such level of veterinary surveillance and ensuring that this degree of surveillance was established and maintained in all flocks would require a substantial resource input.

[26] See SSC opinion of 1998 on Vertical transmission

VI.3. **ELEMENTS OF AN ACCREDITATION SCHEME FOR** *MAINTAINING* **A PROVISIONAL CERTIFICATE OF REPRESENTING A NEGLIGIBLE TSE RISK.**

To maintain a certificate of "TSE negligible risk flock", the flock must be kept closed and a number of conditions must be fulfilled in any case:

VI.3.1. TSE freedom

If a TSE case is detected in a certified TSE-negligible risk flock, the flock can of course no longer been considered as TSE-negligible risk.

VI.3.2. Marking (preferably individual) of all animals and at least indicating the farm of origin. This implies that all movements of animals must be traceable, the farm of origin in case of purchase must be identifiable and that ideally also the parents would be traceable. For animals imported from third countries a special earmark could be used.

VI.3.1. Availability of reliable records ideally computerised, on parents (or at least the dam), movements, purchases, deaths and common grazing:

- all animals and in such a way that the female parent can be individually identified and, if possible, the ram parent also;

- the dam and sire of all animals born in the flock (sire identification is often impractical);

- the dates of each event and all movements into and out the flock and identification of the farms from which animals were brought into the flock and destination of animals, the immediate past residence of any brought in animal for the female parentage and ram (where known). Recording of all temporary acquisitions including hiring of rams.

- all deaths, an with indication of the reasons for death for all animals above 6 months of age;

- common grazing, when used, and identification of the contact flocks. Only flocks of certified TSE-negligible risk status should be allowed .

VI.3.3. Management practices should show that the risk of introduction of infectivity was/is reduced to a negligible level. This implies:

- Trading between holdings should occur only between holdings of equivalent status or from a higher to a lower graded holding.

- Sheep should be prevented from straying in and out of the flock and to prevent directs or close contact with the sheep from another flock. This may require effective fencing of premises.

- Common grazing (simultaneously grazing of animals from different flocks)could only be considered appropriate if no part of the land was used for over-wintering sheep for lambing, or for any other purpose that could lead to more than casual contact between animals from a flock with a different TSE status. Record keeping of the use of common grazing would be required.

- Common pasture (same pasture used by animals from different flocks, but NOT simultaneously) could only be considered if no part of the land was used for over-wintering sheep for lambing and should only be used by animals from holdings of the same TSE status. Record keeping of the use of common pasture would be required.

According to the SSC opinion of 1999 on Vertical transmission, semen from rams from any source do not represent a scrapie risk. This is also valid for BSE, if it is assumed that BSE behaves like scrapie in sheep.

Note: [The presence of some full susceptible sheep, acting as sentinels, is sometimes considered to be an advantage in detecting hidden infections in the flock. However, sentinels are initially (during at least 1 year) healthy carriers of infectivity and could therefore contaminate the flock and the environment before any sign of disease is visible.]

VI.3.4.Testing

As animals in the pre-clinical stage of infection may be present in a flock for several years before a clinical case appears, it is recommended that in a "certified TSE-negligible risk flock" brains from all that have died and ~~at~~ a statistically appropriate number of small ruminants from the flock, that have been slaughtered at an age over 6 months, must be examined in an approved reference laboratory, using at least one immunological test for the detection of PrP^{res} (Western blotting, immunocytochemistry, ELISA). If validated test become available for lymphoid tissue (intestine, tonsils, etc) these should be used by preference as they can detect infectivity earlier in the incubation period. Once available, pre-clinical *in vivo* tests should be applied on a regular basis dependent on the capacity of the test to detect infectivity in the early stages of incubation, but taking into account that peripheral tissues in semi-resistant genotypes do not always accumulate PrP res. Application of testing post mortem on CNS tissues must thus always be incorporated into the scheme.

Once conclusive information on the genotype and TSE susceptibility is available, the extent of this testing scheme as well as the tests to be used and tissues to be tested could be modulated in case of sheep flocks on the basis of the available information on genotype distribution within a flock.

It is clear that even in the total absence of information on the genotypes of the sheep, as it is at present in goats, the maintenance of a status of TSE- negligible risk flock is feasible by testing all the animals above 6 months of age at slaughter or, when available, all animals above 6 months, using an in vivo test and by testing CNS tissue from culled animals post mortem.

VI.4. POSSIBLE SCENARIOS FOR THE ESTABLISHMENT AND MAINTENANCE OF FLOCKS PROVISONNALLY CERTIFIED AS OF "TSE NEGLIGIBLE RISK"

The preceding chapters show the following:

- as a rough estimate, for approx.10% of the scrapie cases, the age at onset is above 5 years,

- TSE infectivity can be present for several years in a flock before infectivity is detected or clinical cases appear,

- the probability of TSE infection developing into clinical disease would also be determined by the genotype composition of a flock,

- it is not proven beyond any doubt that no 100% guarantee that silent carriership occurs in resistant or semi-resistant genotypes and,

- contrary to cattle, there is evidence of maternal (peri-natal) transmission in sheep,

It follows that, *ideally*, a flock could be declared as being of negligible TSE risk only if the prevalence of TSE can be ruled out in a significantly large number of animals of the generation following the "closure" of a flock, that were kept alive for a number of years that corresponds to the upper limits of the incubation period. The data on age of onset show that 90% of the clinical TSE cases in sheep appear at an age below 5 years[27]. So, full certification on the basis of records alone of a flock would probably not be possible within 7 – 10 years and provided a sufficiently large number of animals[28] have been kept alive to exclude the prevalence of TSE infectivity in the flock.

Such full certification is probably not an approach that is readily applicable under real field conditions. The Working Group considers that a practical alternative, which is compatible with field reality and with current knowledge on TSEs in sheep, would be to option for the concept of a "provisional certificate of negligible TSE risk", attributed on the basis of less stringent time criteria but to be applied in combination with other criteria such as testing and genotyping.

Depending upon the available information, time horizons between **0 and 5** years could be envisaged, as illustrated in the scenarios hereafter. They suggest a possible sequential and evolving scheme of certification which recognises the current ability to test for PrP^{Sc} in post-mortem material, the validated and sensitive *in vivo* tests that are likely to become available in a foreseeable future and the advantage that can be taken from genotype information.

VI.4.1. Symbols used

R+:   Reliable flock history records available for at least **5** years indicating a negligible TSE risk originating from different factors as described under

---

[27] For semi-resistant animals, this age would be higher and, moreover, the likelihood to find a TSE case would be lower.

[28] The flock size corresponding to "a sufficiently large number of animals" depends on many factors, as indicated above: genotype composition, age at slaughter, etc.   For flocks entirely composed of "susceptible" genotype(s), the likelihood to find any clinical disease in an infected environment is higher. For flocks entirely composed of "resistant" genotype(s), the likelihood is high to never find any clinical disease and the flock size would therefore need to be very large. However, although the absence of detection of clinical disease in such "resistant" flock during a period of 5 years would not automatically imply total absence of infectivity, one can nevertheless accept that the levels of infectivity, if present, would be very low.

III. (History of TSE/scrapie, feeding, introduction of infection in the flock by environmental, horizontal and vertical transmission). The period of **5** years represents at least the time of the average incubation period of susceptible sheep and goats `and` at least 2 times the shortest incubation time in susceptible sheep. Provided the flock is large enough, there is a probability of approx. 90% that a TSE, should infectivity be present in the flock, would manifest itself in its clinical form within this period. This does not, however, guarantee that no cases at all could emerge later, particularly in more resistant genotypes which will tend to have longer incubation periods.

R- :   Only incomplete or unreliable flock history records available for **5** years.

G+ :   Genotyping is done for all sheep in the flock proving that they are all totally resistant not only to clinical disease but also to sub-clinical infection on the basis of new research findings on genotypic susceptibility to infectivity

G+/-:   Genotyping is done for all sheep in the flock showing that not all sheep are ARR/ARR

G₀: No genotyping or only part of the sheep flock is genotyped. If the latter is the case, then the genotyped animals should preferably be physically separated from the non genotyped, and be considered as a G+ or G+/- category

(Sheep):   Scenario only applicable to sheep flocks

(Sheep and goats):   Scenario applicable to both sheep and goat flocks

VI.4.2. Certification scenarios:

Introductory note: The different time schedules for certification starts in each case from the moment that the herd is fully closed and thus no additional introduction of TSE via live animals, feed, or via environmental, horizontal or vertical transmission is possible from that time.

Many flocks require additional feeding because of poor quality grazing. If a source of food supplements comes from guaranteed MBM-free sources this would be acceptable under the closed-flock definition if traceability of feed and animals is ensured.

Once the flock starts the procedure for being certified "TSE negligible risk," then the testing schemes proposed below are needed and the conditions on marking, recording and management as listed in Chapter VI for maintaining the status should continue.

Scenarios :

1. R+, G+ (sheep) and R-, G+ (sheep)

Provisional certification is possible immediately after closure of the flock, once it is clear that the sheep are all genetically resistant to infection with TSEs.

To maintain certification: regular TSE testing to demonstrate no infection could be undertaken on a statistically suitably sized sample with reliance being placed now on post-mortem tests until in-vivo test procedures become available for older animals e.g. those above 2 years of age at slaughter. Any introduced stock would have to be tested to prove their intrinsic genetic resistance to TSEs.

As most flocks will not be very large, it is likely that testing would have to continue. If a positive was found, certification would need to be reviewed in light of the denominator of accumulated negative tests.

2.   R+ and G+/- (sheep)

Provisional certification would only be possible if new in-vivo TSE testing proved negative in the partially or totally susceptible animals or when a suitable statistically sized fraction of the susceptible genetic stock had undergone post-mortem testing of the spleen, lymph glands, tonsils etc. Regular TSE negative testing of a representative sample of older susceptible above 2 years of age at slaughter (tonsil, spleen, brain) could then be used to maintain certification.

As most flocks will not be very large, it is likely that testing would have to continue. If a positive was found, certification would need to be reviewed in light of the denominator of accumulated negative tests.

3.   R-, G+/- (sheep) and R-, G₀ (sheep and goats)

Provisional certification is possible **5** *years* after closure of the flock and TSE negative testing of all animals above 6 months of age using an *in vivo* test on peripheral tissues (tonsil, blood when validated) and on all the animals at slaughter (tonsil, spleen, brain).

To maintain certification:

- TSE negative testing once a year of all unknown or genotype susceptible sheep above 6 months of age using an *in vivo* test on peripheral tissues (tonsil, blood when validated) and on all the these sheep at slaughter (tonsil, spleen, brain).

- Regularly TSE negative testing of a representative sample (at least 10% per year) of older ARR/ARR sheep above 2 years of age at slaughter (tonsil, spleen, brain). (ARR/ARR are included because absolute certainty about their full resistance is still lacking. Only once this certainty for all breeds is available, the tests for ARR/ARR could be stopped / reduced.][29]. As most flocks will not be very large, it is likely that testing would have to continue. If a positive was found, certification would need to be reviewed in light of the denominator of accumulated negative tests.

---

[29]   See also the SSC Opinion of 30.11.01 on requirements for statistically authoritative BSE/TSE surveys.

4. <u>R+ and G₀ (Sheep and goats)</u>

Provisional certification is possible after closure of the flock and TSE of post mortem material in statistically sufficient numbers have confirmed that no TSE is detectable in spleen, lymphatic tissues and brain. As most flocks will not be very large, it is likely that testing would have to continue. If a positive was found, certification would need to be reviewed in light of the denominator of accumulated negative tests. Once a validated in-vivo test for early infection becomes available and routine testing is feasible then ideally all animals above 6 months of age could be tested using the *in vivo* test. At first it would also be helpful to have confirmatory post-mortem testing.

To maintain certification would require the continued documentation relating to the introduction of any other genetic stock. A system for simple in-vivo TSE testing of these animals before their introduction would provide some indication of their TSE free status, except during the incubation of the TSE. Alternatively confirmation of the originating flock's TSE free status would be needed. In practice further TSE testing would be required in the susceptible host flock to prove that they had not acquired the infection with allowances being made for the incubation time of TSEs and whether reliance was being placed on post-mortem or new in-vivo tests.