OPINION ON THE RISK OF INFECTION OF SHEEP AND GOATS WITH BOVINE SPONGIFORM ENCEPHALOPATHY AGENT.

Adopted by the Scientific Steering Committee

at its meeting of 24-25 September 1998

EXECUTIVE SUMMARY

The Question

On 16 October 1997, the European Commission requested the Scientific Steering Committee "to establish possible ways of transfer of the BSE agent to sheep and goats, identify the critical factors, assess the risk that the BSE agent exists in sheep and goats and evaluate the exposure of humans to the BSE agent through sheep and goats."

The Opinion and its justification

Although there is currently no evidence to suggest that BSE occurs naturally in sheep and goats under field conditions the following evidence suggests that the previous or current geographical risks of exposure of sheep and goats to infection can not be excluded and should be investigated under field conditions.

In the light of the incomplete information available, the working group concludes that it can not be excluded that BSE, once introduced, may be maintained and spread in the sheep and goat population by means of horizontal and vertical transmission.

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.

The Scientific Steering Committee considers that if BSE in sheep and goats exists, the most likely way of introduction has been through infected MBM. It is possible that the BSE-agent has been maintained, propagated and/or recycled by horizontal and vertical transmission in sheep and goats if the agent behaves like scrapie in these species. Maternal transmission is unproven in goats. Other means of transmission are theoretically possible but regarded as extremely unlikely provided current measures are in place and effectively enforced

In the context of the geographical risk of BSE in small ruminants, special attention needs to be paid to the genotypes in the sheep population and the

possibility of horizontal and maternal transmission of BSE in sheep and goats. No information is currently available but it is noted that study of maternal transmission of experimental BSE in sheep is in progress.

Given the existing uncertainties the SSC concluded that whilst BSE has not been identified in sheep and goats under field conditions, the previous or current geographical risks of BSE existence in sheep and goats can not be excluded. The risk of humans being exposed to the BSE agent originates from animals in the pre-clinical and clinical stage of the disease. It can be reduced by effective measures reducing the exposure risk, in particular safe sourcing, exclusion of the potentially most highly infected tissues (age-specific specified risk materials) from processing, reducing the age at slaughter for human consumption and application of validated processing methods with a proven potential to reduce/eliminate any residual BSE-infectivity.

These measures have been previously described by the SSC which considers that there is no scientific reason for a change in that advice.

The list of SRM should be regularly re-evaluated taking account of the results of ongoing epidemiological surveys on BSE in small ruminants and of new scientific data on BSE infectivity distributions in tissues of small ruminants, infectivity and transmission in small ruminants and whether in particular the lymphoreticular tissue should be considered more infective in sheep than they are in cattle.

In order to update this opinion, the SSC urges that EU Member States and other countries should urgently make available toe the European Commission relevant data and information which may be at their disposal.

The Scientific Steering Committee notes that the European Commission has proposed a scrapie control scheme aimed at the eventual elimination of scrapie. The SSC endorses these proposals: the SSC recommends that all EU Member States consider a future scrapie control and eradication programme.

The Scientific Steering Committee finally recommends that high priority should be given to the validation of tests for large scale testing for differential diagnosis of BSE and scrapie.

The Scientific Steering Committee is informed that a number of research projects are currently addressing the issue of BSE in sheep, which may require the present opinion to be updated.

Note: The above opinion of the SSC is based on the report of the working group of the TSE/BSE ad hoc Group, which was accepted by the TSE/BSE ad-hoc group and then by the SSC, following critical discussion and review.

FULL OPINION

Note: Readers should keep in mind that the present report and opinion only addresses the scientific aspects of the risk assessment of the issue (e.g., identification of hazards, levels of infectivity in the starting materials and final products, etc.). The risk management and policing aspects related to the implementation of an opinion, are not dealt with.

Question

On 16 October 1997, the European Commission requested the Scientific Steering Committee "to establish possible ways of transfer of the BSE agent to sheep and goats, identify the critical factors, assess the risk that the BSE agent exists in sheep and goats and evaluate the exposure of humans to the BSE agent through sheep and goats."

Opinion of the SSC

1. On the risk that BSE exists in sheep and goats.

Although there is currently no evidence to suggest that BSE occurs naturally in sheep and goats under field conditions the following evidence suggests that the previous or current geographical risks of exposure of sheep and goats to infection can not be excluded and should be investigated under field conditions.

The experiments of transmission of BSE in sheep can be summarized as follows. Six "negative" line Cheviot sheep[1] were orally challenged with BSE infected brain material. One developed a clinical TSE and BSE infectivity which was identified in brain and spleen. Six "positive" line Cheviot sheep were also orally challenged and 2 of them developed a clinical TSE . In both cases an atypical TSE strain was identified in material from their brain. In parallel, 6 "negative" and 5 "positive" line Cheviot sheep were intracerebrally challenged. From the first group, 5 animals developed clinical TSE. Strain typing was carried out on brain and spleen from one animal which confirmed BSE. In the second group, 3 developed clinical TSE, but transmission of the agent to mice failed in all 3 cases. In the goat experiment, 3 Anglo-nubian goats were orally challenged with BSE infected brain material and 3 were intracerebrally challenged. From the first group, 2 animals developed clinical TSE. Strain typing was carried out on the brain of one animal and the agent identified was an atypical TSE agent. The 3 goats from the second group developed a clinical TSE and for each animal, BSE could be identified in the brain.

In the light of the incomplete information available, the working group concludes that it can not be excluded that BSE, once introduced, may be maintained and spread in the sheep and goat population by means of horizontal and vertical transmission.

Because it has clearly been demonstrated that BSE can be orally transmitted to certain genotypes of small ruminants, and because it is likely that potentially 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 it, even after an effective implementation of a ruminant feed ban.

No appropriate and reliable epidemiological data are available on scrapie incidence to assess whether there might have been a surge in the incidence of scrapie-like disease but derived from BSE infection. Furthermore appropriate surveillance systems with good pathological screening are not of the same quality in all Member States and do

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[1] Negative line Cheviot sheep do not succumb to experimental scrapie following s/c challenge with SSBP 1

scrapie whereas positive line sheep do. It is now known that the positive and negative line sheep have distinctive

and different *PrP* genotypes that explains their response to experimental and natural infection with scrapie.

not allow in most cases the making of proper estimations of the real incidence of TSE in small ruminants. On the basis of data on feeding practices, sheep and goats in many countries have probably been exposed to the BSE agent through MBM.

Further studies are now underway to investigate whether the BSE-agent exists under field conditions in the sheep population in the UK. If this should prove to be the case, then it is also necessary to consider whether the mechanisms that enable scrapie to spread are the same for BSE in sheep and goats. A risk analysis on the basis of the Opinion of the SSC of 23 January 1998 would then give valuable information on the exposure and incident risk of BSE in sheep and goats in different geographical areas.

2) On the possible ways of transfer of the BSE-agent to sheep and goats.

The Scientific Steering Committee considers that if BSE in sheep and goats exists, the most likely way of introduction has been through infected MBM. It is possible that the BSE-agent has been maintained, propagated and/or recycled by horizontal and vertical transmission in sheep and goats if the agent behaves like scrapie in these species. Maternal transmission is unproven in goats. Other means of transmission are theoretically possible but regarded as extremely unlikely provided current measures are in place and effectively enforced.

3) On the critical factors for assessing the risk of the BSE agent being present in sheep and goats.

The SSC on 23 January 1998 set out the parameters needed to define the risk of BSE in specified geographical areas. In addition attention should also to be paid to possible horizontal and maternal transmission of BSE in small ruminants.

The factors contributing to the incident and propagation risks in a geographical area were listed as:

1. Structure and dynamics of the cattle, sheep and goat populations
2. Animal trade
3. Animal feed
4. Meat and bone meal (MBM) bans
5. Specified bovine offals (SBO) and specified risk materials (SRM) bans
6. Surveillance of TSE, with particular reference to BSE and scrapie
7. Rendering and feed processing
8. BSE and scrapie related culling

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.

In the context of the geographical risk of BSE in small ruminants, special attention needs to be paid to the genotypes in the sheep population and the possibility of horizontal and maternal transmission of BSE in sheep and goats. No information is currently available but it is noted that study of maternal transmission of experimental BSE in sheep is in progress.

4. On the risk of exposure of man to the BSE-agent through sheep and goats.

Given the existing uncertainties the SSC concluded that whilst BSE has not been identified in sheep and goats under field conditions, the previous or current geographical risks of BSE existence in sheep and goats can not be excluded. The risk

of humans being exposed to the BSE agent originates from animals in the pre-clinical and clinical stage of the disease. It can be reduced by effective measures reducing the exposure risk, in particular safe sourcing, exclusion of the potentially most highly infected tissues (age-specific specified risk materials) from processing, reducing the age at slaughter for human consumption and application of validated processing methods with a proven potential to reduce/eliminate any residual BSE-infectivity.

These measures have been previously described by the SSC which considers that there is no scientific reason for a change in that advice.

The list of SRM should be regularly re-evaluated taking account of the results of ongoing epidemiological surveys on BSE in small ruminants and of new scientific data on BSE infectivity distributions in tissues of small ruminants, infectivity and transmission in small ruminants and whether in particular the lymphoreticular tissue should be considered more infective in sheep than they are in cattle.

In order to update this opinion, the SSC urges that EU Member States and other countries should urgently make available to the European Commission the following data and information:

– evidence (statistics, figures, documents, articles, ...) of the pattern (amounts, level), source and general uses of meat and bone meal (MBM) in sheep and goat herds in different European countries;

– how MBM was used in the '80s when the BSE concern arose;

– how MBM was used from 1988 to 1997;

– where available evidence on MBM use in particular types of small ruminant flocks in different parts of a country.

– information about ongoing research and surveys on the possible contamination of sheep and goats with BSE.

The Scientific Steering Committee notes that the European Commission has proposed a scrapie control scheme aimed at the eventual elimination of scrapie. The SSC endorses these proposals: the SSC recommends that all EU Member States consider a future scrapie control and eradication programme.

The Scientific Steering Committee finally recommends that high priority should be given to the validation of tests for large scale testing for differential diagnosis of BSE and scrapie.

The Scientific Steering Committee is informed that a number of research projects are currently addressing the issue of BSE in sheep, which may require the present opinion to be updated.

Note: The above opinion of the SSC is based on the attached report of the working group of the TSE/BSE ad hoc Group, which was accepted by the TSE/BSE ad-hoc group and then by the SSC, following critical discussion and review.

ATTACHMENT:

REPORT OF THE WORKING GROUP

SUMMARY CONCLUSIONS OF THE WORKING GROUP:

THE QUESTION:

On 16 October 1997, the European Commission requested the Scientific Steering Committee "to establish possible ways of transfer of the BSE agent to sheep and goats, identify the critical factors, assess the risk that the BSE agent exists in sheep and goats and evaluate the exposure of humans to the BSE agent through sheep and goats."

SUMMARY CONCLUSIONS:

On possible ways of transfer of the BSE agent to sheep and goats

The working group considers that the available scientific evidence shows that BSE could be transmitted to sheep and goats by the oral route. Feeding BSE-infected brain tissue to them has proved this. In how far BSE-infected meat-and-bone meal would have the same effect has not been tested but it must be assumed that this is possible.

On the critical factors

The exposure of small ruminants to potentially infected MBM must be seen as the most critical factor for the assessment of the risk that the theoretically possible transmission has taken place. The working group states that information on this issue is incomplete but that it must be assumed that exposure took place. For a complete assessment of the geographical risk with regard to BSE in small ruminants, the working group recommends to collect the information listed in its opinion of 19 February 1998 on the geographical risk for BSE in cattle, amended by information on genotypes in the sheep and goat populations and information on maternal and vertical transmission.

On the risk that the BSE agent exists in sheep and goats

The working group further considers that the risk that small ruminants have been exposed to potentially contaminated MBM is real and hence that BSE could have been introduced into the sheep and goat population. Even if this risk is strongly reduced since the introduction of the MBM feed ban for ruminants, it can, however, not be excluded that BSE is still prevalent in these species. The reason for this lies in the hypothetical resemblance of BSE in sheep with scrapie in sheep. This would indicate a higher likelihood for vertical (mainly maternal) and horizontal transmission of the BSE agent in sheep and goats as compared to cattle. These routes of transmission could be sufficient to propagate BSE in sheep and goats, even after a complete stop of feeding (BSE-contaminated) MBM. The working group is therefore of the opinion that the BSE agent could still exist in some sheep and goat populations in Europe. For assessing this risk, information on the critical factors (see above) would be needed.

On the exposure of humans to the BSE agent through sheep and goats

If BSE exists in small ruminants, the main vector for human exposure would be via the food chain. By excluding the SRM defined in the Commission Decision 97/534/EC of July 1997 this risk will be considerably be reduced. An update of this opinion, in the light of new scientific results is advisable

as well as regular review in the future. The working group is informed that a number of research projects are currently addressing the issue of BSE in sheep.

General points:

The SSC underlines that information on the pattern, source and general uses of meat and bone meal in sheep and goat herds is essential for a detailed assessment of the risk that BSE is prevalent in sheep and goats and of the risk this could pose to humans. Information on the eight points listed in the opinion of the SSC on the assessment of the geographical risk (19/2/98) would also be needed for that risk assessment. Complete information on ongoing research and surveys on the possible contamination of sheep and goats with BSE would also be a precondition for an eventual update or revision of the present opinion.

The working group finally recommends that high priority should be given to the development and validation of tests for large scale testing for the differential diagnosis of BSE and scrapie.

FULL REPORT OF THE WORKING GROUP

I. TERMS OF REFERENCE

The European Commission on 9 December 1997, requested the Scientific Steering Committee to:

1. **Establish possible ways of transfer of the BSE agent to sheep and goats,**
2. **Identify the critical factors involved in the transfer,**
3. **Assess the risk that the BSE agent exists in sheep and goats and,**
4. **Evaluate the exposure of man to the BSE agent through sheep and goats.**

II. SCIENTIFIC CONTEXT OF THE QUESTION.

At the meeting of the TSE/BSE ad hoc group of 19 December 1997, it was decided to set up a working group on the risk of infection of sheep with BSE, following a request from Directorate-General VI of October 1997 that a scientific committee should assess the risk of transmission to man and animals of TSE agents through use of sheep and goat products.

The request was partly based upon an article by Groschup *et al* (1996) in Neurobiology of Disease 3, 191-195 "Detection of Scrapie Agent in the Peripheral Nervous System of a Diseased Sheep". This paper and the finding of BSE infectivity in the dorsal root ganglia of challenged cattle experimentally, orally infected with infected brain material and clinically affected by BSE (Wells *et al.,* 1998) were, however, taken into consideration by the SSC in its December 9th Opinion on SRMs. Groschup *et al* stated that scrapie infectivity was detected in several peripheral nerves in a single, natural scrapie clinically-affected Suffolk sheep with a titre of 1.5 to 3.0 logs lower than that found in the central nervous system (cerebellum). Finding scrapie infectivity in peripheral nerve tissue was not new as this had been described by Hadlow *et al* for sheep in 1979 and in 1982 and for goats in 1980 but the information on the range and extent of peripheral nerve infection was new. Groschup *et al* concluded that "since muscles are traversed by the nerve tracts tested, mutton of scrapie-diseased animals should not be regarded as being free of scrapie agent."

The authors discussed recent findings on the presence of infectivity in the spleen of one experimentally, orally BSE-challenged sheep that developed clinical disease. They concluded that the infectivity of this tissue was not a feature of the BSE-agent in cattle but was in accord with infectivity being detected in the spleen of sheep clinically affected by scrapie. It is therefore possible that a BSE-infected sheep would also harbour BSE in the peripheral nerves. The article ended by saying that further investigations addressing this question were therefore necessary. It should be noted, however, that Stack *et al (1998)* failed to detect scrapie associated fibrils, another marker for the disease-specific form of PrP and probably infectivity, in 6 tested animals out of 6 N. *sciaticus* from Swaledale sheep with clinical scrapie from one flock, though they were found in parts of the intestine, some lymphoreticular tissues and the CNS. This suggests that the pathogenesis and tissue distribution of scrapie agent in sheep may be variable in different breeds as Hadlow *et al* (1979) also remarked. In fact in one Montadale sheep in the clinical phase of disease they found no infectivity outside the CNS. Varying sensitivity and specificity of present day techniques to detect PrP^{Sc} or infectivity may, however, also be taken into account (Cooley *et al,* 1998). According to van Keulen *et al* (1998), PrP^{Sc} can accumulate in the enteric nervous system of the whole gastro-intestinal tract (from oesophagus to rectum inclusive) of sheep affected by scrapie. This should be seen in the light of Gjevre and Toubro (1998) who reported on the anatomy of processed sheep intestines that (for sausages) where ganglion and nerve cells were preserved after processing.

Historical information

Sheep and goats have suffered from scrapie for centuries but the current distribution, prevalence and incidence of scrapie in the world is largely unknown. TSE agents, especially scrapie agents, are known to exist in a number of different strains that are biologically distinguishable. In particular, all historical and contemporary isolates of scrapie agent from sheep or goats and rodent-adapted laboratory strains of scrapie are clearly biologically distinguishable from isolates from cattle with BSE (Bruce *et al*, 1994 and see below). There is no epidemiological relationship between the historical occurrence of scrapie in sheep and the incidence of CJD in humans. This has led to the widely accepted conclusion that the currently known scrapie strains are not capable under natural conditions of producing disease in humans.

Foster *et al* (1993) showed that BSE could be experimentally transmitted to "positive" and "negative" line cheviot sheep (see Annex 1 for an explanation of these lines) and to goats following either i/c or oral challenge with brain material derived from cattle with BSE. That disease was transmitted was demonstrated by the development of a scrapie like clinical disease, presence of spongiform encephalopathy and/or presence of PrP^{Sc} (by immunoblotting).

Bruce (1994) further showed that the agent strain re-isolated from the brain of one i/c inoculated goat and one i/c inoculated negative line sheep was indistinguishable from BSE. Thus sheep and goats were deemed to be susceptible to BSE following experimental challenge with brain from cattle with BSE.

In a further study, Foster *et al* (1996) showed that two line "negative" sheep from the earlier experiment (one challenged by the i/c and the other by the other oral route) both had BSE infectivity in the spleen and brain i.e the strain of agent re-isolated was the same as the challenge agent in each case.

Unpublished data reveals that not all brains from all sheep and goats in the Foster *et al* (1993) study have been subjected to strain typing but some additional data are nevertheless available. In summary BSE agent has not been re-isolated so far from sheep from the positive line flock though a scrapie-like agent has. This can be explained by the fact that natural scrapie occurs in this line of cheviot sheep and could have been responsible.

In regard to the orally BSE-challenged goats, 2 of 3 developed clinical and pathological signs of a TSE. From one so far examined a TSE-agent was isolated but did not resemble BSE. Since natural scrapie has never occurred in the goat herd it is unlikely that scrapie infection is responsible. It is more likely that mutant strains of agent have been selected during the passage between cattle and goats and/or between goats and mice as there is good evidence for this in experimental rodent scrapie.

Further strain typing of isolates from other sheep and goats from the original Foster et al (1993) study and others are in progress and may shed more light on the issue. However, the conclusions from these studies are that :

- Sheep and goats are susceptible to BSE
- Different *PrP* genotypes show different incubation periods (see below and Annex I)
- At least in "negative" line cheviot sheep the spleen and brain are infected in the clinical phase of disease. This suggests that the pathogenesis of BSE in sheep may resemble that of scrapie in sheep and is independent of the route of challenge.
- The agent strain re-isolated from the orally challenged goat, if unlike either BSE agent or known scrapie agents could have a different pathogenicity for humans than the BSE agent (either higher or lower).
- Failure to isolate the BSE agent strain from a small ruminant does not mean that the BSE agent was not responsible for the disease.
- Unless the agent strain isolated from a small ruminant resembles historical scrapie agent strains (from which there is no apparent danger to humans), such strains should be regarded with the same suspicion as the BSE agent until proven to be harmless.

(Note: In the event that BSE occurs in sheep the origin would be most likely from BSE-infected MBM rather than untreated brain. Studies are in progress to examine experimentally whether produced scrapie infected MBM can infect cattle and cause BSE. In the USA, MBM and tallow derived from naturally scrapie-infected sheep has been fed to cattle that are susceptible to US scrapie infection by the i/c route yet no disease has resulted. In another USA study, US scrapie infected brain also failed to produce clinical disease in orally challenged cattle after 7 years.)

Goldmann *et al*, (1994) have shown that dimorphisms especially in codons 136 and 171 of the ovine *PrP* gene correlated with control of disease incidence and modulation of incubation time. However, in the absence of complete knowledge of the different susceptibilities of different genotypes of sheep and goats to the BSE agent it is reasonable to assume that both scrapie "positive" and "negative" lines of Cheviot sheep and perhaps all goats may be susceptible to BSE by the oral route under certain conditions. It is noted that the dimorphism in codon 142 of the caprine *PrP* gene appears to be associated with different incubation periods in goats, experimentally infected with the BSE agent and two types of scrapie agent including the C-type strain source CH 1641 (Goldman *et al*, 1994). Given that the pathogenesis of BSE infected sheep and goats may be similar to that of scrapie then in theory BSE

infected small ruminants may be misdiagnosed as having scrapie. Simple clinical and pathological analyses cannot distinguish between scrapie and BSE at the present time.

It is also unwise to assume that BSE has not affected sheep and goats on the grounds that scrapie incidence rates have not risen in a country. The assessment of the prevalence of scrapie in sheep or goats is very uncertain and seems to fluctuate markedly depending on compensatory payments for affected animals. Surveillance studies for BSE in sheep and goats are rudimentary or non-existent in most countries and restricted by the lack of rapid and accurate tests to differentiate between BSE and scrapie.

Cattle clinically affected with BSE have not been found to have BSE infectivity in the spleen as judged by bio-assay in mice and cattle by i/c inoculation whereas the spleen of scrapie affected sheep has a relatively high titre of the scrapie agent. Neither has PrP^{Sc} been found in the spleen of cattle incubating experimental BSE (after oral challenge). The tissue distribution of BSE in sheep may therefore parallel that of scrapie raising the possibility of a similar range and level of infectivity in lymphoreticular tissues and indeed of a rate of transmission between sheep akin of that in sheep with scrapie. Horizontal transmission of scrapie in sheep is well recognised and contributes substantially to making the disease endemic. Horizontal transmission of BSE in cattle is very unlikely on the basis of current epidemiological analyses. These observations amplify the working group's concern to exclude the presence of BSE in sheep and goats if it occurs and its request for more detailed studies on the tissue distribution and transmission of BSE in small ruminants.

Apparently, only national programs in the UK and France and EC funded projects are investigating the subject of BSE in sheep. These projects are still in progress.

All EU Member States and third country research institutions are therefore invited to provide the SSC with any additional or new information on research projects in the field of BSE in sheep and goats. This will permit the Scientific Steering Committee to monitor the future developments in that field and to possibly adapt the present report and opinion accordingly.

Most of the UK's MAFF and the EC's "BSE in sheep" studies are in the very early stages or about to be initiated. The 4 EC funded and approximately 16 UK's MAFF and 3 French funded studies include the following subjects:

- Assessing the potential presence of the BSE-agent in cases of sheep with clinical TSE disease and by strain typing using brain pools subdivided into two main *PrP* genotypes, one which has succumbed by the oral route to experimental BSE and one which has regularly succumbed to natural scrapie.

- A pathogenesis study of experimental BSE in sheep. This will use sheep of a selected, known, genotype, and the sequential killing of exposed animals with the assay of tissues in mice.

- A study of maternal transmission of experimental BSE in sheep

- The generation of transgenic mice which highly over-express bovine and ovine prion proteins. They may allow the development of improved bio-assays for BSE and scrapie agent detection.

- The improvement of scrapie control in sheep and goats by a study of host genotypes, TSE isolates and their *in vivo* and *in vitro* interaction. A network of sheep PrP geneticists will be created and improved genotyping techniques will be developed.

- PrP^{Sc} distribution and kinetics in lymphoid tissues of sheep with natural scrapie, and their relationship to sheep *PrP* genotype and scrapie strains.

- Setting up multicentric epidemiological databases and biological sample banks for small ruminant scrapie.

- Development of electrophoresis typing techniques on isolates from TSE infected small ruminants.

The working group noted that during the meetings of 29 October 1997 and of 5 December 1997, the SSC Secretariat invited the sheep and goat meat industry and Member States to provide:

– evidence (statistics, figures, documents, articles) of the pattern (amounts, level), source and general uses of meat and bone meal (MBM) in sheep and goat herds in different European countries;

– how MBM was used in the '80s at the onset of the BSE crisis;

– how MBM was used from 1988 to 1997;

– where available evidence on MBM use in particular types of small ruminant flocks in different parts of a country.

– information about ongoing research and surveys on the possible contamination of sheep and goats with BSE.

Information has only been received from 3 countries so far.

III. ASSESSMENT.

The strategy for the evaluation and risk assessment was defined as follows:

1. Assessment of the risk that sheep and goats have been exposed to the BSE agent and identification of the possible routes of exposure;

2. Identification of the critical factors required for evaluation and risk assessment;

3. Assessment of the risk that the BSE agent exists in sheep and goats, is maintained and could eventually spread by propagation systems;

4. The implications for public health, if BSE in sheep cannot be excluded.

III.1. Assessment of the extent of past exposure and risk of future exposure of sheep and goats to BSE.

The oral route has to be considered as the most probable route under natural conditions if BSE were to occur in sheep and goats. Meat-and-bone-meal (MBM) contaminated with BSE of bovine origin would be the most probable dietary source of infectivity. However, the experiment of feeding contaminated meat-and-bone-meal to sheep and goats has never been done, and all the experimental evidence of transmissibility of BSE to sheep and goats relies on the use of highly infectious, non-processed brain material from clinically affected confirmed cases of BSE which should not enter any food or feed chain in the EU. Such an experiment is now being conducted, but will not be completed before 2006.

In the USA, MBM and tallow derived from naturally scrapie-infected sheep has been fed to cattle that are susceptible to US scrapie infection by the i.c. route, yet no disease has resulted. In another USA study, a brain infected with a US scrapie-isolate also failed to produce clinical disease in orally challenged cattle after 7 years. It

should be noted that more then 20 scrapie isolates exist and the US result does not imply that scrapie can never be transmitted to cattle.

Information on the inclusion of MBM into sheep concentrate feed has been provided by MAFF GB. It is concluded that sheep fed concentrate rations were at risk from any TSE infection in mammalian MBM as this was included in some diets until July 1988 when the practice was prohibited in GB. Flocks with different management and husbandry would have been differentially exposed and this could also have depended on the supplier because not all manufacturers used MBM in sheep rations. Some sheep fed with MBM such as early fat lambs for the Easter trade would present a lower risk if slaughtered for meat at a very young age before the agent, if present, had an opportunity to replicate to a detectable level. Unlike dairy calves most sheep would have been exposed at a later stage of the production cycle as, apart from in flocks maintained for milk production, lambs are suckled and usually do not need supplementary feed until after weaning or until ready for service or parturition. As with cattle, a complete guarantee of freedom from exposure *via* feed cannot be given until the feed ban was completely effective *i.e.* from 1 August 1996 in the UK. BSE risks in sheep and goats in other countries cannot be excluded for several reasons. Firstly the feeding practices are not likely to be substantially different from those in the UK. Secondly, rendering practices were historically also similar at least in some parts of some countries. Thirdly MBM exported from the UK that has been blamed for some cases of BSE in cattle in other countries could also have been fed to sheep or have got into their rations by cross-contamination. Finally, considerable numbers of sheep for direct slaughter, some for breeding and carcass meat for consumption could have contributed infected material to the raw material for rendering. If this is true the only other origin could be from indigenous sources of BSE in cattle (or sheep and goats) that were inadequately inactivated by the then used rendering procedures. The date of operating to the new EC rendering standards, the date of effective enforcement of the feed ban and existence of appropriate and effective SBO or SRM bans would be pertinent.

Maternal and horizontal transmission of scrapie are widely regarded as the reason why once introduced the disease usually becomes endemic in a flock, region or country where rigorous means are not adopted to control the disease. Sheep placenta is widely regarded as a source of infection for sheep and goats and this is supported by experimental evidence (Pattison *et al*, 1972, 1974). It is noted that, until recently, all the experimental evidence relies on this one study completed at a time before PrP and its gene had been discovered and that current research in the UK is in progress to determine the contribution that maternal transmission makes to the natural transmission of scrapie. In a paper accepted for publication; (R.Race, 1998, personal communication), PrP^{Sc} was identified in the placenta of scrapie infected sheep and infectivity in the placenta was confirmed.

Parenteral and oral routes of exposure of sheep and goats could be *via* the use of contaminated pharmaceutical or biological products, keeping in mind the accidental spread of scrapie using a louping ill vaccine in the UK in the 1930s (Gordon, 1946; Greig, 1950). However, this is considered to be unlikely because of the stringent controls applied to the sourcing, production and use of biological and pharmaceutical products. There is no epidemiological evidence that clinical TSE in sheep is linked to the use of these products after the start of the BSE epidemic in 1985 (MAFF, 1996).

The spread of natural and experimental scrapie in sheep, directly by contact in buildings or on pasture (telluric origin), is disputed (Pattison and Millson, 1962; Pattison, 1964; Brotherston, 1968; Dickinson, Stamp and Renwick, 1974). Palsson (1979) reported re-establishment of rida, the Icelandic name for scrapie, in sheep three years after complete depopulation and disinfection of infected farms and restocking with rida-free sheep. Interestingly he also reported that new outbreaks of rida had occurred a few years after bringing in calves and hay from rida-endemic areas. Wiesniewski *et al*, (1996) following concentration of hay mites from five Icelandic sheep farms examined samples by immunoblotting for PrP and one sample was positive. Scrapie was transmitted from concentrated suspensions of mites from 3/5 farms. A total of 10/71 mice succumbed. The authors suggested that mites may serve as vectors for scrapie. However, these studies need to be repeated independently before a hypothesis is generated that might be tested experimentally. Transmission *via* nematode worms is also disputed (Fitzsimmons and Pattison, 1968; Hourrigan, 1990). The working group has not enough data to assess the role of mites and nematodes in the epidemiology of scrapie. The view of the working group at present is that, none is likely to be of importance in regard to BSE transmission to sheep and goats either, partly because they are not regarded as significant risks in regard to cattle where there is no species barrier and partly because the research results so far are disputed or unconfirmed by other laboratories.

III.2. Identification of the critical factors.

Given that BSE-contaminated MBM is the most probable source of exposure of small ruminants to the BSE-agent, assessing the use of MBM in a country or region is clearly important. The critical factors to be considered when assessing the incident and propagation risks for BSE in cattle in a geographical area were listed by the Scientific Steering Committee on 23 January 1998. They are:

- Structure and dynamics of the cattle, sheep and goat population
- Animal trade (import of infected animals and internal animal movements)
- Animal feed (including the import and use of MBM)
- MBM bans and their effective implementation (including possible risks of accidental cross-contamination of small ruminant feed with MBM)
- Specified bovine offals (SBO) and specified risk materials (SRM) bans and their effective implementation
- Surveillance of TSE, with particular reference to BSE and scrapie in accordance with the OIE *International Animal Health Code*
- Rendering and feed processing in accordance with Commission Decision 96/449 EC
- BSE and scrapie-related culling if and when these diseases strike

If an assessment of the geographical risk of BSE in small ruminants becomes necessary, special attention needs to be paid to the genotypes in the sheep population and the possibility of horizontal and maternal transmission of BSE in sheep and goats. No information is currently available but it is noted that study of maternal transmission of experimental BSE in sheep is in progress.

It is noted that the feeding practices of sheep and goats, including the periods during the life of the animals when MBM is fed, may be different for sheep and goats and

bovines. Feeding practices will also vary depending on whether the animals are to be used for meat, wool or dairy purposes.

For biological and pharmaceutical products, reference is made to the guidelines of CVMP (Committee on Veterinary Medicinal Products). Special attention must be paid to the safe sourcing of tissues for these products.

III.3. Assessment of the risk that BSE exists in small ruminants, is being maintained and could eventually spread.

III.3.1. Identification of the existence of BSE in small ruminants by the differentiation between BSE and scrapie.

If BSE occurs in sheep, it is likely to be initiated under similar conditions to those which led to BSE in cattle i.e. the feeding to sheep of BSE-contaminated MBM. Thus, assessment of the potential incidence of BSE in sheep will be related to feeding practices involving the use of MBM and the likelihood of this being derived within the country or by import of infected or contaminated material.

The pathological and clinical properties of BSE and scrapie in small ruminants do not allow their differentiation at the present time. Only biological testing for different TSE agents in defined strains of mice can differentiate scrapie strains from BSE with certainty (Bruce *et al*, 1997). However, this methodology is time consuming and expensive and not suitable for field use.

The appearance of a clinical scrapie-like disease in sheep of *PrP* genotypes in which natural scrapie does not frequently occur may prove to be a potential indicator of the occurrence of BSE but this needs further evaluation.

It has to be noted that 3 sheep out of twelve conclusively developed TSE after oral dosing, *i.e.* showed clinical signs and spongiform encephalopathy. So far, from one the BSE agent was re-isolated from brain and spleen (Foster *et al*., 1996). Work is going on to verify the others. Two out of three goats challenged orally with BSE developed clinical signs and pathology of TSE, but the BSE agent was not re-isolated from the one goat tested. However, the strain isolated from this goat did not resemble the agent strain in the inoculum,.

In another study, nine isolates from sheep with apparent natural scrapie in the UK (including two from a possible feed source of infection) have been biologically strain typed. Two did not transmit to any mouse strain and none of the others showed the characteristic incubation period or lesion profile of the BSE agent. Furthermore as with historical isolates of scrapie the strain types isolated were variable. These data, though small in number, nevertheless are of great significance because isolates originated in natural cases of “scrapie” entirely during the BSE era and in a country where there is likely to have been exposure of sheep to the BSE agent *via* feed. Despite this there is no evidence as yet of naturally occurring BSE in sheep in the UK or anywhere else. Furthermore no reports have been made in the literature on molecular strain typing of disease-specific PrP from sheep or goats and which has a profile like that of nv-CJD (type 4). However, the Sub-Group notes that relatively few isolates of contemporary scrapie from one country (GB) have been biologically strain typed. About 400 are currently being verified but they will still be few compared with the total EU sheep and goat population of over 100 millions.

In view of the insufficient information currently available the working group states that:

- new techniques are clearly needed for the rapid differential diagnosis of BSE and scrapie in live animals, especially in the early incubation phase. Molecular strain typing techniques or methods using monoclonal antibodies which can differentiate BSE and scrapie strains, and are applicable to peripheral tissues such as the tonsils (Schreuder *et al*, 1998), the nictitating membrane (O'Rourke *et al*, 1998), placenta (Race, personal communication) or other tissues would be helpful.

- high priority should be given to the validation of tests for large scale testing and the differential diagnosis of BSE and scrapie in sheep and goats.

- more epidemiological work is needed to establish the true prevalence of Scrapie, including information on genotypes.

III.3.2. Maintenance and spreading of the BSE infection in and amongst sheep and goats.

Little is known at present about the carrier-state in natural scrapie or experimental BSE in these species. The possibility that some genotypes of sheep could express earlier and different clinical signs in response to BSE is also unclear and needs to be clarified. Some limited experimental data indicate that certain *PrP* genotypes of sheep and goats influence the incubation period and possibly occurrence of TSE in these species (Goldmann *et al.,* 1996*).*

No data are available, but if BSE occurs naturally in small ruminants, the mechanisms that enable scrapie to spread may be the same for BSE in these species, notably by maternal and horizontal transmission e.g. *via* the placenta (Pattison *et al*, 1972, 1974). It is noted that an experiment to examine the possibility of maternal transmission of experimental BSE in sheep is in progress in the UK.

In the light of the incomplete information available, the working group concludes that it can not be excluded that BSE, once introduced, may be maintained and spread in the sheep and goat population by means of horizontal and vertical transmission.

Because it has clearly been demonstrated that BSE can be orally transmitted to small ruminants, and because it is likely that potentially BSE-contaminated MBM has been fed to sheep and goats, the working group has to assume that BSE could have been introduced into the European sheep and goat population. If this has been the case it can not be excluded that it continues to exist, even after an effective implementation of a ruminant feed ban.

III.4 Public health and the risk from BSE in sheep and goats.

Should BSE be found to occur in sheep and/or goats under field then the risk to humans would come from:

- eating potentially infected food.

- using pharmaceutical products prepared from potentially infected sheep or goat tissue. or where such tissues were used during their manufacture.

- handling infected tissues, such as placenta, brain, and spinal-cord, by sheep farmers, veterinarians, abattoir workers, and allied workers including those in the pharmaceutical industry processing these tissues.

The risk of being exposed to the BSE agent originates from animals in the pre-clinical and clinical stage of the disease and perhaps from silent carriers (Race and Chesebro, 1998). It can be reduced by effective measures reducing the exposure risk, in particular safe sourcing, exclusion of the potentially most highly infected tissues (age-specific specified risk materials) from processing, reducing the age at slaughter for human consumption and application of validated processing methods with a proven potential to reduce/eliminate any residual BSE-infectivity.

In addition to these measures, which would have an immediate impact on any potential risk, the enforcement of MBM bans for small ruminants and of the rendering procedures used to process mammalian waste as specified in the SSC opinion of 26-27 March 1998 on the safety of meat and bone meal for non-ruminant food-producing animals and its pending opinions on various other uses of MBM, will also contribute to the overall risk reduction strategy.

These measures should be accompanied by measures to control horizontal and vertical transmission and maintenance of the disease in the sheep and goat population.

The development of rapid tests, able to differentiate between Scrapie and BSE in live small ruminants, would assist in achieving effective control.

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ANNEX 1

Experimental BSE in sheep and goats

Foster *et al,* (1993), successfully produced spongiform encephalopathy (SE) in positive and negative line Cheviot sheep and Anglo-Nubian goats. Negative line Cheviot sheep do not succumb to experimental scrapie following s/c challenge with SSBP 1 scrapie whereas positive line sheep do. It is now known that the positive and

negative line sheep have distinctive and different *PrP* genotypes that explains their response to experimental and natural infection with scrapie. (The positive and negative line nomenclature is used here as various relevant publications refer to this description. However, for a full explanation of the relationships see Hunter 1997). Following i/c inoculation with bovine brain derived from cattle confirmed to have BSE, 3 definite out of 5 positive line sheep and 5/6 negative line sheep developed SE. Following oral dosing 2 definite out of 6 positive line and 1/6 negative line sheep developed SE. In regard to goats 3/3 animals challenged i/c and 2 challenged by the oral route developed SE. BSE agent was re-isolated from the brain of each of one i/c and one orally-challenged negative line sheep (Foster *et al*, 1996) and one goat (Bruce *et al* 1994). The *PrP* genotypes of these sheep are now known (see below) and conform to the pattern of genotypes in Cheviot and some other sheep breeds in the UK in relation to the occurrence of natural scrapie (Hunter, 1997).

Pathogenesis of BSE in cattle and sheep

Naturally, clinically affected cattle with BSE have not been found to have BSE infectivity in the spleen following bioassay in mice nor so far, 67 months post i/c challenge in cattle (Dr A Nolan, MAFF GB, personal communication). Infectivity has not been found in the spleen during the course of incubation of experimental, orally induced, BSE (GAH Wells *et al*, 1996). By contrast, sheep and goats consistently show infectivity titres during the incubation period in natural and experimental scrapie (Hadlow *et al*, 1979, 1980 and 1982; Stamp *et al*, 1959, Pattison and Millson, 1960 and Hadlow *et al*, 1974).

Pathogenesis of BSE in sheep

Foster *et al (1996)* discussed recent findings on the presence of infectivity in the spleen of one experimentally, orally BSE-challenged sheep and one i/c-challenged sheep that developed clinical disease. They concluded that replication and accumulation of the infectious agent occurs in the brain and spleen, irrespective of the route of challenge. The presence of BSE-infectivity in the spleen shows that the pathogenesis of BSE in sheep resembles more closely that of natural and experimental scrapie in sheep than of BSE in cattle. Groschup *et al* (1996) concur and state it is therefore possible that BSE-infected sheep would harbour BSE-infectivity in the peripheral nerves. They concluded by saying that further investigations addressing this question were therefore necessary.

***PrP* genotypes of sheep and goats in relation to scrapie and BSE**

Relevant data are given in the publications of Goldmann *et al*, (1994, 1996); Hunter (1996) and Dawson *et al*, (1998). The sheep *PrP* gene codes for a protein of 256 amino acids *via* three DNA bases = 1 codon. In regard to scrapie risk, 5 variant alleles specified by polymorphisms in the amino acids encoded by codons 136, 154 and 171 have been identified (Dawson *et al* 1998). The 5 variant alleles are: ARQ, ARR, VRQ, AHQ and ARH. The first letter determines the amino acid encoded by codon 136, the second by 154 and the third by 171. Genotypes can be homozygous or heterozygous. There is therefore scope for considerable genetic variation between sheep. In practice the amount of variation depends on breed in the range from 3 (*e.g.* in Suffolks) to 15 (*e.g.* in Texels). It is proposed by Dawson *et al* 1998, that allele pairing is described for a particular sheep as two sets of three letters *e.g.* ARR/AHQ that corresponds with the pairing of amino acids for each codon (136, 154 and 171)

i.e., AA/RH/RQ. There is currently no evidence that the dimorphism at codon 154 has an influence on BSE susceptibility.

Experimental oral challenge of six Cheviot, negative line sheep with brain from confirmed cases of BSE resulted in one case of clinical disease to date, occurrence of spongiform encephalopathy and recovery of the BSE agent strain from spleen and brain. The *PrP* genotype was AHQ/ARQ. Similar challenge of six positive line sheep resulted in definite clinical signs and occurrence of spongiform encephalopathy in two, from each of which agents were isolated that were atypical of the BSE agent and probably indicated infection with natural scrapie. The *PrP* genotypes were VRQ/VRQ and ARQ/VRQ.

Experimental i/c challenge of six Cheviot, negative line sheep with brain from confirmed cases of BSE resulted in five definite transmissions with clinical signs and pathology of a spongiform encephalopathy. *PrP* genotypes of these animals were ARQ/AHQ (n=1), AHQ/AHQ (n=1) and ARQ/ARR (n=3) (N.Hunter, personal communication). The incubation periods were substantially longer in the three ARQ/ARR sheep than the other two, indicating the important influence of homozygosity at codon 171. The BSE agent was recovered unchanged from the brain and spleen of the clinically affected AHQ/AHQ sheep and from the brain of the former (isolation from spleen was not attempted). Similar challenge of five positive line sheep resulted in three definite clinical cases with pathology of a spongiform encephalopathy in two. The incubation periods were substantially shorter in the two sheep with *PrP* genotypes VRQ/ARQ than in the single affected sheep with a VRQ/ARR genotype. Inoculation of mice with brain tissue from the former clinically affected sheep did not result in transmission. *Note: general data are from Foster et al, (1996) and Goldmann et al (1994). Other genetic data are from Dr N Hunter by personal communication.*

In regard to the experimental transmission of BSE to Anglo-Nubian goats, three challenged i/c all succumbed to clinical disease and spongiform encephalopathy and the BSE agent was re-isolated from 1/1 tested. Two out of three challenged orally similarly succumbed clinically and developed spongiform encephalopathy. No transmission to mice resulted from the one goat brain tested. (Goldmann *et al*, (1996). *Other data courtesy of Mr J D Foster, Dr M Bruce and Dr N Hunter).*

In regard to the occurrence of experimental BSE in challenged positive line sheep, this is difficult to interpret since natural scrapie occurs in this line of sheep but is non-existent in the goat flock. Thus the occurrence of a clinical neurological disease in positive line sheep challenged by either route with BSE and in goats challenged orally could be interpreted in various ways:

- The sheep were already infected with natural scrapie (goats in this herd have never developed scrapie);
- The challenged animals selected mutant strains in the inoculum that were not the BSE agent;
- The animals were infected with a scrapie-like strain and the BSE strain but the former was predominant and was selected preferentially by the mice or for some unknown reason it not transmit.
- The BSE agent strain may have changed in character by passage through V136 *PrP* genotype sheep and by passage through goats such that it no longer produced BSE agent pattern on subsequent transmission to mice.

All of these possibilities present an unknown hazard. Sheep might be infected with the BSE and the scrapie agent at the same time. Sheep could be infected by a hitherto unknown strain of a "scrapie-like" agent with an unknown pathogenicity for humans.

It is known that the C-type source (a brain pool) of scrapie known as CH 1641 can cause clinical and pathological scrapie in negative line Cheviot sheep when challenged by the i/c route and that the incubation period is longer in these than in positive line sheep. Clinical disease does not occur or is very rare when the experimental pool of A-type strains, SSBP 1, is used and administered by the sub-cutaneous route, though some sheep may succumb after a long incubation if challenged by the i/c route.. Natural scrapie (presumably of A-type strains) has never been recorded in the negative line flock in over 25 years of scrapie research. It is therefore possible, perhaps probable that scrapie and BSE can occur in sheep of the same *PrP* genotype. They might also occur together. Furthermore mutant scrapie strains completely unconnected with the BSE strain could be selected and propagated at any time. Their pathogenicity for man would be completely unknown. It is noted however that in over 250 years until the advent of BSE, this has not happened.

ANNEX 2: NATURAL SCRAPIE IN SHEEP AND GOATS

Classification of tissues by agent titre in Swiss mice and by age, in pre-clinical and clinical cases of Scrapie in Suffolk sheep and in goats [2]

Group	Infectivity Titre (approx. range)	PRE-CLINICAL				CLINICAL	
		SHEEP				SHEEP	GOATS
		≤8 months. (0/16)	10-14 months (8/15) [3]	25 months (1/13)	> 25 months (1/6)	34-57 months (9/9)	38-49 months(3/3)
A	**HIGH [3] 4.0**					Brain Spinal cord	Brain Spinal cord
B	**MEDIUM 3.2 – 4.0**		Colon-proximal, Ileum-distal, LN (RP/MP), Spleen	Colon-proximal, Ileum-distal, LN (RP/MP), Tonsil		Colon-proximal, Ileum-distal, Spleen, Tonsil LN (BM), LN (PF, 1/9 negative), LN (PS, 2/9 negative), LN (PR/MP), (rectum distal+),	Colon-proximal, Ileum-proximal, LN (BM), LN (RP/MP), LN (s.mammary), Pituitary, (Rectum-distal +), Spleen
C	**LOW £ 3.2 or titre unknown**		LN (PS/PF) Tonsil	Brain (medulla/ diencephalon), LN (BM), LN (PS/PF), Spleen		Adrenal, Bone marrow**, Colon-distal, CSF, Liver**, LN (s.mammary x2), Nasal mucosa, Pancreas **, Pituitary, Sciatic nerve, Thymus **,Placenta ** [3]	Adrenal, Colon-distal, CSF Nasal mucosa, Sciatic nerve, Thymus
D	**Undetec-table**	Ileum, LN (PS/PF) LN (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, Fetus, Heart, Kidney, Lung, Mammary gland, Muscle-skeletal, Ovary, Saliva, Salivary gland, Sem. Vesicle, Testis, Thyroid, Uterus	Blood clot, Bone marrow, Faeces, Kidney, Mammary gland, Milk, Muscle-skeletal, Ovary, Salivary gland, Serum (see report), Uterus

(-/-) (Number positive / number examined)
* = Log_{10} mouse intracerebral LD/50 per 30 mg tissues
\+ = Not assayed but high content of lymphoreticular tissue
° = negative in other studies
** = trace or exceptional
PF = Prefemoral
PS = Prescapular

[2] After Hadlow et al. (1979, 1980, 1982), Pattison *et al.* (1964, 1972), Groschup et al. (1996). Regarding DRG: see text.

[3] Techniques for the determination of infectivity become more and more sensitive. The age range may go below 10 months. In individual cases, tonsil infectivity has been detected in lambs of 16 weeks. Placenta has been placed in Group C, but titres are unknown.

RP = Retropharyngeal
MP = Mesenteric/portal
CSF = Cerebro-spinalfluid
LN = Lymph node
BM = Bronchomediastinal