EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B1 - Monitoring and dissemination of scientific opinions

Scientific Steering Committee

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

EXECUTIVE SUMMARY

The European Commission requested the Scientific Steering Committee (SSC) to

(1) pre-emptively carry out risk assessments corresponding to a range of circumstances (scenarios) should BSE be confirmed to occur in the sheep population under domestic conditions.

(2) present an updated state of affairs regarding tissue infectivity distribution on the basis of the most recent results of the ongoing experiments on BSE in small ruminants,

(3) identify and list the criteria to be fulfilled to conclude that the BSE/vCJD strain has been isolated from sheep

1. The SSC's opinion on the situation regarding BSE in small ruminants remains unchanged from the position adopted by the SSC on 24-25 September 1998, namely that one "*has to assume that BSE could have been introduced into the sheep and goat population because it has clearly been demonstrated* [by experiments] *that BSE can be orally transmitted to certain genotypes of small ruminants and because it is likely that BSE-contaminated MBM has been fed to some sheep and goats".* There is no evidence to confirm that BSE is present in small ruminants. However, the extent of observations to detect its presence is very limited and we do not at present have adequate means (by monitoring, testing, etc.) to confirm beyond reasonable doubt a diagnosis of BSE in sheep directly or indirectly exposed to the BSE agent.

The SSC considers that it cannot at this stage assess the risk to the human population from the possibility that BSE exists in small ruminants under normal conditions. There are too many variables and unknowns for which additional information is needed. For example, it is not known whether horizontal transmission between sheep is as important as transmission by infected feed. A first conclusion is that there is an urgent need to begin collecting the information required for an adequate assessment of the likely prevalence of BSE in small ruminants.

The SSC considers that a number of possible scenarios could be listed, ranging from the situation that the exposure to infected feed is considered to be unlikely, to the scenario that BSE in small ruminants has been confirmed under natural conditions and that there are related outbreak(s) of cases over a wide geographic area.

The SSC considers the SRM list proposed in its opinion of April 2000 to be valid for the time being. When more comprehensive data on BSE infectivity in sheep is available, the SSC will revisit this conclusion.

The SSC proposes that rapid tests should be developed for differential diagnosis of TSEs in small ruminants. There is further a need to significantly improve surveillance and culling strategies and to accelerate the introduction of a system of individual identification of sheep and of criteria for the determination of the TSE-free status of flocks. A start must be made for the collection of data useful for the assessment of the risk of BSE in small ruminants at the national level, but also at the level of management types and other local features (e.g., farming practices). The SSC further recommends EU wide national programmes of breeding towards a genetically fully resistant sheep population.

2. The SSC pre-emptively lists a number of additional measures that will need to be considered should there be strong indications or evidence of BSE actually being present in domestic flock. These refer to specified risk materials, culling strategies and generalised testing of animals.

The detailed scientific report attached to the full opinion provides an update of the state of affairs regarding tissue infectivity distribution on the basis of the most recent results of the ongoing experiments on BSE in small ruminants. They seem to indicate that BSE, experimentally transmitted to sheep, is likely to show a disease development comparable to scrapie in sheep. This would imply that the list of specified risk materials that must be excluded from consumption, should BSE in small ruminants be likely, would be more comprehensive than in cattle.

3. Regarding the criteria to be fulfilled to conclude that the BSE/vCJD strain has been isolated from small ruminants and the possible positive identification of BSE in small ruminants, the SSC considers that, at the moment, the complete protocol of strain typing by mouse bioassay experiments is the only reliable method for detecting/confirming BSE infectivity in sheep. This protocol is long and presently takes 2 years to be completed. No other clinical or pathological features can conclusively confirm the presence of BSE although there are preliminary indications that some new tests may be able to do so.

The SSC points out that a tentative list of currently available tests and tests under development was presented in the SSC Opinion on *Criteria for the diagnosis of clinical and pre-clinical TSE disease in sheep*, adopted on 13-14 April 2000.

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I. TERMS OF REFERENCE

On 24-25 September 1998 the Scientific Steering Committee (SSC) adopted its opinion on *"The risk of infection of sheep and goats with Bovine Spongiform Encephalopathy agent."* and on 13-14 April 2000 its opinion on *"Specified risk materials of small ruminants"*.

Following the adoption of these opinions, the European Commission requested the SSC to (1) pre-emptively carry out risk assessments corresponding to a range of circumstances (scenarios) should BSE[1] be confirmed to occur in the sheep population under natural conditions,(2).present an updated state of affairs regarding tissue infectivity distribution on the basis of the most recent results of the ongoing experiments on BSE in small ruminants and (3) identify and list the criteria to be fulfilled to conclude that the BSE/vCJD strain has been isolated from sheep.

The request is a logical follow-up of a number of SSC opinions indicating that, although there is currently no evidence to suggest that BSE occurs naturally in sheep and goats under field conditions, there are a number of reasons for concern, for example:

- the difficulties in relation to surveillance and eradication of scrapie/BSE in sheep;
- the risk of infection of small ruminants with BSE;
- the uncertainties related to breeding sheep to be resistant to scrapie/BSE;
- the difficulties in diagnosing clinical and pre-clinical TSE disease in sheep and to differentiate TSE agent strains.

The present opinion is largely based on an in-depth evaluation by the TSE/BSE *ad hoc* Group of a detailed report prepared by a special Working Group and subsequent discussion by the Scientific Steering Committee at its meeting of 8-9 February 2001. The report is attached to this opinion.

Scope of the present opinion:

Annex 1 provides the background to and history leading to the submission to the SSC of the above questions.

For the purpose of the present opinion, small ruminants includeonly sheep and goats. Because much more experimental and field information is available for sheep, the assessment has been initially and primarily carried out for sheep. However, the conclusions will be broadened to goats by taking into account whatever information is available.

It is noted that only sheep and goats have been experimentally challenged and succumbed to BSE infection. Scrapie occurs naturally in sheep, less frequently in goats and moufflon. Maternal transmission (which occurs in sheep) has not been confirmed as occurring in goats.

[1] Regarding the difference between presence of a PrP(res) in a tissue and this tissue also being infectious: see the SSC opinions of 13-14 April 2000 on *Oral exposure of humans to the BSE agent: infective dose and species barrier* and on *The Safety of ruminant blood with respect to TSE risks*

It is further noted that the agent causing scrapie, the expression of clinical disease in scrapie-affected sheep, cannot currently be distinguished from BSE by any means other than biological strain typing of the agent responsible. In the present report, it will be assumed as a reasonable work approach that, in a given infected animal, in general, the BSE agent behaves in sheep and goats like the scrapie agents in sheep and goats. Classical scrapie is present as a persistent disease with a variable within-flock incidence.

III. ANSWERS TO THE QUESTIONS

Following analysis and discussion of the attached detailed report, the Scientific Steering Committee proposes the following answers to the 3 questions:

III.1 Pre-emptive risk assessment corresponding to a range of circumstances (scenarios) should BSE be confirmed to occur in the sheep population under natural conditions.

Preamble:

The Scientific Steering Committee considers that it cannot at this stage assess the risk to the human population using the range of circumstances presented in the detailed report. There are too many variables and unknowns for which additional information would be needed. There is not a precise diagnostic procedure widely available to detect BSE in small ruminants. Data is lacking on the likely prevalence of exposure to the likely source of infection, the quality of surveillance for both TSE and BSE in the affected population and the actual occurrence of sheep to sheep transmission of BSE if this occurs.

The SSC considers that its opinion of 24-25 September 1998 regarding BSE in sheep remains valid namely that one "*has to assume that BSE could have been introduced into the sheep and goat population because it has clearly been demonstrated* [by experiments] *that BSE can be orally transmitted to certain genotypes of small ruminants and because it is likely that BSE-contaminated MBM has been fed to some sheep and goats"*. There is no evidence to confirm that BSE is present in small ruminants. However, the extent of observations to detect its presence is very limited and we do not at present have adequate means (by monitoring, testing, etc.) to confirm a diagnosis of BSE in sheep directly or indirectly exposed to the BSE

A number of possible scenarios could be addressed, ranging from the situation that the exposure to infected feed is considered to be unlikely, to the scenario that BSE in small ruminants would have been confirmed under natural conditions and that there are outbreak(s) of cases with epidemiological links between each other over a whole geographic area.

The identification of definitive BSE in sheep or goats (if based on biological strain typing) is likely to be retrospective by a number of years. In the meantime a level of uncertainty and potential or actual risk may exist until the new situation is assessed and new measures to control any actual or potential risk are in place and effective.

Several SSC opinions already contain recommendations with regard to the risk should BSE in small ruminants be present and to avoid exposure of small ruminants to infectivity. In addition the SSC wishes to offer a number of recommendations.

III.1.1. Diagnosis of BSE in small ruminants.

The SSC considers that the current numbers of TSE cases in small ruminants that are submitted to a diagnostic test or assay for BSE is far too limited as compared to the total number of small ruminants in the European Union. In fact, currently only 178 isolates from UK sheep have been inoculated into mice (for biological strain typing) and an additional 12 isolates from other European countries have been inoculated giving a total of 190 isolates that have been inoculated in UK. Results so far indicate that no BSE agent was present.

The SSC recommends:

- That a sufficient number of testing facilities to carry out these tests is guaranteed;
- That research efforts are undertaken to further develop and validate possible other tests that are more rapid;
- That the number of such tests is drastically increased in all EU countries where scrapie occurs so as to permit reliable conclusions to be possible;
- That the laboratories that have the appropriate expertise, protocols and material share these with other laboratories in other countries;
- That a "ring-test" programme be launched to guarantee, whenever possible, harmonisation between laboratories.

III.1.2. Scenario: BSE in small ruminants is not excluded.

Even if BSE is not found in small ruminants, the SSC recommends the following actions, (some of them are already being applied fully or partly in some Member States):

With respect to human exposure protection:

Specified risk materials. The SSC refers to the SSC opinions of 13-14 April 2000 *listing the specified risk materials in small ruminants.* In that opinion, it considered that: "*at present, in the absence of evidence that BSE is present in any national small ruminant flock:*

- *The list of tissues and materials that possibly pose the highest risk includes: skull (including the brain, pituitary gland, dura mater, eyes and tonsils) and spinal cord of all small ruminants above 12 months and spleen of small ruminants of all ages.(…)* .
- *The potential infectivity of the intestine and lymph nodes of sheep (experimentally) infected with BSE is underway and results are urgently needed to improve the risk assessment."*

Meanwhile, some incomplete additional results on PrP(res) distribution in tissues as well as for infectivity have become available (See Annex 2). The SSC considers the SRM list proposed in its opinion of April 2000 to be valid for the time being. When

more comprehensive data on BSE infectivity in sheep is available, the SSC will revisit this conclusion.

Culling. The SSC recommends a culling programme to be implemented. Culling schemes should basically lead to two effects:

1. a reduction of future number of clinical cases*;*
2. a reduction of present prevalence of sub-clinical infection, which would reduce the human exposure risk to products derived from infected animals.

Various options may be considered to achieve these goals. Some are illustrated in the attached detailed report.

Tracing and certification. The SSC recommends the acceleration of the introduction of a system of individual identification of sheep (ideally to enable tracing of the parents). The SSC also recommends that criteria for the determination of scrapie- and TSE-free status of small ruminant flocks be developed.

<u>With respect to general measures</u>:

Surveillance:

- Introduction of an improved surveillance system, composed of the following two components facilitating the quantitative estimate of the occurrence of TSE in small ruminants:
    a. TSE-in-small-ruminants surveillance that includes active surveillance of potentially exposed populations, awareness campaigns, etc., as outlined in the attached report.
    b. After validation of available rapid post mortem tests for TSE in small ruminants, testing of defined tissue (retropharyngeal lymphnodes, tonsils, other) of statistically sound numbers of slaughtered animals of a specified age (to be defined according to ongoing research[2]) from selected populations, for the presence of a TSE agent.
- Accelerate the development and validation of new rapid TSE tests and of differential BSE/other TSE diagnostic tests in small ruminants, both for confirmatory purposes and to verify the presence of PrP-res in live animals.
- Strain typing by mouse bio-assay for at least one isolate from as many incidents of TSE in small ruminants as possible and reasonably feasible, especially if the genotype of the sheep codes for resistance.
- Start a programme of genotyping of all small ruminants (giving priority to the populations considered at the highest potential risk).

Risk assessment. Start the collection of data useful for the assessment of the risk of BSE in small ruminants at the national level, but also at the level of management types and other features (e.g., farming practices).

It is however expected that the availability of exploitable data will be limited for most countries The assessment of the possible presence of risk of BSE in small ruminants or

[2] So far, PrP(res) has first been detected in susceptible genotypes between 10-14 months after inoculation at 6 months of age, in non visceral tissues and tonsils and 22 at months after inoculation in CNS tissue.

the extent of such risk if BSE in small ruminants were confirmed, will therefore most likely and in the first place have to be based on the outcome of testing programmes with validated differential BSE: Scrapie tests and of reliable surveillance.

Breeding and genotyping of sheep. The SSC recommends that projects within the EU of breeding towards resistance (meaning that BSE latency can be excluded) should be initiated, using TSE resistant rams, in accordance with the SSC opinion "Breeding for scrapie resistance" of 22-23 July 1999.

Information. Information campaigns on TSEs in small ruminants for all involved people.

With respect to research, the SSC recommends to launch additional research where needed. The TSE/BSE ad hoc Group considers that an essential role should be played by the "TSE/BSE research network" established on 15 December 2000 by the Research Directorate General on suggestion by the Council of Ministers.

III.1.3. BSE in small ruminants is likely but not confirmed

If BSE in small ruminants is likely (including circumstantial evidence from any domestic flock) but not confirmed in a domestic flock, the SSC reiterates its view that the most recent data on tissue infectivity obtained from experiments on BSE in small ruminants should be assessed with a view to the possible extension of the list of tissues to be considered as specified risk materials. At the same time culling, tracing and certification should be reinforced and other pro-active actions such as those listed in section III.1.4. may be considered.

III.1.4. BSE in small ruminants is confirmed

Should there be evidence of BSE being actually present in any domestic flock, further actions may include:

- Identify which risk level is applicable on the basis of the approach outlined in the attached report.
- Culling/destruction of all suspects; by "suspects" is meant
  - > the confirmed case, its offspring and other traceable relatives (including birth cohorts);
  - > all the animals with genotype pointing at TSE-susceptibility, both in the affected and in the contact flocks.

  Restrict movements of the remaining resistant animals to the slaughterhouse and prohibit the use of any part of a restricted animal for human consumption until declared fit for human consumption after TSE testing by the Competent Veterinary Authority.

  Depending upon the extent of the risk, the culling should be applied to the whole exposed population at flock level only, or for a given management type, or possibly for a whole region (*e.g.* a hill area where co-grazing is practised) or country.
- Detailed epidemiological analysis according to the scheme specified in Annex 3.

- (If feasible: rapid genotyping of all animals in the affected flock and the flocks linked to it.)
- Repopulation should be done from uninfected (certified scrapie and TSE-free) flocks or with genetically fully resistant animals; (this will also reduce the exposure of sheep and man but may have impact on the reporting likelihood.)
- Use resistant rams for breeding in affected flocks;
- Possibly: disinfection of affected premises with a disinfectant appropriate for TSEs as illustrated in the detailed report.

The SSC considers that the risk assessment that will be carried out should a first case of BSE in small ruminants be found should take into account the general prevalent underreporting of TSE in small ruminants.

III.2. Update of the state of affairs regarding tissue infectivity distribution on the basis of the most recent results of the ongoing experiments on BSE in small ruminants,

The main recent findings can be summarised as follows:

BSE agent has been experimentally transmitted to susceptible sheep and goats following oral challenge. Infectivity and PrP-res have been detected in the spleen, most other lymphoreticular tissues and the CNS but variably depending on the stage of incubation and *PrP* genotype. A Summary of the pathogenesis of experimental BSE in sheep carrying the ARQ and ARR allele is attached as annex 2. This summary is based on the limited research results available in this field. From experiments done with sheep of the Romney breed it appears that some ARQ homozygous sheep of this breed have no detectable PrP-res in peripheral tissues after neuro-invasion has occurred.

No infectivity nor PrP-res has so far been detected in any tested tissue of ARQ/ARR or ARR/ARR animals orally challenged with BSE up to 2 years post challenge. On the assumption that PrP-res and infectivity are correlated this would suggest that if these animals are infected, the titre of infectivity in a substantial period (and up to 2 years) following challenge is lower (or undetectable) in peripheral tissues of these genotypes than in 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. Unless titrations are done the titre of infectivity (if any) will not be possible to assess. Thus a quantitative risk analysis for these tissues may not be possible unless no infectivity is detected in a statistically significant number of animals.

III.3. Identification and listing of the criteria to be fulfilled to conclude that the BSE/vCJD strain has been isolated from sheep.

The characteristics that could potentially be used to distinguish TSE affected animals at higher risk of being infected with the BSE agent from other TSE affected animals include: clinical presentation, *PrP* genotype, age, breed, type of animal (milk or meat), histopathology, biochemical analysis, flock history and potential exposures to other TSE affected flocks and infected feed. There are currently insufficient data available on the characteristics, including the clinical or pathological presentation of BSE in sheep to allow individual TSE affected animals that are more likely to be infected with the BSE agent to be identified.

At the moment the complete protocol of strain typing by mouse bioassay experiments is the only method for detecting/confirming BSE infectivity in sheep; no other clinical or pathological features can conclusively confirm presence of BSE (i.e. distinguish between BSE and scrapie agents) although there are indications that some new tests may be able to do so. However, recent data indicate that in case of concurrent infections with scrapie strains and BSE agent, the BSE agent might be masked by scrapie agent in the mouse bioassay.

A tentative list of currently available tests and tests under development is presented in the SSC Opinion on *Criteria for the diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical determination of TSE agent strains*, adopted on 13-14 April 2000.

In the detailed report, a list of factors which will target cases for further testing is presented and discussed.

ANNEX 1. BACKGROUND: SUMMARY OF THE SSC OPINIONS ON TSE IN SHEEP.

1. In the Report of the Scientific Veterinary Committee on *Surveillance of transmissible spongiform encephalopathies* adopted 11 June 1997. It was noted that "*Following the experimental oral transmission of BSE to sheep and goats there are theoretical possibilities that sheep and goats could be infected naturally via feed (MBM) by the BSE agent*" In respect to surveillance for scrapie/TSE in sheep a passive and active surveillance programme was recommended, taking into account that scrapie (and BSE, if BSE in sheep behaves like scrapie in sheep), is a flock disease.

2. The minimum criteria to be met by scrapie eradication programmes are described in the *Report on the Standards for EC funding of Eradication Programmes for Scrapie* of the Scientific Committee on Animal Health and Animal Welfare, adopted 23 June 1998. This report considered the hypothetical infection of sheep by the BSE agent and recommended that such programmes should be extended to all TSEs in small ruminants. The recommendations in the report are based on the then current scientific knowledge on scrapie but it was concluded that, if BSE were confirmed to occur in sheep, the entire flock and its contacts should be destroyed.

   Criteria were defined so that progress in eradication programmes should be expected, with the ultimate aim of eradicating the causative agent. These criteria may be summarised as follows: animal identification, movement recording, disease reporting, inspection of holdings, auditing of records, checking of cull animals, effective farm management, action on farm when disease confirmed, publicity about the disease, compensation payments, adequate diagnostic resources and a reference laboratory for standardisation of test techniques and strain typing.

3. In its opinion of 24-25 September 1998 on *The risk of infection sheep and goats with Bovine Spongiform Encephalopathy agent*, the SSC concluded :

   "Because it has clearly been demonstrated that BSE can be orally transmitted to certain genotypes of small ruminants, and because it is likely that BSE-contaminated MBM has been fed to some sheep and goats, the Scientific Steering Committee has to assume that BSE could have been introduced into the sheep and goat population. Therefore it can not be excluded that the risk could persist, even after an effective implementation of a ruminant feed ban.

   On the basis of data on feeding practices, sheep and goats in many countries have probably been exposed to the BSE agent through MBM. It is noted that the feeding practices, e.g., the age and extent of MBM feeding of sheep and goats, are different from cattle. These will also vary depending on whether the animals are to be used for meat, wool or dairy purposes."

4. In its opinion of 27-28 May 1999 on "*Actions to be taken on the basis of (1) the September 1998 SSC Opinion on the Risk of infection of sheep and goats with the BSE agent and (2) the April 1999 SEAC Subgroup report on research and surveillance for TSEs in sheep"*, the SSC proposed immediate actions concerning the improvement, strengthening and acceleration of the implementation in all EU Member States of an epidemio-surveillance system for TSE in sheep, and testing of a statistically representative number of sheep for the presence of TSE. Furthermore research on modelling of BSE in sheep, validation of large scale testing methodology for TSE in

sheep and creation of an effective network between research groups was proposed. In the medium and long term, full implementation of high quality epidemio-surveillance systems, improvement of the methods and speed of large scale applicable differential diagnosis of BSE and scrapie on *post mortem* samples as well as development of *in vivo* tests for TSE diagnosis and differential scrapie-BSE diagnosis was proposed.

5. In the SSC opinion of 22-23 July 1999 on "*The policy of breeding and genotyping of sheep, i.e. The issue whether sheep should be bred to be resistant to scrapie*", the SSC concluded that, if BSE has infected sheep, which is not certain today, and if BSE transmits and behaves in sheep in a manner similar to scrapie, for which there are some experimental indications, then a similar strategy for BSE and scrapie should be adopted. The following steps as a preliminary to the introduction of scrapie-resistance breeding programmes were recommended:
   - At the level of the EU: analysis of the breeding programmes that are presently ongoing in a number of European countries and establishment of a register with the resistant breeds per country and their corresponding genotypes.
   - At the level of each EU country: start the genotyping of large numbers of animals, in order to acquire a view on the distribution of the various genotypes in the national flocks. This would provide a first step towards the estimation of the repartition of genotypes involved in resistance against scrapie and, possibly, BSE.

6. The possibilities for TSE diagnosis in sheep were presented in the SSC Opinion on *Criteria for the diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical determination of TSE agent strains*, adopted on 14.04.00.

7. The SSC opinion of 13-14 April 2000 on *Specified risk materials of small ruminants* stresses that if BSE in sheep or goats was to occur under natural conditions in a region or a country, wherever this risk occurs in an animal or flock no tissues that are likely to contain BSE infectivity should enter any food or feed chain.

8. In its opinion of 26-27 October 2000 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), the SSC concluded that *"Confirmation is needed on two major points i.e. identification of the agent (BSE or not) and the origin of the transmission. Pending confirmation, the data in this experiment are new to the extent that they 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. As these preliminary data 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 only be considered a tentative evidence of the transmissibility of the BSE agent through blood."*

ANNEX 2: EXPERIMENTAL BSE IN SHEEP: DISTRIBUTION OF INFECTIVITY BY INCUBATION STAGE AND *PRP* GENOTYPE AND STAGE OF INCUBATION.

INFECTIVITY TITRE	PRE-CLINICAL		CLINICAL	
	ARR/ARR, ARR/ARQ	**ARQ/ARQ**	**ARR/ARR, ARR/ARQ**	**ARQ/ARQ**
HIGH				**Brain** Spinal cord Spleen
MEDIUM		Spleen Lymph nodes Tonsil		Lymph nodes Tonsil
LOW				
PrP-res DETECTED BUT INFECTIVITY NOT TITRATED		spleen Lymph nodes Intestine Forestomachs abomasum		Intestine Forestomachs abomasum
NOT DETECTABLE	Brain, Spinal cord, Spleen Lymph nodes, Tonsil			

Notes: The summary table is based on the limited research results currently available in this field. Full literature references are provided in the attached report. The table should be used with caution since it relates to experimental, and not natural BSE in sheep, some data are incomplete and some experiments are on-going. Nevertheless it may serve as a guide to the degrees of risk that may exist. The Table should be updated as new results come forward.

No PrP-res has so far been detected in ARQ/ARR or ARR/ARR animals inoculated with BSE up to 2 years post challenge. On the assumption that PrP-res and infectivity are correlated this would suggest that if these animals are infected the titre of infectivity in the years immediately following challenge is lower (or undetectable) in peripheral tissues of these genotypes than in more susceptible genotypes.

ANNEX 3: EPIDEMIOLOGICAL ANALYSIS OF A 'BSE IN SMALL RUMINANTS'-CASE.

Detailed epidemiological tracing on and tracing back should allow to define a range of probabilities in respect to the origin of the cases (maternal, direct or indirect contact, drugs or vaccines, etc). This epidemiological inquiry should include information such as:

> flock data: feed history, geographical co-ordinates, distance to other flocks, flock history, veterinarian(s), sheep handlers, reproduction techniques, type of flock (meat, fibre, dairy), other species in the holding;

> animal data: identification, detailed history of the case, genealogy (ascendant, descendants, collaterals, cohorts);

> inventory of the holding : scheme of the holding(s)

> sales (tracing on);

> purchase (tracing back)

> mortalities;

> supplies of the holding (feed, insemination, embryos, drugs);

> inquiry in the holding of origin;

> cartographic analysis;

> Final evaluation, integrating and combining all above elements.

> Genotyping and testing for PrP-res presence of all animals in the culled herd.

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B1 - Monitoring and dissemination of scientific opinions

Scientific Steering Committee

PRE-EMPTIVE RISK ASSESSMENT SHOULD BSE IN SMALL RUMINANTS BE FOUND UNDER DOMESTIC CONDITIONS.

AS DISCUSSED BY THE TSE/BSE AD HOC GROUP MEETING AT ITS MEETING OF 1 FEBRUARY 2001.

SUBMITTED TO THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF

8-9 FEBRUARY 2001

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TABLE OF CONTENTS

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I. TERMS OF REFERENCE

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Following the adoption of these opinions, the European Commission requested the Scientific Steering Committee to (1) present an updated state of affairs regarding tissue infectivity distribution on the basis of the most recent results of the ongoing experiments on BSE in small ruminants, (2) identify and list the criteria be fulfilled to conclude that the BSE/vCJD strain has been isolated from sheep and (3) pre-emptively carry out risk assessments corresponding to a range of circumstances (scenarios) should BSE be confirmed to occur in the sheep population under domestic conditions.

The request is a logical follow-up of a number of SSC opinions indicating that, although there is currently no evidence to suggest that BSE occurs naturally in sheep and goats under field conditions, there are a number of reasons for concern, for example:

- the difficulties in relation to surveillance and eradication of scrapie/BSE in sheep;
- the risk of infection of small ruminants with BSE;
- the uncertainties related to breeding sheep to be resistant to scrapie/BSE;
- the difficulties in diagnosing clinical and pre-clinical TSE disease in sheep and to differentiate TSE agent strains.

Scope of the present report:

Small ruminants include, for the purpose of the present report, only sheep and goats. Because much more experimental and field information is available for sheep, the assessment has initially and primarily been carried out for sheep. However, the conclusions will be broadened to goats by taking into account whatever information is available.

It is noted that only sheep and goats (of small ruminants) have been experimentally challenged and succumbed to BSE infection. Scrapie occurs naturally in sheep, less frequently in goats and moufflon. Maternal transmission (which occurs in sheep) is not confirmed to occur in goats.

It is further noted that infectivity with the agent causing scrapie, the expression of clinical disease in scrapie-affected sheep, cannot currently be distinguished from BSE by any means other than biological strain typing of the agent responsible. In the present report, it will be assumed as a reasonable work approach, in general, that the BSE agent behaves in infected sheep and goats like the scrapie agents in sheep and goats. Classical scrapie is present as a persistent disease with a variable within-flock incidence.

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II. DATA ON INFECTIVITY (EXPERIMENTAL CHALLENGE OF SHEEP WITH BSE AGENT)

BSE agent has been transmitted to both sheep and goats by the i/c and by the oral routes (Foster, Hope and Fraser, 1993). Furthermore, in contrast to cattle where BSE infectivity has not been recorded in the spleen even after i/c inoculation of cattle (GAH Wells, personal communication), sheep with experimental BSE do show such infectivity (Foster *et al*, 1996). This suggests that the pathogenesis of BSE in sheep (and possibly goats) may align more with the pathogenesis of scrapie in these species than with that of BSE in cattle. There is further support for this notion derived from recent studies (Jeffrey et al, 2001a; 2001b) of the distribution of PrP-res in lymphatic tissues of sheep experimentally infected with the BSE agent.

In regard to consumers, the risk tissues for natural BSE agent in sheep and goats, if it occurred, would be similar to the scrapie-infected tissues. These tissues are listed by the WHO (WHO, 1997) and the SSC (SSC Opinion of 13-14 April 2000 on *Specified risk materials of small ruminants*.)

Annex 1 elaborates further on tissue infectivity and genotype. Annex 2 provides a summary classification of tissues by infectivity status in pre-clinical and clinical cases of natural and experimental scrapie in sheep and goats. Annex 3 summaries, for experimental BSE in sheep, the current knowledge on the distribution of infectivity by incubation stage and PrP genotype and age/stage of incubation.

The main recent findings can be summarised as follows:

BSE agent has been experimentally transmitted orally to susceptible sheep and goats, infectivity is detected in spleen, most other lymphoreticular peripheral tissues and the CNS but variably depending on the stage of incubation and *PrP* genotype. A Summary of BSE in sheep pathogenesis results in sheep carrying the ARQ and ARR allele is attached as annex 3. However, some ARQ homozygous sheep have no detectable PrP-res in peripheral tissues after neuro-invasion has occurred.

No PrP-res has so far been detected in ARQ/ARR or ARR/ARR animals inoculated with BSE up to 2 years post challenge. On the assumption that PrP-res and infectivity are correlated this would suggest that if these animals are infected, the titre of infectivity in the years following challenge is lower (or undetectable) in peripheral tissues of these genotypes than in 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. (NOTE: unless titrations are done the titre of infectivity (if any) will not be possible to assess. Thus a quantitative risk analysis for these tissues may not be possible unless no infectivity is detected.)

III. CRITERIA AND DIAGNOSIS.

The characteristics that could potentially be used to distinguish TSE affected animals at higher risk of being infected with the BSE agent from other TSE affected animals include clinical presentation, *PrP* genotype, age, breed, type of animal (milk or meat), pathology, biochemical analysis, flock history and potential exposures to other TSE affected flocks and infected feed. There are currently insufficient data available on the characteristics, including the clinical or pathological presentation of BSE in sheep to

allow individual TSE affected animals that are more likely to be infected with the BSE agent to be identified.

At the moment the complete protocol of strain typing using various inbred mouse strains (or possibly using transgenic 'bovine' mice in the future) is the only method for detecting/confirming BSE infectivity in sheep[3]; no other clinical or pathological features can conclusively confirm presence of BSE (i.e. distinguish between BSE and scrapie agents) although there are indications that some new tests may be able to do so. However, recent data (Baron and Biacabe, 2001; see Annex 6) indicate that in case of concurrent infections with scrapie strains and BSE agent, the BSE agent might be masked by scrapie agent in the mouse bioassay.

A tentative list of currently available tests and tests under development is presented in the SSC Opinion on *Criteria for the diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical determination of TSE agent strains*, adopted on 13-14 April 2000. It is noted that biological strain typing using in-bred strains of mice is effective at distinguishing the BSE agent from scrapie strains but that the method is not practical for routine use. On the criteria for differential molecular/biochemical aetiological diagnosis of TSE disease in sheep it was concluded that conclusive differential biochemical strain identification is not yet possible. Fragment size and glycoform analysis adds to prion characterisation although on its own it is not sufficient for strain typing TSE isolates from sheep. Indeed, no current test is able to conclusively define strains of TSE agents.

Factors which will target cases for further testing

The most useful criteria for targeting natural TSE in small ruminant cases that may be BSE from scrapie cases, is the occurrence of BSE or scrapie in the region and the exposure history of the flock in which the case is found. For example, if 'scrapie' is found in animals that could have been exposed to BSE-infected material and are in a closed flock, in a region where scrapie has not previously been identified, it would be worth targeting affected animals in this flock for mouse bioassay.

If any characteristics that could distinguish TSE-affected animals that may be infected with BSE from other TSE-infected animals are identified these characteristics could be used to target control policies to the most appropriate animals. The main factors that would target a TSE case for further testing for BSE are:

1. The flock history with regard to TSE (especially if scrapie-like disease is found in a BSE region where scrapie previously did not occur), including also flock and animal movements and flock management.
2. The feeding history and practices of the small ruminant (flock) in which TSE ~~BSE~~ is found;

Annex N° 6 elaborates further on the above factors.

[3] It cannot be excluded that there exists a scrapie strain with the same transmission characteristics as BSE. This would be picked up by the complete mouse bio-assay protocol, which includes the transmission characteristics (e.g., incubation period), the distribution of lesions in the brain and the glycoform pattern.

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The SSC opinion, adopted on 13-14 April 2000, provides a comprehensive report on the criteria for diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical diagnosis of TSE agent strains is available as an.

IV. SCENARIOS

To date, no BSE has been diagnosed in domestic flocks of small ruminants. However, on experimental grounds and because possibly contaminated MBM has been fed in the past to small ruminants, the possibility that this could occur cannot be excluded. An initial list of sources representing the highest levels of possible risk has therefore been identified in various SSC opinions, the control of which would significantly reduce the possible exposure of humans to the BSE agent should BSE in small ruminants be present but undiagnosed.

IV.1. THE RANGE OF CIRCUMSTANCES TO BE CONSIDERED SHOULD BSE BE CONFIRMED TO OCCUR IN THE SHEEP POPULATION UNDER NATURAL CONDITIONS.

The two principal questions to be addressed are:

- the likely risk of infection being transmitted to man if BSE is found in sheep;
- How this risk could be reduced.

The first of these questions includes consideration of the strength of the species barrier between sheep and man for the BSE agent, the likely prevalence and distribution of BSE infection in sheep, the likely duration of the epidemic in sheep and the level of infection likely to be present in various sheep tissues.

The second includes consideration of the likely effectiveness, compliance with and enforcement of control measures.

IV.1.1 The major determinants of risk to humans are:

- The current prevalence of BSE infection in sheep
- The likely future prevalence of BSE infection in sheep (is it increasing, reducing or being maintained at the same level)
- The extent to which the human population is exposed to the infection in sheep

In a given situation there are various facts that need to be taken into account in assessing the likely current and future prevalence of infection in sheep and the likely exposure of the human population to this source of infection. There are some facts that are not dependent on the particular situation. These will apply in all situations and the level of knowledge about these facts will determine how accurately we can assess the risk to humans, these scenario-independent facts include;

- Routes and rates of transmission and what determines how rates of transmission vary
- Distribution of infection in tissues by species, genotype and stage of incubation
- Efficiency of diagnostic tests used by species, genotype and stage of incubation
- The nature of genotype-regulated susceptibility to BSE in sheep

There are other scenario-dependent facts and those which should be considered when trying to assess the three major determinants of risk for humans listed above:

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IV.1.2 Facts that should be taken into account when assessing the likely current prevalence of BSE infection in sheep.

- The prevalence of the likely source of infection for cases of BSE in sheep (detailed epidemiological investigation of affected flocks should be used to determine the likely source of infection for sheep);
- If BSE in sheep is found: the number of BSE affected flocks/animals detected;
- The effectiveness of surveillance in regions where BSE is detected in sheep (both the efficiency of surveillance for detecting TSE in sheep and the efficiency of surveillance to determine whether the TSEs detected in sheep are scrapie or BSE should be considered. The efficiency of surveillance for TSE in sheep will depend on farmer awareness, number of neurological submissions, consequences of reporting, diagnostic tests used, identification of sheep. The efficiency of surveillance for determining whether TSE in sheep is BSE will depend on number of animals tested and efficiency of tests).

In summary the likely prevalence of BSE infection in sheep will be higher if:

a) the prevalence of exposure of sheep to the likely source of BSE in sheep is high;
b) the number of BSE affected flocks/animals detected at the same time is high;
c) the efficiency of surveillance for TSE in sheep was/is low, because as a result the number of secondary cases may have increased.

IV.1.3 Facts that should be taken into account when assessing the likely future prevalence of BSE in sheep.

The following elements need to be considered when assessing the likely future prevalence of BSE in sheep:

- The likelihood of transmission within affected flocks is likely to depend partly on the management within affected flocks. How accurately this likelihood in any particular scenario can be assessed will depend on the level of knowledge of scenario-independent information i.e. the routes and rates of transmission in different circumstances.
- The likelihood of transmission between affected flocks which depends on movement of sheep (or other contaminated or infected materials, including feed) from affected to other farms, contact with sheep from affected farms and the density of the sheep population.
- The genotype distribution (hence the genotype regulated level of resistance) in the affected population. This distribution is scenario-independent. (Note: One could imagine a region where the genetic susceptibility of flocks/individuals is high and another region where it is low; these would then become two separate scenarios that could significantly affect the risk and incidence of transmission to man.)
- The presence, date of introduction and level of enforcement of MBM in feed and SRM bans (including the likelihood of cross-contamination) Effective identification and destruction of clinically suspect animals, effective rendering controls and effective SRM bans are beneficial in reducing risk to small ruminants if a feed ban is only partially effective, but scientifically become much less important once a feed ban is completely effective since everyone agrees that the most likely route of *initial* exposure is *via* MBM. It is therefore important to know the date that a feed ban was

first introduced and its effectiveness, and the date when it could for practical purposes be deemed to be completely effective. These dates may be widely separated and the second can only be determined retrospectively after a further mean incubation period is complete.

- Feeding practices for sheep in a region, country, farming system, etc.;
- Culling policy for affected flocks. How effective a culling policy will be will depend on the nature of the culling carried out, the efficiency of this culling policy. (The detailed Working Group report further elaborates on this issue.)
- The efficiency of surveillance for TSE and BSE in sheep. (The detailed Working Group report further elaborates on this issue.)

In summary the likely future prevalence of BSE infection in sheep will be higher if:

a) Natural transmission of BSE occurs in sheep;
b) the management in affected flocks is likely to encourage transmission within flocks (particularly intensive management at lambing time).
c) the management of affected flocks is likely to encourage transmission between flocks. The risk is higher for/in a densely populated area with movement of sheep from affected to unaffected flocks.
d) the proportion of susceptible genotypes in the population is high.
e) the MBM and SRM bans in place do not prevent exposure of the sheep population to infection in feed.
f) MBM is fed to breeding sheep particularly at young ages.
g) culling policy is likely to allow infected sheep to remain in affected flocks
h) the quality of surveillance is such that affected flocks would remain undetected.
i) There is no attempt to increase the genetic resistance of the flock by selection and use of homozygous resistant rams.

IV.1.4 Facts that should be taken into account in assessing the likely exposure of human population to any infection that is present in sheep.

The following elements need to be considered when assessing the likely exposure of human population to any infection that is present in sheep:

- the movement of sheep from affected farms to slaughter house;
- the presence of an SRM ban and its level of enforcement;
- the culling policy for affected flocks;
- the genotype distribution of affected animals;
- the strength of the species barrier between small ruminants and man for the BSE agent[4].
- local on-farm management / birth assistance.

[4] In theory it could be that, if the prion theory is correct, no form of sheep PrP is pathogenic for humans (*cf* bovine PrP is not pathogenic for hamsters or chickens). Alternatively the barrier could be lower than between cattle and man or somewhere in between. Because the prion hypothesis has yet not been proven beyond any doubt and because there may be another component to the agent, one has to be rather cautious in the approach and probably opt for the second alternative.

In summary the exposure of the human population to infection in sheep is likely to be higher if:

- infected animals from affected flocks are likely to enter the slaughterhouse
- SRM bans in place are incompletely effective in preventing infected material getting into human food chain and especially if the ban does not trap all tissues that contain detectable infectivity
- culling within affected flocks is unable to reduce the level of infection
- there is a high proportion of susceptible animals within affected population.

IV.2. BASIC SCENARIOS

The Working Group considers that it cannot at this stage assess the potential risk to the human population in each scenario, as there are too many variables and unknowns for which additional information is needed. Each scenario should therefore be assessed using the criteria listed above to determine the likely risk of transmission to man. It is for example difficult to accurately assess at this stage the likely prevalence of BSE in sheep because data is lacking on the likely prevalence of exposure to the likely source of infection, the quality of surveillance for both TSE and BSE in the affected population and the actual occurrence of sheep to sheep transmission if this occurs. Also, the assessment of likely prevalence of BSE in sheep given that a single case of BSE in sheep had been identified may vary considerably depending on the prevalence of exposure of sheep in the affected population to the most likely source of BSE (infection in feed).

A first conclusion is thus that there is a need to start collecting the information required for a correct assessment of the likely prevalence of BSE in sheep in a country or region and according to the flock and management type, should it be found under natural conditions.

The identification of definitive BSE in sheep or goats (if based on biological strain typing) is likely to be retrospective by a number of years. In the meantime a level of uncertainty and potential or actual risk may exist until the new situation is assessed and new measures to control any actual or potential risk are in place and effective. The Working Group proposes, for the time being, the following scenarios to be envisaged:

Scenario A: The exposure to infected feed is considered to be unlikely.

Scenario B: No BSE is found in small ruminants, but exposure to infected feed is thought to be likely.

Scenario C: No BSE is found in small ruminants, but the presence of BSE in small ruminants is considered to be probable.

Scenario D: BSE in small ruminants has been confirmed under natural conditions. The cases are isolated, with no epidemiological link between them

Scenario E: BSE in small ruminants has been confirmed under natural conditions. The outbreak(s) of cases is (are) restricted to certain flocks or situations (e.g. vaccine, farming system)

Scenario F: BSE in small ruminants has been confirmed under natural conditions. The outbreak(s) of cases is (are) over a geographic area and there exist

epidemiological links between them. This link may be direct (e.g., feed, pasture land, travel, import/export, maternal risk enhancement, …) or indirect (e.g., survival of the agent in a population several years after the implementation of such measures.

In its opinion of 24-25 September 1998, the SSC concluded that "*Because it has clearly been demonstrated that BSE can be orally transmitted to certain genotypes of small ruminants, and because it is likely that BSE-contaminated MBM has been fed to some sheep and goats, the Scientific Steering Committee has to assume that BSE could have been introduced into the sheep and goat population. Therefore it can not be excluded that the risk could persist, even after an effective implementation of a ruminant feed ban."*

The Working Group considers that this conclusion is still valid and that there are no sufficient arguments to consider that either BSE agent in small ruminants is probable or that BSE agent is present in the small ruminant populations. For the latter hypothesis there is no evidence. Against the scenario that the presence of the BSE agent in small ruminants is probable, the following arguments can be listed:

- The experimental conditions under which sheep and goats have been challenged with the BSE agent are most likely not representative for domestic feeding conditions;
- There is no epidemiological evidence of increased scrapie incidence attributable to exposure to infected feed or occurrence of BSE in cattle, although the quality of the epidemiological surveillance for TSEs in small ruminants in most Member States is considered to be poor;
- There seems to exist no link in the UK between the incidence of BSE and scrapie on the same farm (see also Annex 6).

The Working Group therefore concludes that at present, the most likely scenario is that, where possibly contaminated MBM was fed to small ruminants, they are likely to have been exposed to the BSE agent (Scenario B) and that there is no evidence for the scenario of the presence of BSE agent in small ruminants being probable (Scenario C).

In the sections hereafter, the above scenarios B-F are further elaborated on.

IV.2.1. Scenario B - Current situation: small ruminants are likely to have been exposed to the BSE agent but there is no evidence for Scenario C (the presence of BSE agent in small ruminants being probable).

The risk aspects linked with this scenario have been dealt with in a number of previous SSC opinions:

- The SSC has addressed, in its 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*, the advantages resulting from implementing a programme of progressive replacement of sheep genotypes by scrapie resistant, or at least much less susceptible, genotypes. The opinion addresses the use of TSE-resistant rams in all flocks to increase genetic resistance of national population.
- In its opinion of 13-14 April 2000, the SSC listed the specified risk materials in small ruminants. In its opinion of 24-15 June 1999 on "Fallen stock", the SSC considered that small ruminants with a TSE are unfit for further processing.

Currently, the only definitive method for confirming that BSE in sheep is present, is by submitting test material for biological strain typing in a panel of mice appropriately composed for this purpose. The Working Group considers that the current numbers of TSE cases in small ruminants that are submitted to biological strain typing in mice is far too limited as compared to the total number of small ruminants in the European Union. In fact, currently only 178 isolates from UK sheep have been inoculated into mice (for biological strain typing) and an additional 12 isolates from other European countries have been inoculated giving a total of 190 isolates that have been inoculated in UK. The Working Group therefore recommends:

- That the number of such tests is significantly increased in all EU Member States where scrapie occurs;
- That the laboratories that have the appropriate expertise, protocols and material share these with other laboratories in other countries;
- That a "ring-test" programme be launched to guarantee, whenever possible, harmonisation between laboratories.

In addition, the following pro-active actions may be recommended:

- In order to obtain a good quantitative estimate of the occurrence of TSE in small ruminants, and to monitor the incidence of disease in the future, it is recommended to improve the current surveillance system, as follows::
  A; surveillance that includes active surveillance of potentially exposed populations, awareness campaigns, etc.), as outlined in Annex 8 to the present report. To increase the chance to identify BSE cases in sheep, target populations could be identified for an active surveillance, on the basis of risk factors involved i.e. dairy sheep, genotypes, birth cohorts and offspring of BSE cases, exposure to MBM etc.
  b. testing, with rapid validated *post mortem* tests, of statistically sound numbers of targeted slaughtered animals, for the presence of a TSE agent. The statistical credentials of such programme would need to be established. Sample size and design would depend upon the incidence levels to be detected and upon expected precision and reliability of the results. The Working Group considers that it would not be sufficient to deploy such testing programme on fallen animals alone, as, for small ruminants, the level of declaration of fallen animals is low. However, presently there is no validated rapid post mortem test available for use in sheep.

- Accelerate the development and validation of new rapid TSE tests and of differential BSE/other TSE diagnostic tests in small ruminants, both for confirmatory purposes and to verify the presence of PrP-res in live animals. Establish an inventory of what tests and techniques are currently available and how far they are harmonised and have provided comparable results.
- Strain typing by mouse bioassay should be carried out for at least one isolate from as many incidents of TSE in small ruminants as possible and reasonably feasible.’ To date, no confirmed natural BSE case in sheep or goats has been reported, however the risk that BSE has been introduced in [certain] flocks of small ruminants cannot be excluded on theoretical grounds. It seems therefore essential that from now on an effort should be made to determine the strain of agent responsible for each outbreak. If all

isolates from confirmed TSE outbreaks in small ruminants were submitted to strain typing this would achieve the objective[5].

- Recommend countries to start collecting data useful for the assessment of the risk of BSE in small ruminants at the national level, but also at the level of management types and other features (e.g., farming practices). These data should then possibly be used to develop a geographical 'BSE in sheep' risk assessment (GBSR) comparable to the geographical BSE risk assessment for cattle (GBR). (Note: It probably would be important to first collect data regarding those type of sheep at highest risk and fed high protein diets such as milk sheep. Surveillance could target these subpopulations. As it was seen with BSE, dairy cattle were the most likely to be affected.

  It is however expected that the availability of exploitable data will be limited for most countries. The assessment of the possible presence of risk of BSE in small ruminants or the extent of such risk if BSE in small ruminants were confirmed, will therefore most likely and in the first place have to be based on the outcome of testing programmes with validated differential BSE: Scrapie tests and of reliable surveillance.

- Individual identification of sheep (ideally to enable tracing of the parents), for example using special earmarks or other methods.

- Develop criteria for the determination of scrapie and TSE-free status of small ruminant flocks; definition and introduction of an accreditation scheme for scrapie and TSE-free status. Development and implementation in each Member State of accreditation schemes for scrapie/TSE free status.

- Strict control of SRM and ;MMBM bans;

- Start a programme of genotyping of all small ruminants (giving priority to the populations considered at the highest potential risk).

- Start EU wide national programmes of breeding towards a genetically fully resistant sheep population (meaning that BSE latency can be excluded), using TSE resistant rams, in accordance to the SSC opinion on "Breeding for scrapie resistance" of 22-23 July 1999[6].

- Use of TSE-resistant rams in all flocks with scrapie and possibly others to increase genetic resistance of national population.

- Culling scheme. Various options exist, including for example: The emphasis here might be adjusted to removal of susceptible sheep on the one hand whilst building up resistant genotypes on the other

  a. Option:

     > Culling and testing of the TSE-case and its offspring and the birth cohort (including those in in-contact flocks).

[5] Many might claim that this is impractical. But the UK, which has the largest sheep population in the EU, reports only about 600 scrapie cases *p.a.* and some of these are multiple incidents so the number of outbreaks is likely to be smaller. The number of bioassays might become manageable as a result of careful selection and by the use of RIII mice only. It is noted that 43 isolates from several European countries are being strain-typed by mouse bioassay as part of an on-going EU research project FAIR -CT97-3305. These isolates are from France, Germany, The Netherlands, Ireland, Greece, Iceland, Norway and Cyprus. The strain typing of these isolates is carried out by 3 labs: INRA (FR), CVL (UK) and FRCVDA (DE). The potential, but unambiguous confirmation of a BSE case in a small ruminant would thus require at least an additional 18-24 months following the identification of a suspect TSE case.

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> replace the rams in the affected flock by ARR/ARR genotyped rams;

> Restrict the movements of the animals from the affected and in-contact flocks to other flocks of a higher status (unless ARR/ARR and carrier status disproved) or to flocks of an equal or lower status unless tested negative by a validated *in vivo* test);

>When slaughtered for consumption: apply a rapid TSE diagnosis test on all animals from the affected flock.

b. Option:

> 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 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);

– Launch additional research where needed. The TSE/BSE ad hoc Group considers that an essential role should be played by the "TSE/BSE research network" established on 15 December 2000 by the Research Directorate General on suggestion by the Council of Ministers.

IV.2.2. Scenario C - there is evidence for the scenario of the presence of BSE agent in small ruminants being probable

The possible reasons for such scenario becoming probable are various. One could mention, for example, a sudden and/or unexplained increase in 'scrapie' incidence in a given region where feeding of feedstuffs containing ruminant proteins was common practice; the elements in the diagnosis of the presence of a TSE agent point to a so far unknown scrapie agent; appearance of new, so far not-described clinical signs that cannot be attributed to a classical scrapie; etc.

The Working Group considers that under this scenario, the most recent data on tissue infectivity obtained from the experiments on BSE in small ruminants, should be assessed with a view of possible further exposure risk reduction. More precisely, the possible risks with respect to BSE agent from the following tissues should be assessed on the basis of the than available scientific data:

- The whole digestive tract;
- The large and small intestine;
- The forestomachs and the abomasum ;
- The intestine-associated lymph nodes;
- The large lymph nodes that become/are visible when cutting the animal;
- Spleen;
- The retro-pharyngeal lymph nodes;

- The lymph nodes, dorsal root ganglia, thymus & nerves.

IV.2.3. BSE in small ruminants has been confirmed (Scenarios D, E or F)

The assessment which scenario D, E or F is likely to be correct should BSE in small ruminants be confirmed under natural conditions, should be based on (non exhaustive list):

- *Epidemiological analysis* of the case. Detailed epidemiological tracing on and tracing back should allow to define a range of probabilities in respect to the origin of the cases (maternal, direct or indirect contact, drugs or vaccines, etc). This epidemiological inquiry should include information such as:
  - > flock data: feed history, geographical co-ordinates, distance to other flocks, flock history, veterinarian(s), sheep handlers, reproduction techniques, type of flock (meat, fibre, dairy), other species in the holding;
  - > animal data: identification, detailed history of the case, genealogy (ascendant, descendants, collaterals, cohorts);
  - > inventory of the holding : scheme of the holding(s)
  - > sales (tracing on);
  - > purchase (tracing back)
  - > mortalities;
  - > supplies of the holding (feed, insemination, embryos, drugs);
  - > inquiry in the holding of origin;
  - > cartographic analysis;
  - > Final evaluation, integrating and combining all above elements.
  - > Genotyping and testing for PrP-res presence of all animals in the culled herd.

- *Flock and movement history*. Detailed inquiry of the records should identify the risks by contact within and between flocks and by maternal or other ways of transmission and should lead to a thorough clinical examination and testing by a (possible in future) *in vivo* TSE test of all in contact animals. The results of these tests will give a realistic estimation of the prevalence of the infection and the transmission ratio within and between flocks if rapid sensitive tests are available, capable to detect BSE infection early in the incubation period.
- *Feeding practices*. The most likely way for the initial introduction of the BSE infection into the sheep population is through the feeding of BSE infected MBM. The amount of MBM fed to the sheep and goat population and the *risks of cross-contamination* before and after the ban are therefore directly related to the probability that the BSE case is caused by BSE infected feed and to the number of BSE infected sheep in the exposed flocks. (Note: However, experience has shown that is not always possible to obtain an accurate and complete feed history. Also, the amount of cross contamination is difficult to estimate**.)**

  Houston *et al* (2000) report on how a high volume blood transfusion from sheep to sheep can transmit a BSE-like illness within the same species and that infectivity can be transmitted from blood taken during the asymptomatic incubation period of the disease of the donor sheep. The SSC, however, in its opinion of 26-27 October

2000[7], considered that "*confirmation was needed on two major points i.e. identification of the agent (BSE or not) and the origin of the transmission. Pending confirmation, the data in this experiment are new to the extent that they show that the exchange by transfusion of (400 ml of) whole blood taken during the incubation period of a sheep* [experimentally] *infected with the BSE agent can transmit disease to a healthy sheep. This ovine model adds to data obtained in mouse and hamster models of scrapie and* [rodent-adapted] *human TSE. As these preliminary data 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 only be considered a tentative evidence of the transmissibility of the BSE agent through blood* [transfusion]."

If confirmed, the Houston *et al* (2000) data would show that the above remarks could also be applied to the feeding of ovine or caprine blood or blood products (or access to these from pasture on which such blood has been spread).

There is currently no knowledge as to the extent of inclusion of any blood products in small ruminant feed. However, waste blood may be sprayed or distributed on to grazing land.

- *Culling scheme* in place. The efficacy of a culling scheme to eliminate BSE infected animals is related to the further spread of the infection by both feed (direct by MBM or indirect by cross-contamination of feed), horizontal and maternal transmission.
- Level of *surveillance*. The quality of the epidemio-surveillance system as for the efficacy to detect clinical, pre-clinical and exposed animals will determine the capability to detect the initial MBM caused case(s) and the prevalence of infected or exposed animals resulting from the initial MBM caused case(s).
- [Non-] availability of diagnostic tests. The availability, rapidity, sensitivity and specificity of diagnostic tests to detect in the future *in vivo* (e.g., tonsils, third eyelid, blood, milk, …) as early as possible in the incubation period BSE infected sheep, would greatly enhance the efficacy of the epidemio-surveillance system and of the culling scheme.
- *Routes of transmission*. The efficacy of the different routes of transmission (e.g., feed, lateral, vertical and maternal transmission, vaccines, environment) will determine the number of animals infected by MBM and the number of animals infected by other routes. The relative efficacy will determine the extent of the epidemic and the scenario to be applied.

[7] 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 on 26-27 October 2000.

If for example the efficacy of transmission through BSE-infected MBM is very low, but once introduced, the efficacy through horizontal and maternal routes is high, the scenario to be applied will be influenced by the point in time that the first case is detected. If it is detected early before lambing, without secondary transmission, scenario **C** could be proposed, but if it is likely that the first case is already a secondary case resulting from horizontal or maternal transmission scenario **D**, or in the absence of scrapie/BSE differentiating tests, scenario **E** could be proposed.

- *Genotype regulated resistance* against disease. The efficacy of the transmission within a flock and between flocks could be influenced by the genotype: if for example ARR/ARR is shown to encode for full resistance against BSE disease, and the BSE case occurs in a susceptible animal of a flock composed of mostly ARR/ARR animals, a more restrictive scenario could be proposed.

Reactive actions (should BSE in a small ruminant be confirmed) may include:

The TSE/BSE *ad hoc* Group considers that the action taken could vary by genotype if infectivity varies with genotype.

- Identify which scenario is likely to be applicable (C, D or E).
- On risk tissues: see Scenario C.
- Culling/destruction of all suspects; by "suspects" is meant
  - > the confirmed case, its offspring and other traceable relatives (including birth cohorts);
  - > all the animals with genotype pointing at TSE-susceptibility, both in the affected and in the contact flocks.

  Restrict movements of the remaining resistant animals to the slaughterhouse and prohibit the use of any part of a restricted animal for human consumption until declared fit for human consumption after TSE testing by the competent veterinary authority.

  Depending upon the extent of the risk, the culling should be applied to the whole exposed population at flock level only, or for a given management type, or possibly for a whole region (*e.g.* a hill area where co-grazing is practised) or country.
- Detailed epidemiological analysis according to the scheme specified above;
- (If feasible: rapid genotyping of all animals in the affected flock and the flocks linked to it.)
- Repopulation, to be be done from uninfected (certified scrapie and TSE-free) flocks **or** with genetically fully resistant animals; (this will also reduce the exposure of sheep and man but may have impact on the reporting likelihood.)
- Use resistant rams for breeding in affected flocks;
- Disinfection of affected premises with a disinfectant appropriate for TSEs (see Chapter on Environmental cleaning).

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V. DISCUSSION OF SOME RISK REDUCING MEASURES OTHER THAN EXCLUDING RISK TISSUES FROM THE FEED AND FOOD CHAINS

Preamble:

There are countries without the [TSE in small ruminants] infection, flocks without the disease, there are a substantial and growing number of resistant sheep in some populations and within the range of genotypes there are some that, if infected, do not show significant or detectable PrP-res in peripheral tissues at least before the stage of neuroinvasion *i.e.* on average about half way through the incubation period. There are others that do appear to have widespread distribution of infectivity like the Suffolk sheep examined by Hadlow *et al* (1982). Suffolk sheep in the clinical phase of disease had maximum brain titres ranging from 3.9 – 6.5 mouse i/c LD_{50}/30 mg of tissue. However, in their 1979 paper (Hadlow *et al* 1979) some other breeds examined had a more ~~very~~ restricted distribution of infectivity like BSE in cattle, the maximum brain titres in the clinical phase of disease were lower than in the Suffolks and ranged from 1.5 – 4.7 mouse i/c LD_{50}/30 mg of tissue. It might be that ideally a sheep population could eventually be divided into flocks where there is no infection, those where there is infection but no disease, and those where there is both. In the last two categories it might then be possible to divide the sheep therein into totally resistant animals, partially resistant animals (in which tissue distribution on a time and tissue basis might resemble BSE) and susceptible animals where if they are infected there is likely to be a wide distribution of infectivity at early ages after exposure.

It can be accepted that the risk that BSE entered in the small ruminant population is proportional to the prevalence of BSE in cattle and the amounts of possible contaminated MBM that may have been fed to the small ruminants. The risk that BSE entered the small ruminant population should therefore be assessed also using information from the SSC's assessment of the geographical BSE risk (GBR) and all available information on feeding of contaminated MBM to small ruminants. But, this criterion can only be temporary as also other "BSE-in-cattle"-independent factors play a role, for example the management type and its intensity which will determine risk factors such as feeding practice, flock transhumance, pre-natal infection, direct on indirect (e.g., soil) transmission between animals, etc.

V.1 CULLING

a. Culling schemes should basically lead to two effects:
   3. a reduction of future number of clinical cases;
   4. a reduction of present prevalence of sub-clinical infection, which would reduce the human exposure risk (HER) to products derived from infected animals.

   However, historical culling schemes (other than those adopted for imported sheep retained in quarantine) have been operated without eliminating scrapie, certainly in the short term and sometimes in the long term (>10 years). Such schemes have mostly been operated without the application of genetic knowledge. By contrast application of modern genetic techniques (see below) shows much greater promise for eliminating scrapie (and presumptively BSE if it occurs). Thus, the group wishes to emphasise that adoption of this approach should be strongly considered as currently the best for optimising the reduction and eventual elimination of TSE in sheep in the EU. It is however appreciated that this method of scrapie control is

dependent upon eliminating TSE infection and disease if human health is to be maximally safeguarded. Care must be taken to ensure that sheep resistant to disease are not under any circumstances able to carry or transmit infection. Research to investigate this aspect is underway.

In the short term, a culling system should influence the number of infected and infectious sheep being slaughtered affecting chiefly the processing risk. This processing risk can be defined as the probability that within a given period, an infected animal or material thereof enters the food and/or feed chain e.g. is processed either in a slaughterhouse or directly in a rendering plant with a view to be used as food or feed. It is assumed that, combined with a high quality surveillance system, an appropriate culling system should reduce substantially the processing risk (PRRreduc).

In the long term, an efficacious culling system should contribute to the decline and shortening of an epidemic (EPIDdecl). This decline could be over an extended period.

b. A question is if and to what extent a given culling system can contribute to effects 1 and/or 2. On the basis of an analysis made by Wilesmith and Ryan (updated July 1999) for BSE in bovines, it was concluded that the incidence of BSE after the selective cull has not been reduced significantly, but they also concluded that if the cull had been initiated earlier in the UK epidemic the effect would have been significantly higher. This illustrates that the efficacy of different measures for PRRred and EPIDdec depends largely on the time of implementation. Moreover, the effect of the different measures depends also largely on the extent of the epidemic. Durand *et al* (1999) showed the probability to detect incubating, and thus fully infectious cohort animals is only significant when the individual contamination probability is medium (0,5) or high (0,9), and this probability is directly related to the infection rate. So, if BSE in sheep spreads like scrapie it can be concluded that the chance to eliminate fully infectious or incubating animals by flock, cohort and offspring culling is likely to be higher than in case of BSE in cattle as the proportion of infected sheep in an infected flock or cohort is likely to be higher. However, in case of BSE in small ruminants, offspring and cohort culling alone will not be very efficacious in totally eliminating the infection from a flock. For BSE in cattle, cohort culling is in most cases considered to be the more cost-effective approach. (see SSC opinion on BSE-related culling in cattle of 14-15 September 2000).

c. In the absence of sensitive pre-clinical tests, capable of detecting animals early in the incubation, the cohort testing will detect only animals in the later stage of the incubation. Negative results should therefore be interpreted carefully.

d. Another, not negligible, aspect of different culling policies is the Animal Welfare aspect and the public perception of the scientific rational behind the strategy, although in the case of BSE a number of uncommon features play an important role in the concern of the public (always fatal disease, painful death, uncommon “new” disease, scientists seem to face a lot of uncertainties, involuntary exposure, large scale exposure, young people affected, no treatment available).

e. The question may be raised of resistant sheep possibly being carriers (and thus possibly excretors) of infection. In its 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 the SSC concluded that "the possibility that sheep may harbour a latent scrapie infection exists and if so, that they could pass an infection to other sheep. It is however expected that the background levels of infection in a resistant sheep population are significantly lower than in a susceptible population."

f. For culling to be successful animal identification is required with complete possibilities for tracing. Furthermore where significant co-mingling occurs any policy of slaughter may have to take into account the effects on the environment (elimination of grazing by sheep on hills) and the effects on the whole sheep system e.g. movement of sheep from hill flocks to lowland flocks. These systems differ in different countries. The timing of culling in relation to pregnancy and lambing may be particularly important. *PrP* genotyping may also be important and even more so in rams if 'resistant' ARR/ARR.

g. In conclusion, all data indicate that culling of the whole herd or the whole cohort (with the exception of resistant ARR/ARR animals) in case of BSE in sheep is likely to be more efficacious than in BSE in cattle because besides the effect on recycling in food and feed chain, the elimination of each BSE in sheep case avoids horizontal and maternal spread of infection and could significantly add to the two aimed effects: PRRred and EPIDdec. Each culling system should be evaluated in respect to the effect on the willingness to declare cases unless large scale testing systems become available, capable of detecting all clinical and, ideally, all pre-clinical cases.

h. Changing from a very drastic culling system to a less severe system (only the affected flock with cohort and offspring culling) could improve significantly the number of declared clinical suspected cases, as shown in Switzerland after the change from whole herd culling policy for cattle BSE to cohort culling in June 1999. Official authorities could be reluctant to take the risk of detecting a significantly higher number of BSE cases in sheep, if each new case has an impact on their export position or on internal sheep meat prices.

i. If there is a positive effect on current prevalence an effect on future incidence can be expected as well. However, whereas the effect of culling on future incidence can only be a reduction or maybe no effect at all, in principle, prevalence can be increased by culling as well. This might happen if culling criteria don't fit the real source of infection and lead to an over-proportional elimination of uninfected animals.

   To have positive effects culling criteria should therefore be defined in regard to the conditions under which the epidemic is happening (MBM exposure, recycling of SRM in feedstuff, horizontal, maternal or vertical transmission etc.) to fit the most likely source of infection. In case of proven MBM exposure it would be of critical importance to know if the cases are restricted to the exposed animals, or if there is already secondary spread in the flock. Problems arise if BSE in sheep should occur under sporadic form like BSE in cattle with little clustering of infection in the population so that precise identification of presumably exposed cohorts becomes difficult and the amount of culling would have to be large to eliminate a high proportion of exposed or infected animals.

j. To describe the possible future epidemic itself and the effects of culling schemes in terms of prevalence detailed population data are required to refer to. This seems to be extremely difficult if not impossible at present for BSE in sheep as even epidemic

curves showing relative incidence for scrapie are not available for most if not all EU Member States.

V.2 SURVEILLANCE AND FLOCK AND ANIMAL MOVEMENTS

a. The effect of a culling system is directly linked to the quality of the epidemiosurveillance system. For a good surveillance system, a number of conditions are required such as an awareness scheme for farmers and veterinarians, passive surveillance based on the reporting of a significant number of suspects, full compensation for all animals slaughtered (suspects, cohorts, flock, offspring, embryos), notification of cases, identification and registration of all susceptible sheep, standard laboratory protocols for pre-clinical and clinical diagnosis, etc. However, one should be aware of a possible negative effect of the culling policy on the willingness of sheep farmers and veterinarians to declare suspected cases. The pressure on farmers to hide the one BSE case which may lead to the destruction of his whole flock, or even the all the flocks in a region, can only be decreased. Only when the existence of sub-clinical infected or secondary fully infectious cases in the late incubation can regularly be demonstrated in affected flocks, can the pressure on farmers to hide the one BSE case which can lead to the destruction of his whole flock or even the all the flocks in a region, can be decreased. The same can be argued if export licence is dependent on the freedom from BSE of all flocks in a region. To avoid the hiding of cases, and as experienced in the USA (L.Detwiler, pers.comm.), anonymous large scale testing of fallen stock, emergency slaughter or other target populations as well as large application of pre-clinical tests, once they become available, is indicated.

b. In Annex 8 the characteristics of a high quality passive and targeted active surveillance system for scrapie and BSE in small ruminants are considered. In the absence of rapid tests to differentiate BSE from other TSE agents in both the live animal[8] and post mortem, an epidemio-surveillance system to detect emerging BSE in sheep should be able to provide, in addition to the outline given in Annex 1:
   - detailed documentation on the use of MBM in small ruminants from early 90s onwards, and close monitoring of the exposed flocks;
   - a genotyping of all TSE cases in sheep and goats in order to identify an abnormal range of TSE in genotypes known to be linked to scrapie resistance (presently, only ARR/ARR animals are thought to have very low risk).

c. It is noticed that no appropriate and reliable epidemiological data are available for the different Member States on scrapie incidence to assess whether there might have been a surge in the incidence of scrapie-like disease but derived from BSE infection. Epidemiological work is therefore urgently needed in each Member State, in order to establish the true prevalence of clinical TSE in small ruminants and to identify the prevalence of different genotypes in different breeds, related to scrapie/BSE susceptibility.

V.3. INDIVIDUAL IDENTIFICATION AND PERMANENT MARKING OF ALL SHEEP

[8] For example by using peripheral tissues such as the tonsils, mandibular ganglia, the nictitating membrane, placenta or other tissues.

Obligatory individual identification or marking of all sheep is essential, otherwise there is no way to properly trace sales, purchases, movements etc. in case such would be necessary.

V.4. USE OF GENETICS

On the use of genetics as tool for risk reduction with respect to TSEs in sheep, the SSC has adopted on 22-23 July 1999 an opinion on the *Policy of breeding and genotyping of sheep, i.e. The issue of whether sheep should be bred to be resistant to scrapie.*

V.5. ENVIRONMENTAL CLEANING.

Annex 10 provides an outline of the aspects covered by environmental cleaning.

V.6. INFORMATION CAMPAIGNS

It is considered that appropriate information campaigns with regard to TSEs in small ruminants, addressed to all people involved (farmers, butchers, slaughterhouses, meat industry, etc., effectively contributes to risk reduction.

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VI. LITERATURE:

Andréoletti, O., Berthon, P., Marc, D., Sarradin, P., Grosclaude, J., Keulen, L., Schelcher, F., Elsen, JM., Lantier, F., 2000. Early accumulation of PrP Sc in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie.*Journal of General Virology* (2000), 81, 3115–3126..

Baron, T.G.M., Biacabe, A-G., 2001. Molecular analysis of the abnormal prion protein during co-infection of mice by Bovine Spongiform Encephalopathy and a scrapie agent. Journal of Virology, **75 (1):** 107-114.

Benestad S. L., Thu, B., Schönheit, J., Tranulis, M. A. and Bratberg, B., 1999. Naturally occurring scrapie in a presumed resistent PrP genotype. In: *Characterization and diagnosis of prion diseases in animals and man. Tübingen, 23 - 25.09.1999*, edited by A. Aguzzi, J. Collinge, D. Dormont, M. H. Groschup, H. Kretzschmar, and C. Weissmann, 1999, p. Poster 74.

Bratberg, B., Benestad, S., Knutsen ,G., 2000. Resultater fra overvåkings og kontrollprogrammet for skrapesjuke hos sau – histopatologi (Results from surveillance- and control programme for scrapie in sheep – histopathology). Husdyrforsøksmøtet Ås-NLH Norway 15-16.02.2000, Proceedings ISBN 82-7479-008-1, 173-176.

Bratberg, B., Krogsrud, J., Benestad, S.L., Thu, B., 2000. Første verifiserte besetningsutbrudd av scrapie hos norskfødt sau. (First verified outbreak of scrapie among indigenous sheep in Norway). Norwegian, English summary. *Nor.Vet.Tidsskr.* 112 (5): 376-380.

Clark A.M., Dawson M. and Scott A.C. (1994). Scrapie associated fibrils in found-dead sheep. Veterinary Record, 134, 650

Ersdal, C., Benestad, S.L., Tranulis, M., Ulvund, M.J., 2000. Histopathology and detection of PrPSc in brain and retropharyngeal lymph node in a Norwegian sheep flock with scrapie. Symposium on Prion Diseases and Related Processes. Les pensieres, Veyrier-du-Lac, France, 12-16 Nov 2000. Abstract, poster, p. 158.

Ersdal, C., Benestad, S.L., Tranulis, M., Ulvund., M.J., 2000. Histopathology and detection of PrPSc in brain and retropharyngeal lymph node in a Norwegian sheep flock with scrapie. Int. Workshop on TSE Decontamination and Safety Issues, Merieux Foundation Lyon France, Nov. 2000. Abstract, poster, p. 158.

Hadlow, W.J., Race, R.E., Kennedy, R.C. and Eklund, C.M. 1979. Natural infection of sheep with scrapie virus. In: Slow Diseases of the Nervous System, Vol 2. S.B. Prusiner and W.J. Hadlow Eds. Academic Press, New York. Pp 3-12.

Hadlow W.J., Kennedy, R.C. and Race R.E., 1982. Natural infection of Suffolk sheep with scrapie virus. J. Inf. Dis. 146, 657-664.

Hagen, G., Thorud, K.E., Hoel, K., Støvring. M., 2000. Skrapesjuke og andre overførbare spongiforme encefalopatier: Forvaltningsmessige forhold i Norge. (Scrapie and other transmissible spongiform encephalopathies. Administrative handling). In Norwegian, English summary. *Nor.Vet.Tidsskr.* 112 (5):381-385.

Heggebø, R., Press, CMcL., Gunnes, G., Ulvund, M.J., Tranulis, M., Landsverk T., 2000. Distribution of prion protein in lymphoid tissue of lambs experimentally exposed to the scrapie agent. Int. Workshop on TSE Decontamination and Safety Issues, Merieux Foundation Lyon France, Nov. 2000. Abstract, poster, p. 159.

Heggebø, R., Press, CMcL., Gunnes, G., Ulvund, M.J., Tranulis. M., Landsverk. T., 2000. Distribution of prion protein in lymphoid tissue of lambs experimentally exposed to the scrapie agent. Symposium on Prion Diseases and Related Processes. Les pensieres, Veyrier-du-Lac, France, 12-16 Nov 2000.Abstract, poster, p. 159.

Hopp, P., Ulvund, M.J., Bratberg, B. 2000. Skrapesjuke hos sau i Norge. Historikk og epidemiologi. (Scrapie in Norway.History and epidemiology). In Norwegian, English summary. *Nor.Vet.Tidsskr.* 112 (5):368-375.

Hourrigan,J., Klingsporn, A., Clark; W.W., de Camp, M., 1979. Epidemiology of scrapie in the United States. In: Prusiner SB, Hadlow WJ (Eds.), Slow transmissible diseases of the nervous system. Academic Press, New York, I:pp 331-356.

Jeffrey, M., Martin, S., Barr, J., Chong, A., Fraser, J.R., 2001. Onset of accumulation of PrPres in Murine ME7 Scrapie in relation to pathological and PrP immunohistochemical changes. Accepted for publication (J.Comp.Path.)

Jeffrey, M., Ryder, S., Martin, S., Hawkins, S.A.C., Terry, L., Berthelin-Baker, C., Bellworthy, S.J., 2001. Oral BSE agent challenge of sheep. 1. Onset and distribution of disease specific PrP accumulation in brain and viscera of Romney sheep. Accepted for publication (J.Comp.Path.)

Sigurdarson, S., 1991. Epidemiology of scrapie in Iceland and experience with control measures. In: *Sub-acute spongiform encephalopathies. Proceedings of a seminar in the CEC Agricultural Research Programme, held in Brussels, 12-14 November 1990*, edited by R. Bradley, M. Savey, and B. Marchant, 3300 AA Dordrecht:Kluwer Academic Publishers, 1991, p. 233-242.

Tranulis, M.A.., Osland, A., Bratberg, B., Ulvund M.J., 1999. Prion protein gene polymorphisms in sheep with natural scrapie and healthy controls in Norway. *J.Gen.Virol.* 80 (Pt 4):1073-1077.

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OTHER SCIENTIFIC OPINIONS AND REPORTS THAT ARE RELEVANT TO THE QUESTIONS:

Report of the Scientific Veterinary Committee on *Surveillance of transmissible spongiform encephalopathies.* Adopted on 11 June 1997.

Report of the Scientific Committee on Animal Health and Animal Welfare *on the Standards for EC funding of Eradication Programmes for Scrapie*. Adopted on 23 June 1998.

SSC Opinion on *The risk of infection sheep and goats with Bovine Spongiform Encephalopathy agent*. Adopted on 24-25 September 1998

SSC Opinion on "Actions to be taken on the basis of (1) the September 1998 SSC Opinion on the *Risk of infection of sheep and goats with the BSE agent* and (2) the April 1999 *SEAC Subgroup report on research and surveillance for TSEs in sheep*. Adopted on 27-28 May 1999.

SSC Opinion on The policy of breeding and genotyping of sheep, i.e. The issue whether sheep should be bred to be resistant to scrapie". Adopted on 22-23 July 1999.

SSC Opinion on *Criteria for the diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical determination of TSE agent strains.* adopted on 13-14 April 2000.

SSC Opinion on *Specified risk materials of small ruminants.* Adopted on 13-14 April 2000.

SSC Opinion on *BSE-related culling.* Adopted on 14-15 September 2000.

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ANNEX 1: EXPERIMENTAL CHALLENGE OF SHEEP WITH BSE AGENT: TISSUE INFECTIVITY AND GENOTYPE.

Based on PrP-res detection described in the available incomplete studies of experimental BSE in Romney sheep (Jeffrey *et al*, 2001a), the tissue list might need to be modified for different *PrP* genotypes. In this study, sixty one Romney sheep of three different *PrP* genotypes (ARR/ARR, ARR/ARQ and ARQ/ARQ) were orally dosed with cattle brain tissue obtained from confirmed cases of natural BSE. Twenty one-ARQ homozygous sheep were dosed at 6 months of age, divided into groups and sequentially necropsied. Only one sheep from each of two groups of four sheep necropsied at 4 and 10 months post inoculation (mpi) had disease specific PrP accumulation within single lymph nodes. At 16 mpi disease-specific PrP accumulations were detected in some viscera, in spinal cord and in brain of 2/4 sheep. Of the sheep killed at 22 mpi, three had widespread disease-specific PrP in all tissues examined but in two sheep PrP accumulation was confined to the CNS. Clinical onset was observed between 20-26 mpi. Three sheep killed with advanced clinical disease had widespread PrP in viscera, brain and spinal cord. One sheep remains healthy. Unchallenged ARQ/ARQ controls were negative for PrP. Twenty ARR homozygous sheep and twenty ARQ/ARR heterozygous sheep were divided into groups of four animals and dosed. None[9] of these sheep have yet shown immunohistochemical evidence of PrP accumulation following necropsy at 1 and 2 years post challenge. These results confirm that ARQ/ARQ Romney sheep are susceptible to BSE challenge. The sites at which disease specific PrP accumulation was first detected in these sheep suggest that there is a variable point of entry of infection into the host and rapid spread through the lymphoreticular system once infection has been established. In contrast to BSE in cattle most sheep show disease specific PrP accumulation within viscera. In this context the pathogenesis of experimental BSE in most ARQ/ARQ Romney sheep does not appear to be different from that in natural scrapie. However, it is important to note that in some ARQ/ARQ Romney sheep, neuroinvasion may apparently occur in the absence of detectable PrP accumulation within the viscera or the peripheral nervous system. There may be differences in the *rates of neuroinvasion* and *targeting* to *visceral cell types* when patterns of PrP accumulation are compared to natural sheep scrapie.

Note: the most 'susceptible' ARQ/ARQ Romney sheep appear to exist as two sub-types where one subtype has no detectable visceral PrP^{Sc} at stages leading up to and beyond neuroinvasion as detected by immunohistochemistry. If PrP^{Sc} detection was consistently a proxy for infectivity and the reason for the existence of these two sub-types could be established (and was detectable by a test) the list of tissues containing infectivity might be less extensive in this sub-type than in natural scrapie of sheep and goats as listed by WHO and the SSC. Depending on the frequency of this phenomenon occurrence in the population at large, TSE risk management procedures could be modified accordingly. Indeed, if the infectivity studies in natural and experimental (orally-induced) scrapie of sheep and goats were repeated

[9] Scrapie has been reported in one Japanese ARR/ARR scrapie sheep, but confirmaton of this case is lacking However large numbers of scrapie sheep are being genotyped in Europe and US, so the one ARR/ARR is becoming very low as a percentage of total (=in the whole world) of scrapie sheep, possibly lower than 0.1% and dropping fast, as the numbers of scrapie sheep genotyped increases in epidemiology studies.

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with a full knowledge of the *PrP* genotypes it might be possible to determine TSE-risk tissues for each genotype. This could lead to the development of a range of options to enable management of any TSE risk, depending on the *PrP* genotype of sheep and the agent under consideration.

ANNEX 2. NATURAL AND EXPERIMENTAL SCRAPIE IN SHEEP AND GOATS: CLASSIFICATION OF TISSUES BY INFECTIVITY STATUS IN PRE-CLINICAL AND CLINICAL CASES (Number positive/number examined)

Infectivity titre	Pre-clinical				Clinical	
	Sheep				Sheep	Goats
	<8M (0/16)	10-14M (8/15)	25M (1/3)	>25M (1/6)	35-57M (9)	38-49M (3)
High ?4.1-6.5			Brain Spinal cord		Brain Spinal cord	
Medium ?3.2-4.0		Colon-proximal Ileum-distal LN (RP/RM) Spleen	Colon-proximal Ileum-distal LN (RP/RM) Tonsil		Colon-proximal Ileum-distal LN (BM) LN (PF 1/9-ve) LN (RP/MP) (Rectum-distal) + Spleen Tonsil	Colon-proximal Ileum-distal LN (BM) LN (PS/PF) LN (S/mammary) Pituitary (Rectum-distal) + Spleen
Low ?<3.1		LN (PS/PF) Tonsil	Brain (medulla / diencephalon) LN (BM) LN (PS/PF) Spleen		Adrenal Bone marrow ** Colon-distal CSF Liver ** LN (S/mammary x2) Pancreas ** Pituitary Sciatic N # Thymus **	Adrenal Colon-distal CSF Lung ** Nasal mucosa Sciatic N Thymus
Titre unknown TO BE COMPLETED	Tissues not listed in any box have not been tested. These tissues include the oesophagus and all stomachs.					

Infectivity titre	Pre-clinical				Clinical	
	Sheep				Sheep	Goats
	<8M (0/16)	10-14M (8/15)	25M (1/3)	>25M (1/6)	35-57M (9)	38-49M (3)
Infectivity tested but not found	Ileum LN (PS/PF) NL (RP/MP) Thymus Tonsil Spleen	Blood clot Brain (medulla) Colon-distal Faeces LN (BM) Serum	Adrenal Brain (cortex mid-brain) Colon-distal LN (S/mammary) Nasal mucosa Salivary glands Spinal cord Thymus	Colostrum	Blood clot Foetus Heart Kidney Lung Mammary gland Muscle-skeletal Ovary Placenta 0 Saliva Salivary glands Seminal vesicle Testis Thyroid Uterus	Blood clot Bonne marrow Faeces Kidney Mammary gland Milk Muscle skeletal Ovary Salivary gland Serum Uterus

* Log10 mouse intracerebral LD50/30mg tissue
\+ not assayed but high content of lymphoreticular tissue
0 +ve in other studies on scrapie (Pattison *et al* 1972, 1974)
** trace or exceptional
# wider range of nerves positive in scrapie (Groschup *et al* 1996)
BM bronchomediastinal
MP mesentric/portal
PF pre-femoral
PS pre-scapular
RP rectopharyngeal
CSF cerebro-spinal fluid
LN lymph node

ANNEX 3: EXPERIMENTAL BSE IN SHEEP: DISTRIBUTION OF INFECTIVITY BY INCUBATION STAGE AND PRP GENOTYPE AND AGE/STAGE OF INCUBATION.

INFECTIVITY TITRE	PRE-CLINICAL		CLINICAL	
	ARR/ARR, ARR/ARQ	**ARQ/ARQ**	**ARR/ARR, ARR/ARQ**	**ARQ/ARQ**
HIGH				**Brain** Spinal cord Spleen
MEDIUM		Spleen Lymph nodes Tonsil		Lymph nodes Tonsil
LOW				
PRP(RES) DETECTED BUT INFECTIVITY NOT TITRATED		spleen Lymph nodes Intestine Forestomachs abomasum		Intestine Forestomachs abomasum
NOT DETECTABLE	Brain, Spinal cord, Spleen Lymph nodes, Tonsil			

Notes: The summary table is based on the limited research results available in this field. Full literature references are provided in the attached report. The table should be used with caution since it relates to experimental, and not natural BSE in sheep, some data are incomplete and some experiments are on-going. Nevertheless it may serve as a guide to the degrees of risk that may exist. The Table should be updated as new results come forward.

No PrP-res has so far been detected in ARQ/ARR or ARR/ARR animals inoculated with BSE up to 2 years post challenge. On the assumption that PrP-res and infectivity are correlated this would suggest that if these animals are infected the titre of infectivity in the years immediately following challenge is lower (or undetectable) in peripheral tissues of these genotypes than in more susceptible genotypes.

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ANNEX 4: CURRENT INFORMATION ON TRANSMISSION

1. Transmission of BSE in small ruminants

Scrapie is the clinico-pathological expression of a TSE of sheep and goats which is endemic in many countries and has been recognised as a single disease entity for over two hundred years. Different "strains" of the scrapie agent have been identified by their biological properties on transmission to inbred mice, but the segregation of such extant strains from a newly introduced TSE strain (e.g. BSE), potentially introduced into sheep, on the basis of the presenting disease in sheep, is problematic and not possible on current information.

In contrast to our understanding of conventional infectious agents, aetiological typing of TSE agents has so far been achieved only on criteria of disease phenotype (clinico-pathological features) and the biological characteristics of the agents on transmission. The identification and definition therefore, of an aetiologically novel form of TSE in sheep can come only from the recognition of a phenotype of "scrapie" (TSE of sheep) distinct from the range of known presentations of scrapie and the confirmation that the agent isolated from that disease phenotype has biological properties on transmission to mice which are either like those of the BSE agent or differ in some substantial respect from historical and contemporary isolates of scrapie agents. Even then, the justification for the introduction of an aetiologically specific terminology for "scrapie" would require experience of multiple cases of the new phenotype. Analogies can be drawn with the identification and separate classification of vCJD from sporadic and other forms of CJD.

A further difficulty arising in the identification of a novel aetiological form of scrapie comes from the long established observation that the biological properties of TSE agents may change on transmission in a new host species.

2. Ways of transmission

2.1. Natural transmission

a. Vertical Transmission: transmission of disease 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. Thus no TSE equivalent to familial CJD in man has been reported in any other animal species. TSE infection has not been reported in semen or ova though there have been very few studies (see below). For transmission *via* embryos, see below.

Transmission by semen. Semen from cattle with clinical BSE does not contain detectable infectivity (Fraser and Foster, 1994) and epidemiological studies (Wilesmith, 1994) support this view. Furthermore infectivity was not found by bioassay of ovine semen from a ram with scrapie, in 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. It is looking increasingly clear that bovine embryos derived from BSE affected cows inseminated with semen from healthy bulls and bulls with BSE do not transmit BSE even though the experiment is not quite complete (Wrathall, 2000). This author also interprets the results of two other 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 is remains unclear whether scrapie can be experimentalky transmitted by embryos.

b. **Horizontal (lateral) Transmission**: transmission of disease from one animal to another 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 in natural sheep scrapie could be a form of horizontal transmission.

It is known that the sheep placenta could be infected (Pattison *et al* 1972, 1974, Onodera *et al* 1993, Race, Jenny and Sutton, 1998) and that it can transmit disease to other sheep and goats by the oral route (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.

2.2.Other forms of transmission

a. **Via Feed**. It is entirely possible that scrapie could be transmitted by infected feed. This is because scrapie (Pattison *et al* 1972, 1974) and BSE (Foster, Hope and Fraser, 1993) are transmissible to both sheep and goats by the oral route. Furthermore, MBM is the vehicle for the transmission of BSE to cattle (Wilesmith *et al*, 1988, Wilesmith, Ryan and Atkinson, 1989) and MBM has been fed historically to sheep and goats. This potential source of infection would be the one most likely to have established BSE in small ruminants and demands strict management of the risk.

b. ***Via vectors*** The evidence for transmission of scrapie via vectors is limited but 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 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. Laplanche *et al* 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.

c. Environmental transmission

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-free flocks in fenced areas of Iceland.

2.3.Iatrogenic exposure

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[10].

[10] There is at present not enough evidence for the transmission of BSE via vaccines in cattle, humans or other species, despite widespread usage of bovine materials in these biological products. The hypothesis of transmission of BSE via vaccines in cattle, humans or other species, is presently being evaluated by a working group of the Scientific Steering Committee. However, it is not certain that it will be possible to draw firm conclusions.

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ANNEX 5. GENETIC INFORMATION ON BSE IN SHEEP

Numerous studies have proven relationship between *PrP* genotypes and scrapie risk in sheep. 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 observation (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).

Data concerning resistance of ARR/ARR sheep to BSE are limited (Goldmann et al, 1994) but experiments are progressing in different countries. The possibility that ARR/ARR may carry BSE infectivity is even less known (*in vitro* assays). On the contrary, the other *PrP* genotypes are more or less susceptible to scrapie and BSE and animals may carry infectivity (or at least show PrP^{Sc} accumulation) early in their life.

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 side, 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 only either 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, the possibility of some reduction in risk to human population from the control of genetic structure of populations or flocks because of the lower level of infectivity in those animals. A reduction in clinical cases should occur.

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ANNEX 6. CRITERIA AND DIAGNOSIS.

1. Feeding history of the small ruminant (flock).

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 are likely to be due to initial feed exposure to BSE-infected MMBM. 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 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 double bow namely, preventing initial exposure and preventing propagation.

It follows that if MMBM has never been fed there is a very low risk of BSE being responsible. If the BSE agent was responsible, subsequent TSE/prion cases could be due either to exposure to infected MMBM (recycling) or to sheep to sheep transmission of BSE, though this has not yet been confirmed to occur even in experimental BSE. It is crucial to find out if experimental BSE in sheep has the ability to be transmitted naturally between sheep (directly and/or indirectly). If it does not, and there is no other route of exposure than feed, practical and effective measures to control BSE risks and eliminate infection can be devised and could become completely effectively in a relatively few years.

By contrast, the origin of scrapie historically is unknown but the disease is perpetuated by sheep to sheep transmission and possibly 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 (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 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 - details unknown, vectors etc., resulting in both horizontal and, in sheep only, 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.

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Probably due to lack of focus on concentrates fed to small ruminants, information on the origin of protein used in concentrates has been difficult to obtain. Although information has been requested from the EU countries (1997) on amount, source and general use of MBM for sheep and goats in the 80's at the onset of the BSE crisis, and later, very few countries (only 3) responded (SSC Sept.1998). In Britain, mammalian MBM was included in some diets until July 1988, when the practice was prohibited. However, accidental cross contamination of small ruminant diets could have occurred in the UK 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.

Bearing in mind that sheep seem to be more susceptible to scrapie during the first months in life (Hourrigan et al. 1979), and that tracing possible infective bovine MBM has proven difficult in the various countries, increasing risk factors would thus be:

- Concentrates possibly containing contaminated MBM has been/is being fed to adult sheep or goats;
- Concentrates with contaminated MBM has been or is being fed to lambs or goat kids.

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 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.

Small ruminant holdings with cattle, past or present, known to have had BSE, or having had the possibility of being fed (infectious) RMBM at one time, are also at risk (feeding of same concentrates, cross contamination of feeds). Analysis of data on notifications of BSE and scrapie in UK reveals that 200 farms 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 observation). 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 how many sheep generations, infectivity may be silently carried in less susceptible or more resistant genotypes of sheep and goats.

Sheep management and feeding:

Sheep 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 is most common statistically, are usually given concentrates at least during late pregnancy and early lactation (often from six weeks before lambing until a month after, dependent on 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 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.

Management in goats:

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.

2. Flock history with regard to scrapie.

Introduction of scrapie into a country or an area has often been associated to imports of foreign sheep, or purchase of a certain ewe or ram. In a scrapie-infected flock previously devoid of clinical scrapie, the disease can be come overt several years after the introduction of an uninfected 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 turns up in a new flock, it is often very difficult to trace back the origin of infectivity.

The example of Norway

The very first scrapie case in indigenous Norwegian sheep was detected in 1981, in a small and rather isolated flock (26 heads) with limited contacts to other flocks through breeding and grazing. During the three preceding years, the owner could, however, recall that four sheep had exhibited ataxia and moderate itching (Bratberg et al. 2000). When the second

case appeared in a similarly isolated flock 4 years later, no connections to the first flock could be traced. During the succeeding years, with a small epidemic-like occurrence of the disease in southwestern counties, 800 kilometres from the first two flocks, no connections were found to them (Ulvund *et al,* 1996). Less than half of these southwestern flocks had traceable contacts between each other (ewes, rams, common pastures etc, Hopp *et al,* 2000). The pattern of sudden appearance in rather isolated flocks has been repeated several times. The incidental breeding of susceptible genotypes during a time-period, some trade, and increased infection pressure through clinical cases, may have led to the small "epidemic-like" occurrence. However, numerous preceding years with no history of purchasing animals, and no clinical cases, may be indicative of carrier states or other routes, in fact through several generations and many years. Actually, the disease may have been introduced to Norway with sheep imported from GB around the turn of the 19th century (Hopp *et al,* 2000). This also brings to mind that the possibilities of discovering clinical cases are good in Norway, as most flocks are small and are kept indoors throughout winter so observation is frequent and good.

In Norway, clinical and *post mortem* studies of sheep in affected flocks that have been compulsorily slaughtered reveal all grades of prevalence of PrP (to be published). Scrapie has also been found in more resistant genotypes (AHQ/AHQ) of sheep in flocks with coexistent, 5-6 years old, clinically normal, susceptible sheep (Tranulis *et al,* 1999). These more resistant types have been somewhat clinically atypical (ataxia only, circling, no or little pruritus) and with atypical brain lesions (Benestad *et al,* 1999).

A recent two-year summing up of a national surveillance- and control programme for scrapie, launched in 1997, comprising both passive and active surveillance, showed that it was the targeted surveillance which led to discovery of three (atypical) scrapie cases, i.e. cases which probably not would have been detected with no such monitoring. The active surveillance with annual HP-monitoring (medulla) of around 5900 sheep above 3.5 years at slaughter**,** has yielded no verified scrapie cases so far. Sheep with ≥2 intraneuronal vacuoles in medulla has been found in 1,1 and 0,6 % of the samples, all, however, has been negative on IHC. (Bratberg *et al,* 2000). In Norway, with a low scrapie incidence (less than 0.3% of the flocks), targeted surveillance has therefore yielded best results in detecting scrapie. In Iceland, sheep sent to slaughter from one flock showed distinct symmetrical vacuolation for 5 consecutive years before a clinical case was seen (Sigurdarson, 1991).

Besides the use of contaminated MBM, movement of scrapie-infected sheep is historically by far the most important means of introducing scrapie infection into a country or flock. 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).

3. Flock management

a. 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 free 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. Another circumstance could be when genetic factors, as known in the case of scrapie, increase

the incubation period of infected ewes beyond their commercial life-span. 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. Annex 2 lists some of the specific elements of a scheme with respect to the monitoring the risk of introduction and spread of BSE by movement of flocks and animals.

b. Placenta can be infectious for over year before clinical signs develop in the sheep. Sequential pregnancies may show consistent infectivity/PrP or not in placenta perhaps related to the genotype of the placenta that in turn results partly from that of the ram. in the late incubation or clinical stage. 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.

4. Diagnostic tests currently available or under development for TSE

Note: A comprehensive report on the criteria for diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical diagnosis of TSE agent strains is available as an SSC opinion, adopted on 13-14 April 2000.

Some tests are currently available for the confirmation of TSE. The methods which offer the greatest potential for early identification of *infected* rather than *affected* animals currently rely on the detection of PrP(res) which is commonly accepted to be a proxy for the presence of infectivity. Some of these methods offer more potential for strain definition than others. Until the pathogenesis of natural scrapie is clearly defined, and the variation (if any) in pathogenesis as a result of agent strain or host genotype is known, it is difficult to define the most appropriate test/tissue combination for any particular diagnostic circumstance.

The most clearly defined/developed methods available so far are summarised below.

- **Histopathology**. Is very specific, but compromised by autolysis and poor sampling. Of no value in identifying pre-clinical or asymptomatic animals. Full histopathology profiling may offer some scope for strain differentiation (work in progress, VLA) but would be labour intensive and expensive. No specific changes are detectable in the LRS with this method.
- **Scrapie-associated-fibrils (SAF).** Less sensitive and specific for diagnosis of clinical cases compared to tests such as PrP-IHC, but results available rapidly.
- **Western Blotting.** Is a rapid and sensitive PrP detection method. Currently applied routinely to TSE suspects in UK, where it performs with a similar sensitivity and specificity to IHC. In experimental laboratory conditions it offers the potential for strain differentiation using glycoform analysis, but the rapid methods currently used for routine diagnosis do not show any apparent variation in glycotypes from diverse sources of ovine TSE. Some indication that differential proteinase K digestion patterns

may offer some strain differentiation potential. Methods under development for application to LRS.

- **Immuno-histo-chemistry.** Is a sensitive and specific PrP detection system which yields additional information on the nature of the PrP deposit and it's neuroanatomical / LRS and cellular tropisms. Variations in staining patterns may inform on strain variations. (Indications that this may vary also with antibody used - no detailed study of the potential significance/consistency/application of this available as yet). Recent work on mice (Jeffrey et al, 2001b) seems to indicate that Immuno-histo-chemistry may be more sensitive than Western Blot, especially in determining the presence of PrP(res) at an earlier stage in the incubation period.
- **Immuno-capillary-electrophoresis (ICE):** Also a PrP detection system , with apparently much greater sensitivity than those listed above but still falls short by about 3 logs for the detection of infectivity by bioassay. Method still experimental, and not validated for large-scale applications. Would only have strain-differentiating properties if Mab binding affinities were shown to differ with strain.
- **Rapid *post mortem* tests.** 3 tests can be listed for the time being. An evaluation programme of 5 additional tests has been launched by the European Commission at the end of 2000.

  a) **The ENFER test**- a chemiluminescent ELISA. Performed well in EC evaluation for confirmation of clinical disease. Appears to be a good rapid 'screen'. No scope for strain differentiation.

  b) **The BIORAD test** - a 'sandwich' immunoassay. Performed well in EC evaluation. High sensitivity. Would only have strain-differentiating properties if Mab binding affinities were shown to differ with strain.

  c) **The PRIONICS** test. Consists of a Western Blot assay using a monoclonal antibody.

- **The DELFIA** test - a 'sandwich' immunoassay (time resolved Dissociation-Enhanced Fluoro-Immuno Assay).
- **CDI -** work by Safar has shown apparent strain-related differential **Mab** binding affinity between experimental strains - may have implications for the strain-definition potential of all immunologically-based assays if it can be reproduced and extended/defined in field isolates.
- **Bioassay** - very slow and prohibitively expensive. Only useful in laboratory (or exceptional diagnostic) circumstances. Currently the only 'definitive' strain typing method. Relies on strains having the ability to cross the species barrier from sheep/goats to mice in the first place. It is already known that not all scrapie isolates from sheep transmit to mice.
- **Other metabolic markers** - many groups are assessing the value of other markers in blood, urine and CSF. Nothing has as yet been found which shows clear promise for practical diagnostic screening in sheep.

Other tests are under development, for example: PET blot (Schulz-Schaeffer, 2000); Immuno-PCR; Fluorescence Correlation Spectroscopy (Bieschke J *et al*, 2000, PNAS, 97, 5468-5473.); Bio-assays with transgenic mice.

On the criteria for diagnosis of clinical disease and pre-clinical TSE infection in sheep, it was concluded that microscopic examination of the brain does not guarantee the confirmation of diagnosis in all clinically suspect scrapie and that additional tests should be applied (*e.g.* SAF, IHC, Western Blot, Dot Blot, PET blot) but they need further evaluation. To maximise the possibility of diagnosis of TSE in clinically affected and pre-clinically infected sheep the following procedures/methods should be included: all clinical suspect cases or found dead animals above 1 year (FD) showing SE with classical histopathology (HP) alone, all clinical suspect cases of TSE or FD positive with immunohistochemistry or Western Blot/Dot Blot (WB/DB) alone and all cases of preclinical TSE or FD scoring positive with histopathology plus one positive IHC or WB/DB test. SAF results should always be confirmed by an additional test (IHC, HP or WB/DB). Results should always be confirmed by an additional test (IHC, HP or WB/DB). A preclinical or FD histology negative but both IHC and WB positive would be considered as a TSE. Some breeds in the US who do indeed not appear to develop well discernable lesions. (L.Detwiler, pers.comm., January 2001)

Later work on mice (Jeffrey et al, 2001b) seems to indicate that Immuno-histo-chemistry may be more sensitive than Western Blot, especially in determining the presence of PrP(res) at an earlier stage in the incubation period.

Note: Recent research results[11] from Baron and Biacabe (personal communication; 2001, in print) suggest that, at least as far as attempts at molecular strain typing are concerned, the presence of a scrapie strain may, in some conditions, hide the presence of BSE if the animals have been infected by both strains. This raises the theoretical possibility that, following characterization of scrapie strains in sheep, the presence of BSE in sheep, if it occured as a coinfecting minor strain, may remain undetected.

5. Note on: Concurrent infections of sheep with natural scrapie and BSE

TSE strains are routinely isolated from their natural hosts and biologically characterized by serial passage in different PrP genotypes of mice. Frequently more than one TSE strain can be identified. However it is not known whether these mouse-passaged strains represent two separate strains present in the donor sheep or were selected by mice from mutants adapted to the experimental hosts. It has been shown that two experimental strains can infect and replicate simultaneously in mice [Dickinson, 1972 #220; Dickinson, 1975 #164]. TSE strains can also change their properties (e.g. see below, 87A - ME7 model) on serial passage.

From his findings Prusiner *et al* (pers.comm, August 2000; paper in preparation) advance the *hypothetical* scenario that "it is possible that low levels of the BSE 'prion

[11] Baron and Biacabe (2001, in print) describe the molecular analysis of the abnormal prion protein in mice which were infected by both BSE and a scrapie strain, previously adapted to mice. Both strains used for these experiments had been described to kill the mice following similar incubation periods, when inoculated alone by the same route, and with comparable titers of infectious agents into their brains at the terminal stage of the disease. When mice were inoculated at the same time by both strains, the molecular features of PrP res (glycoform ratios and apparent molecular masses of the unglycosylated protein) in brain at the terminal stage of the disease were undistinguishable from those of the strain which was expected to lead to the highest level of PrP res in brain. A BSE profile was only observed when mice were inoculated by BSE intra-cerebrally, whereas scrapie was inoculated by a peripheral route. In contrast, a scrapie profile was found when both BSE and scrapie were inoculated by the same intra-cerebral route at the same time, including when challenge by a higher dose of BSE was used.

strain' are actually endemic in the scrapie sheep population, but that the BSE prions never surface as such because their presence is marked by the more rapidly growing sheep scrapie strain. Any unusual selective treatment, such as the change in rendering process, could remove the less dangerous sheep scrapie strain and allow the BSE strain to accumulate and spread to the cattle population". They conclude that "BSE prions in sheep may thus have been there all the time at very low levels that pose no significant risk to humans but unusual circumstances might have allowed them to spread either through the sheep or cattle population and accumulate to levels hazardous to humans."

Recent research results[12] from Baron and Biacabe (personal communication; 2001, in print) suggest that, at least as far as attempts at molecular strain typing are concerned, the presence of a scrapie strain may, in some conditions, hide the presence of BSE if the animals have been infected by both strains. This raises the theoretical possibility that, following characterization of scrapie strains in sheep, the presence of BSE in sheep, if it occured as a coinfecting minor strain, may remain undetected.

There is no direct evidence available which shows that two separate TSE strains can affect sheep simultaneously, but the above data from experimental mouse models suggest that it is possible. Conclusion: It is a theoretical possibility that an individual sheep could be infected concurrently with both with one or more scrapie agents and with the BSE agent.

[12] Baron and Biacabe (2001, in print) describe the molecular analysis of the abnormal prion protein in mice which were infected by both BSE and a scrapie strain, previously adapted to mice. Both strains used for these experiments had been described to kill the mice following similar incubation periods, when inoculated alone by the same route, and with comparable titers of infectious agents into their brains at the terminal stage of the disease. When mice were inoculated at the same time by both strains, the molecular features of PrP res (glycoform ratios and apparent molecular masses of the unglycosylated protein) in brain at the terminal stage of the disease were undistinguishable from those of the strain which was expected to lead to the highest level of PrP res in brain. A BSE profile was only observed when mice were inoculated by BSE intra-cerebrally, whereas scrapie was inoculated by a peripheral route. In contrast, a scrapie profile was found when both BSE and scrapie were inoculated by the same intra-cerebral route at the same time, including when challenge by a higher dose of BSE was used.

ANNEX 7: STABILITY OF AGENT STRAINS

1. BIOLOGICAL STABILITY OF AGENT STRAINS; CHANGE IN STRAIN PROPERTIES OF BSE ON SHEEP PASSAGE.

When TSE isolates are passaged into a new host species the incubation period for the primary passage is usually longer than for subsequent passages in the recipient species. (It may be longer or shorter than the incubation period in the donor species). Titres at primary passage in the new host are usually lower than in the donor species and subsequent passages in the new host. There may also be differences in the intensity and distribution of pathological lesions: histological lesions are sometimes more focal and more intense in primary passage recipients.

There are several reasons for these phenomena: Firstly, transmission across a species barrier is inefficient; thus titres are lower and incubation periods are lengthened. This may be because the donor tissue is more readily recognised as foreign by the recipient's immune system. Many primary inocula will not establish infection directly in the CNS but require processing/replication in peripheral organs first. In some cases there may be a change in the properties of the agent, an adaptation to the new host and specifically to the new host's PrP sequence. In addition a new strain can arise within an infected animal from the original infecting strain. For example mice infected with the 87A strain of scrapie can continue to yield 87A if the agent is passaged at high dilution but will tend to yield a new strain, ME7, if passed at low dilution (Bruce, M. E. & Dickinson, A. G. (1987). Biological evidence that scrapie agent has an independent genome. *Journal of General Virology* **68**, 79-89). In such cases there may be a much greater shortening of incubation periods and possibly a change in the histopathological presentation to a more diffuse, widespread pathology.

Implications for humans: It can be concluded that on transmission to sheep, BSE may or may not significantly change its properties with respect to its phenotypic characteristics and its ability to infect man. The only evidence that informs this assessment is the experimental transmission of BSE to sheep and then to mice. In this experiment the phenotypic properties of sheep passed BSE in mice were indistinguishable from those of BSE passed directly from cattle (Bruce, M. E., McBride, P. A., Jeffrey, M. & Scott, J. R. (1994). PrP in pathology and pathogenesis in scrapie-infected mice. *Molecular Neurobiology* 8, 105-12), and similar to BSE passed through man (as vCJD) and then characterised in mice. This evidence therefore suggests that there was little change in the BSE agent during these transmissions. However these data only refer to a single pass through sheep and it remains a possibility that BSE may alter its properties on serial passage in sheep. Such studies are ongoing but no results are available yet. It is also possible that different *PrP* genotypes of sheep may interact with BSE in different ways, possibly leading to different phenotypic properties. If the BSE agent were to change its properties on passage through sheep there is a possibility that its ability to affect humans may be altered. However it would be unwise to assume that any such change in host susceptibility might take place.

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2. Molecular stability of the agent; persistence in the environment

Compared with conventional microorganisms, TSE agents are much more difficult to inactivate by both chemical and physical methods. Many procedures achieve small to moderate degrees of inactivation but do not achieve sterility. The only procedures that are currently considered to be completely effective under worst-case conditions are a) exposure for an hour to a sodium hypochlorite solution containing 20,000ppm of available chlorine (Taylor *et al*, 1994) or b) combining autoclaving at 121^{o}C with prior or simultaneous exposure to sodium hydroxide (Taylor *et al*, 1997). With regard to partially-inactivating procedures, it appears that any inactivating effect is achieved within a relatively short space of time, and that prolonged exposure does not necessarily enhance the decontamination process. For example, when the 263K strain of hamster-passaged scrapie agent was autoclaved at 134^{o}C, the infectivity titre was reduced by seven logs during the first twenty minutes of the "sterilisation" process but no further titre reduction was observed in the remaining "resistant subpopulation" when the autoclaving time was extended to one hour (Taylor *et al*, 1998). Also, when the 301V strain of mouse-passaged BSE agent was exposed to 1M sodium hydroxide for only one minute at room-temperature (Taylor *et al*, 1999), the degree of inactivation achieved was at least equivalent to that achieved with other TSE agents using 2M sodium hydroxide for periods of up to two hours (Taylor *et al*, 1994).

As far as the survival of TSE agents in the general environment is concerned, the information presented above would point to the conclusion that some degree of inactivation is likely to occur through their exposure to natural environmental factors such as heat, irradiation, chemicals, proteolytic enzymes etc. However, it is likely that significant levels of infectivity will survive, and there is some evidence to support this. For example, it has been shown that CJD-infected brain-tissue is still highly infectious after it has been left at room-temperature for 28 months (Tateishi *et al*, 1987); scrapie agent survives in a desiccated state for at least 30 months (Wilson *et al*, 1950); some scrapie infectivity survives three years internment in the ground (Brown & Gajdusek, 1991). Also, epidemiological studies in Iceland suggest that there may have been relatively longterm survival of scrapie agent on pastures (Palsson, 1979).

Because of the high degree of resistance of TSE agents to inactivation, the precautionary assumption must be that infectivity can survive in the general environment for long periods of time but it is unknown how long this period might be. Formol-fixed brain-tissue from TME-infected mink was still infectious after six years of storage at room–temperature (Burger & Gorham, 1977). However, it has been shown that the exposure of TSE agents to physical or chemical procedures (including formalin) that fix proteins, rather than destroy them, protect infectivity from inactivation by heat (Taylor, 2000). Such exposures are also likely to enhance the resistance of TSE agents to other methods of inactivation, but this remains unproven.

The overall conclusions are that TSE infectivity that has not been exposed to any "protein-fixing" process is likely to be progressively degraded over an indefinable period of time in the general environment, whereas TSE infectivity exposed to "protein-fixing" procedures is likely to survive for extremely long periods that are also indefinable.

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References

Burger, D., Gorham, J.R., 1997. Observation on the remarkable stability of transmissible mink encephalopathy virus. *Research in Veterinary Science* **22**, 131-132.

Palsson, P.A., 1979. Rida (scrapie) in Iceland and irts epidemiology. In S. B. Prusiner and W. J . Hadlow Eds. Slow transmissible diseases of the nervous system. Volume 1. Academic Press, New York, pp. 357-366.

Sigurdarson, S., 2000. Scrapie eller rida i Island. Norsk Vet. 112, 408- 413.

Tateishi, J., Hikita, K., Kitamoto, T. & Nagara, H., 1987. Experimental Creutzfeldt-Jakob disease: induction of amyloid plaques in rodents. In: *Prions: Novel Infectious Pathogens Causing Scrapie and Creutzfeldt-Jakob Disease* (eds. Prusiner, S.B. & McKinley, M.P) pp. 415-426. New York; Academic Press.

Taylor, D.M., 2000. Inactivation of transmissible degenerative encephalopathy agents: A review. *Veterinary Journal* **159**, 10-17

Taylor, D.M., Fernie, K., McConnell, I., 1997. Inactivation of the 22A strain of scrapie agent by autoclaving in sodium hydroxide. *Veterinary Microbiology* **58**, 87-91.

Taylor, D.M., Fernie, K., McConnell, I., Steele, P.J., 1998. Observations on thermostable populations of the unconventional agents that cause transmissible degenerative encephalaopthies. *Veterinary Microbiology* **64**, 33-38.

Taylor, D.M., Fernie, K., Steele, P,J., 1999. Boiling in sodium hydroxide inactivates mouse-passaged BSE agent. *Astracts of a Meeting of the Association of Veterinary Teachers and Research Workers.* Scarborough,29-31 March 1999, p.22.

Taylor, D.M., Fraser, H., McConnell,I., Brown, D.A., Brown, K.L., Lamza, K.A., Smith, G.R.A., 1994. Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Archives of Virology* **139**, 3313-326.

Wilson, D.R., Anderson, R.D., Smith, W., 1950. Studies in scrapie. *Journal of Comparative Pathology* **60**, 267-282.

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ANNEX 8: SOME CONSIDERATIONS ON SURVEILLANCE FOR BSE IN SHEEP.

A high quality passive and targeted surveillance system for scrapie and BSE in small ruminants should allow to identify at maximum (i) suspect clinical scrapie and BSE cases; (ii) suspect pre-clinical scrapie and BSE cases; (iii) the flocks at higher risk from scrapie and TSE (exposed, infected, incubating animals not detectable by pre-clinical test methods) and (iv) emerging BSE or non-scrapie TSE in sheep

1. General requirements for a TSE -surveillance system in small ruminants

All surveillance systems should at least enable the detection of suspected and confirmed TSE cases. This implies that notification should be mandatory. It may also imply an appropriate compensation for all animals slaughtered in the frame of TSE-surveillance and TSE-control measures (suspected, affected, farm culling).

2. Special requirements

2.a. Passive surveillance on clinically suspicious animals

To guarantee that all clinically affected animals are identified, a surveillance system would have to fulfil the following conditions:

1. Continuous recognition of clinical signs of scrapie and BSE in small ruminants, through continuous education, training and awareness raising for sheep and goat holders, veterinarians and other concerned persons.
2. Guarantee that suspected animals, i.e. animals showing signs of disorders for which scrapie or BSE can not be excluded, can in no way disappear without efficient clinical examination via slaughterhouses (especially emergency slaughter), rendering plants, burial or slaughter at home.
3. Regular (at least 2 times a year) scrapie and BSE oriented inspections of the flock by the official veterinary officer or practitioner and pre-slaughter inspection with a view to support points 1 and 2 above and auditing of flock records to account for all sheep born or brought into the flock. Also individual identification allowing identification of flock and female parent and possibly also at a later time, PrP genotype e.g. by colour coding.
4. Specific scrapie and BSE oriented pre-slaughter inspection for all emergency slaughter.
5. To all suspect cases a quality assured test system is applied in national reference laboratory for TSE according the criteria described in the SSC Opinion of 13-14 April 2000 “The criteria for diagnosis of clinical and pre-clinical TSE disease in sheep and for differential biochemical diagnosis of TSE agent strains”.
6. If epidemiological data on the “real” incidence of neurological and other disorders in sheep and goats for which scrapie and BSE can not be excluded, i.e. all suspected cases, would be available, it would be possible to estimate the number of brains that must be examined in order to verify, with a pre-defined level of confidence, the presence or absence of TSE (this means microscopic examination only).

However, it should be understood that in any case all suspect cases have to be analysed with regard to TSE. The normal incidence of neurological and other disorders compatible with scrapie or BSE in a given country or geographical area should be defined on the basis of:

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- historical data such as autopsy reports in veterinary faculties, official laboratories etc., concerning at least the foregoing ten years with data on symptomatology, age and laboratory results and
- a field inquiry including a statistical valid proportion of sheep and goat flocks

In defining these normal incidence animals for which scrapie or BSE could be excluded on the basis of age, incubation period or symptoms should not be taken into account.

2.b. Active surveillance

2.b.1. Active surveillance targeting exposed animals around clinical scrapie or BSE cases

For an effective active surveillance, able to identify the exposed animals around clinical scrapie or BSE- cases, the following conditions should be met:

1. Identification and registration of all susceptible sheep and goats kept under controlled conditions by:

   1.1. individual identification (ear tags, chips)

   1.2. centralised data bases including or allowing to establish movement records

   1.3. it would be an advantage if herd and/or animal history records would be kept - on farm or centralised, which would include, for example, information on feeding, treatments, and clinical examination results.

2. Once they become available the following should be applied:

   2.1. Fast post-mortem testing: Individual testing (ELISA, Western blot, etc.) of CNS and tonsil tissue of slaughtered or dead sheep and goats with the aim to identify animals before appearance of clinical symptoms or to identify clinical cases, not recognised in the passive surveillance. Tests described in the preliminary report of 24 June 1999 “The evaluation of tests for the diagnosis of transmissible spongiform encephalopathy in bovine”, could be useful, but need further validation for pre-clinical and clinical scrapie and BSE diagnosis in small ruminants.

   2.2. Ante-mortem testing: Individual testing of other tissues (cerebrospinal fluid, peripheral nervous tissues, lymphoid tissue such as tonsil or mandibular ganglia, third eye lid, white blood cells, etc) on all living animals with the aim to identify animals before appearance of clinical symptoms.

3. Identification and testing (if available with the help of the aforementioned tests) of:
   - all offspring and cohort animals of a scrapie or BSE case; and
   - all sheep and goat above 6 months of the affected herd with post mortem tests as described under 2.b.1.2.2.1. and under 2.a.5.

2.b.2. Active surveillance, targeting risk populations defined by other than incidence, e.g. flocks fed with ruminant MBM, animals imported from TSE affected countries/regions/flocks and fallen stock and emergency slaughter.

1. Fallen stock and emergency slaughter. According to SSC opinion **, all small ruminants above 1 year entering the rendering plant as fallen stock or being considered as emergency slaughter and for which a TSE cannot be excluded, should be considered as a suspect TSE case and tested as described 2.a.5. or using another

validated fast post mortem testing, also applicable on autolysed material, would provide confirmation or information.

2. Imported animals. All animals imported from scrapie and/or BSE affected countries/regions or flocks represent a potential risk.
3. Animals exposed through feed. Efforts have to been made to define, identify and monitor potentially exposed flocks or flocks being at risk to be infected by the BSE-agent through feed independent of the appearance of clinical scrapie or BSE cases.

The effect of different quality levels of scrapie/BSE -surveillance systems on risk reduction could be summarised as follows:

1. A system meeting all requirements listed under 1 and 2 with the exception of the application of fast post mortem tests and/or ante mortem TSE tests as long as no approved tests are available for small ruminants would be able to provide a complete image of the epidemiological scrapie/BSE -situation of the country, can significantly reduce HER and animal infection risk and will be able to detect the emergency of BSE in sheep. This would correspond with the highest level of risk reduction (highest quality epidemio-surveillance):
2. A system meeting the requirements listed under 1, 2.a, 2.b.1, 2.b.2.2 and 2.b.2.3 would be able to provide an appropriate but not fully complete image of the epidemiological scrapie/BSE situation of the country, can reduce the HER and animal infection risk to some extent and will detect the emergency of BSE in sheep.
3. A system meeting only the requirements of 1 and 2.a would not be able to provide an appropriate image of the epidemiological scrapie/BSE -situation of the country, could not reduce the HER and animal infection risk and will not detect the emergency of BSE in sheep.
4. A system not fulfilling the conditions under 1 and only partly the conditions listed under 2.a would be of poor quality: it could provide a wrong image of the epidemiological scrapie/BSE-situation of the country, could even increase the HER and animal infection risk by providing a wrong impression of safety and will not detect the emergency of BSE in sheep.

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ANNEX 9: SOME SPECIFIC ELEMENTS OF A SCHEME WITH RESPECT TO MONITORING THE RISK OF INTRODUCTION AND SPREAD OF BSE BY MOVEMENT OF FLOCKS AND ANIMALS

1. Marking of all animals and indicating the farm of origin; individual marking with a special earmark of animals imported from third countries.

2. Recording, ideally computerised, of
   - all animals and in a way the female parent to be individually identified and if possible ram parent;
   - 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, with indication of reasons for death for all animals above 6 months of age;
   - common grazing, when used.

3. Management of the ewe flock in a way that it is either completely closed in the female line or that breeding females and embryos originate only from flocks of equivalent or higher BSE status.

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

   Under the terms of Council Directive 91/86/EEC rams and semen may come from any source, if it is assumed that BSE behaves like scrapie in sheep.

   Effective fencing of premises, to prevent sheep from straying in and out of the flock and to prevent directs or close contact with the sheep from another flock.

   Common grazing could only provided that 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.

   Common pasture only to be used by animals from holdings of the same BSE status. Recorded keeping of the use of common grazing.

ANNEX 10: ENVIRONMENTAL CLEANING: SOME EXEMPLES

a) Measures on the affected holding

The carcasses of the culled animals are treated as high-risk waste (133 °C, 20 min, 3 bar). The meat and bone meal thus obtained is burned or otherwise destroyed.

In order to possibly eliminate the infectious agent from the holding and depending on the possibility of satisfactory cleaning and disinfecting, houses where small ruminants have been kept may be torn or burned, or most often subjected to sanitation measures including:

- removal of manure;
- removal and burning of all wooden materials and other material that has been in direct contact with the sheep and goats (flooring, walls, drinking basins etc);
- cleaning and disinfecting of remaining indoor areas;
- painting of at least the bottom 1,5 m of the walls of the building, including windows;
- fitting of new concrete floors, doors, walls etc according to the condition on the individual farm.

b) Sanitation measures are also to be taken on outside areas, including:

- changing of the upper layer of stone/tarmac on surrounding roads used by the animals
- painting of the outside wall of relevant buildings
- ploughing and/or burning of grass on grazing areas
- fitting of new fences on areas that have been in contact with sheep

Sheep may at the earliest be reintroduced on the farm two years after the completion of the sanitation programme. Grazing areas that cannot be satisfactorily decontaminated must be kept free from sheep, normally for a period of five years.

Compensation is paid to the owner, covering the value of the herd and the expenses related to the sanitation measures.

c) A sanitation manual

Should be worked out containing more detailed practical recommendations on cleaning, disinfecting, painting and use of flame in rooms where animals have been kept, adjacent rooms (for feeds, offices, other), rooms for manure, outdoor walls, feed-stores, tools and equipment, pastures (home fields, cultivated pastures, outlying fields), use of pastures during times of fallow, treatment of roads/farmyards, areas where sheep have been lying, and handling of fences).