REPORT OF THE MEETING OF THE
OIE AD HOC GROUP ON BOVINE SPONGIFORM ENCEPHALOPATHY
(With the participation of the World Health Organization)
Paris, 2-3 May 1996

The OIE Ad hoc Group on Bovine Spongiform Encephalopathy (BSE) met at the Office International des Epizooties (OIE) headquarters on 2-3 May 1996. A World Health Organization (WHO) representative participated in the meeting.

The purpose of holding the meeting was:

1. To review the recent developments in BSE incidence, epidemiology and research,
2. To review the new United Kingdom (UK) and European Union (EU) regulations on BSE,
3. To review the new WHO recommendations on BSE,
4. To evaluate the evidence of risks posed by BSE to human health,
5. To evaluate the *International Animal Health Code* chapter and supporting document regarding BSE,
6. To produce conclusions and recommendations.

The OIE Ad hoc Group on BSE had last met on 1-2 September 1994, and the resulting chapter on BSE of the *International Animal Health Code* was adopted at the 63rd General Session of the OIE (1995). A WHO Consultation on public health issues related to animal and human spongiform encephalopathies was last held on 2-3 April 1996.

Participants in the meeting are listed in Appendix I. The Agenda adopted is given in Appendix II.

Dr W.H.G. Rees chaired the meeting and Dr W.G. Sterritt was the co-chairman.

1. **Opening**

Participants were welcomed by Dr J. Blancou, Director General of the OIE, who outlined the background and purpose of the meeting.
2. Review of new developments in bovine spongiform encephalopathy incidence, epidemiology and research

Representatives of countries in which BSE has been diagnosed to date each briefly reviewed current data on the occurrence of the disease in an opening session on BSE epidemiology. Basic statistics are presented in Appendix III.

Cattle exported from the UK have succumbed to BSE in: Canada, Denmark, Falkland Islands, Germany, Ireland, Sultanate of Oman, Italy and Portugal. All of the affected animals appear to have been exposed to infected animal protein products prior to leaving the UK. No secondary spread from these imported cases has been documented.

Four countries other than the UK (France, Ireland, Portugal and Switzerland) have experienced BSE cases in native cattle. Veterinary controls have been instituted in all of these countries. Epidemiological investigations regarding affected native cattle showed potential exposure to rendered animal protein products in most of the BSE cases. Cases born after the date of introduction of the ruminant feed ban (BAB cases) have been reported in the UK (>26,000), Switzerland (9) and Ireland (3).

No other country has experienced BSE with the incidence observed in the UK. Cases not apparently linked to feed exposure include some BAB cases in Switzerland, which introduced a ruminant feed ban in December 1990, suggesting the possibility of alternative routes of transmission, albeit infrequently. Imported meat and bone meal was probably the source for most cases of BSE. In Switzerland, the specifications of the raw products for producing meat-and-bone meal (MBM) have been modified by prohibiting the inclusion of skulls and spinal cords from adult cattle. Only MBM complying with this standard can be imported.

Two different epidemic patterns were noted – one increasing to a peak and then declining in response to control measures – and the other exhibiting a steady incidence over several years (see table 1 in the supporting document). The decline of the epidemic in the UK was continuing as reported previously. As the average age of cattle with BSE increased the positive confirmation rate decreased as expected from about 85% to 80%.

In regard to the BAB cases in the UK, which now totalled over 26,000, the major source of infection appeared to be leakage in the specified bovine offals (SBO) ban as it related to animal feeds and cross contamination of ruminant diets by feeds prepared for pigs and poultry. The rules had been progressively tightened and the most recent legislation (April 1996) prohibited the use of mammalian protein for feeding any species of farm animal, horses or fish.

There was no evidence of maternal transmission of BSE in any country. No infectivity had been found by bioassay in mice in semen or in embryos or the uterine flushings used to collect them from confirmed cases of BSE using International Embryo Transfer Society (IETS) protocols. Neither had BSE transmitted to recipient cows or to offspring up to 4 years old derived from these embryos transferred using the IETS protocols. The results to date of embryo transfer experiments in sheep in the UK and United States of America (USA) were noted.

It was reported that Ireland and the UK had identified a small number of cases of BSE in imported animals from continental Europe which had occurred such a short time after importation that exposure was almost certainly in the country of origin.

Different views were expressed on the usefulness of entire herd slaughter when a case of BSE was diagnosed.
3. **Review of the new United Kingdom and European Union regulations on bovine spongiform encephalopathy**

The new European Union (EU) regulations were described. Currently, there was a temporary restriction on exports of cattle and cattle products (except milk, hides and skins) from the UK. However, previous European Commission (EC) decisions controlling these items were still in place. France and Ireland reported the introduction of an SBO ban and Switzerland and the UK reported adjustments to their existing SBO bans.

The Council of Agriculture Ministers of the EU concluded on 27 March 1996 that the new levels of protection against BSE within the EU should include processing of all waste animal protein at a temperature of at least 133°C, at a pressure of 3 bar for 20 minutes or another process which gives equivalent guarantees.

The EC Scientific Veterinary Committee had concluded on 26 April 1996 that bovine semen was safe and that gelatin and tallow from bovine sources were also safe for consumption and other purposes, provided that certain conditions of manufacture were achieved. These conclusions would be given to the EC Standing Veterinary Committee for their approval.

4. **Review of the new World Health Organization recommendations on bovine spongiform encephalopathy**

The WHO report of 2-3 April 1996 was discussed. There was some discussion on the meaning and interpretation of recommendation No. 3, section 2.2, which reads as follows:

"Countries should not permit tissues that are likely to contain the BSE agent to enter any food chain (human or animal)."

The WHO Representative explained that this should be interpreted in light of:

a) the BSE status (e.g. presence or absence of the disease or unknown status) and BSE risk factors prevailing in the country (e.g. presence or absence of scrapie in sheep, use of MBM in ruminant feed, etc.)

b) the nature of the BSE control and/or prevention measures, if any, which have been taken, and

c) according to whether MBM, live animals and animal products had been imported from BSE infected countries in the past.

In all countries where there is an absence or inadequacy of BSE surveillance and monitoring, an ascertainment of the BSE status and of BSE risk factors should be carried out to help decision-making.

When appropriate, a country list of 'tissues most likely to contain infectivity' mentioned in the above report should be determined by taking account of the above as well as the following information and the level of reassurance thought to fit the country situation:

- in naturally infected cattle, detectable BSE infectivity has only been found in central nervous system (CNS) tissues;

- the list of SBOs given in the WHO report dated May 1995 (e.g. brain, spinal cord, tonsil, thymus, spleen and intestines - from duodenum to rectum - from animals aged more than six months);

- the extended list of 'specified bovine materials' adopted recently in the UK adding to the above SBO list some tissues from cattle aged less than six months (e.g. thymus and intestines) and some organs or parts of the carcass of cattle aged more than six months (e.g. whole head and vertebral column).
It was recommended that the OIE develop a model of minimum standards for surveillance of BSE. It was noted that there will be difficulty in implementing some of the WHO recommendations without further information.
5. Evaluation of the risks posed by bovine spongiform encephalopathy to human health

The current situation in regard to Creutzfeldt-Jakob disease (CJD) and in particular the new variant form (V-CJD) was reported. There were now 11 confirmed cases of the V-CJD in the UK and 1 in France. A direct link between BSE and CJD has not been demonstrated, though BSE may be a possible explanation for this variant. Several lines of enquiry were being followed, including a request for neurologists in the UK to refer all suspect cases to the CJD Surveillance Unit. There was no excess number of referrals to date. Transmission studies in transgenic mice were underway and transmission studies in conventional mice (for strain typing) were imminent. If the variant is caused by BSE, it is probable that patients in the UK had been exposed to SBO in food before a ban had been put in place (November 1989). At present, definitive information about the length of the incubation period (assuming the disease to be transmissible) was lacking, making it difficult to predict future events.

CJD surveillance worldwide gained new importance and was in progress in Australia, Canada, New Zealand, South Africa and the USA as well as in several European countries. No prediction could be made about the future number of cases of V-CJD.

The pathogenesis of BSE in cattle was discussed and at present it seemed to follow the pattern of transmissible mink encephalopathy (TME) in mink, and not that of scrapie, with minimal evidence for replication within the lymphoreticular system during the early and middle part of the incubation period.

The risk to man (if the BSE agent was a human pathogen) would mostly likely occur from exposure to high titre CNS tissues. The transgenic mouse study suggested that the cattle to human species barrier was greater than the cattle to mouse species barrier. Furthermore, the comparative bioassay of cattle tissues in mice and cattle suggested the infectivity in non-central nervous tissues (including muscle) would be \( >100,000 (10^5) \) less than that of brain, making it clear that if exposure to CNS tissues was avoided the risks would be very low.


Sub-groups were appointed to review the two documents taking account of the days' discussions and to suggest amendments, if necessary. These were further discussed by the participants. The resulting proposed amended version of the International Animal Health Code chapter on BSE is in Appendix IV. Double underlining the text indicates additions or modifications and small type in brackets indicates deletions. The amended supporting document is in Appendix V.

7. Conclusions and recommendations

The epidemic of BSE in the UK continues to decline in the manner expected. The large number (>26,000) of BAB cases in the UK is attributed to leakage of the bans and in particular to cross contamination of ruminant feed with diets containing ruminant protein included in pig and poultry diets.

There is no evidence for maternal or horizontal transmission, although they cannot be totally excluded as rare events.

Trading in semen, embryos, milk, gelatin or tallow from bovine animals from any country is regarded as safe provided the recommendations in the Code have been adopted.

Since 29 March 1996, the UK has prohibited the feeding of mammalian protein to all farmed animals, including horses, poultry and fish, and its use as agricultural fertiliser. There should, therefore, be no risk of infection from feed after 30 April 1996.
In the opinion of the Group all countries should conduct surveillance and monitoring for BSE. This should include examination of cattle with clinical neurological signs followed by histopathological examination of the brain as described in the *Manual*.

The cases of V-CJD are a matter for concern and continued research is strongly supported in order to determine the origin of the disease and the mode of transmission, if any. WHO through its regional offices is strengthening surveillance of V-CJD in all its Member States, especially outside Europe.

If BSE causes V-CJD the most likely source of infection would be from infected central nervous system tissue. In countries at risk from BSE care should be taken when dressing cattle carcasses to avoid cross contamination of parts of the carcass used for consumption with any of these tissues.
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Agenda

1. Opening
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4. Review of the new World Health Organization recommendations on bovine spongiform encephalopathy
5. Evaluation of the risks posed by bovine spongiform encephalopathy to human health
7. Conclusions and recommendations
### NUMBER OF REPORTED CASES OF BSE WORLDWIDE

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**Note:**
- For the years before 1995:
  - Great Britain, Northern Ireland and Jersey: figures by date of confirmation; Guernsey: figures by date of slaughter; Isle of Man: figures by date of service of restriction notice on suspected case.
- For 1995 - 1996:
  - United Kingdom: figures by date of service of restriction order.

(a) Data for Ireland: as of 30 April 1996
(b) Data for France: as of 30 April 1996
(b) Data for Portugal: as of 29 April 1996
(b) Data for Switzerland: as of 19 April 1996
(b) Data for United Kingdom: as of 31 March 1996.

**In addition, the following countries have reported cases only in imported animals (date of initial detection)**

- Germany: 4 cases (02/92, 02/94, 04/94, 05/94)
- Canada: 1 case (11/93)
- Denmark: 1 case (07/92)
- Falkland Islands: 1 case (1989)
- Italy: 2 cases (10/94)
- Oman: 2 cases confirmed in 1989
Appendix I (contd)
SECTION 1.1.

DEFINITIONS

For the purposes of this Code:

*Meat-and-bone meal*

... means the protein product obtained when waste animal tissues are treated by heat (rendered), and includes any intermediate protein product.

CHAPTER 3.2.13.

**BOVINE SPONGIFORM ENCEPHALOPATHY**

(BSE)

Article 3.2.13.1.

Bovine spongiform encephalopathy (BSE) is a nervous disease of adult cattle. There is no evidence that the disease is contagious. It is an individual animal disease, not a herd disease. BSE has a long *incubation period* measured in years, and arose from feeding contaminated ruminant protein.

The BSE status of a country can only be determined by continuous surveillance and monitoring. The minimum requirements for effective surveillance are:

1) compulsory notification and clinical investigation of suspect cases;

2) laboratory examination of brain material from clinically suspect animals which are slaughtered or which die, in accordance with the diagnostic techniques set out in the *Manual (B83)*;

3) registration of suspected and confirmed cases.

Each confirmed case should be registered and reported as a separate outbreak.

In the absence of surveillance data, the status of a country must be considered as unknown.

The following Articles recommend conditions under which cattle and bovine products can with safety be traded for human and animal consumption, and other uses.

Article 3.2.13.2.

*Veterinary Administrations* can authorise without restriction the import or transit through their territory, directly or indirectly, of *semen*, milk, milk products, hides and skins originating from healthy animals from countries
where BSE has been reported, and by-products, such as gelatin, collagen and tallow, produced by processes which inactivate any residual BSE infectivity.
Appendix IV (contd)

Article 3.2.13.3.

The following Articles do not apply to countries in which all cases of BSE have been clearly demonstrated to originate directly from the importation of live cattle or embryos from countries where BSE has been reported, provided that the disease is made notifiable and clinically suspected animals are slaughtered, investigated and, if disease is confirmed, completely destroyed.

Article 3.2.13.4.

When importing from countries with a low incidence of BSE, or of unknown status, Veterinary Administrations should require:

for cattle

the presentation of an international animal health certificate attesting that:

1) the disease is compulsorily notifiable;

2) affected cattle are slaughtered and completely destroyed;

3) suspect heifers or cows close to calving are isolated;

4) the feeding of meat-and-bone meal [protein product] derived from ruminants [over six months of age to cattle] to ruminants has been banned;

   [The tissues referred to above are: brain, spinal cord, thymus, tonsils, spleen and intestine (from duodenum to rectum);]

5) cattle selected for export:

   a) are identified by a permanent mark enabling them to be traced back to the dam and herd of origin;

   b) are not the first generation progeny of BSE suspect or confirmed females.

Article 3.2.13.5.

When importing from countries with a high incidence of BSE, Veterinary Administrations should require:

for cattle

the presentation of an international animal health certificate attesting, in addition to the requirements set forth in Article 3.2.13.4., that animals for export:

[were born at least three years after the introduction of the ban referred to in paragraph 4) of that Article, unless, on documentary evidence, it can be certified that the animals had at no time in their life received proteins from ruminants (other then milk or milk products) in feed.]

1) either were born after the date on which an effective ban on the use of ruminant meat-and-bone meal in feed for ruminants has been applied; or

2) were born, raised and had remained in a herd in which no case of BSE had ever been confirmed, and which contains only cattle born on the farm or coming from a herd of equal status.
Appendix IV (contd)

Article 3.2.13.[6]

When importing from countries with a low incidence of BSE, or of unknown status, Veterinary Administrations should require:

_for fresh meat (bone-in or deboned) and meat products from cattle_

the presentation of an _international sanitary certificate_ attesting that:

1) the disease is compulsorily notifiable;

2) _ante mortem_ inspection is carried out on all bovines over 18 months of age;

3) affected cattle are slaughtered and completely destroyed;

4) the meat products do not contain brain, eyes or spinal cord from cattle born before the date on which the feed ban referred to in paragraph 4) of Article 3.2.13.4, was introduced.

Article 3.2.13.[8]

When importing from countries with a high incidence of BSE, Veterinary Administrations should require:

_for fresh bone-in meat [(bone-in or deboned) and meat products] from cattle, excluding tissues listed in Article 3.2.13.1,._

the presentation of an _international sanitary certificate_ attesting that:

1) the disease is compulsorily notifiable;

2) _ante mortem_ inspection is carried out on all bovines over 18 months of age;

3) the tissues listed in Article 3.2.13.12, are removed from all cattle at slaughter and destroyed;

4) [3][4] nervous and lymphatic tissues exposed during the cutting process have been removed from carcasses of cattle born before, or within three years after the introduction of the ban referred to in paragraph 4) of Article 3.2.13.4.

5) affected cattle are slaughtered and completely destroyed;

5) the cattle from which the meat originates:

   a) were less than 30 months old at the time of slaughter; or

   b) were born after the date on which an effective ban on the use of ruminant meat-and-bone meal in feed for ruminants has been applied; or

   c) were born and had only been kept in herds in which no _case_ of BSE had been recorded.

Article 3.2.13.8

When importing from countries with a high incidence of BSE, Veterinary Administrations should require:

_for fresh deboned meat and meat products from cattle_
the presentation of an *international sanitary certificate* attesting that the conditions in Article 3.2.13.7. apply or alternatively that:

1) the disease is compulsorily notifiable;

2) *ante mortem* inspection is carried out on all bovines over 18 months of age;

3) the tissues listed in Article 3.2.13.12. are removed from all cattle at slaughter and destroyed;

4) affected cattle are slaughtered and completely destroyed;

5) nervous and lymphatic tissues exposed during the cutting process have been removed and destroyed.

Article 3.2.13.[6].9

When importing from countries with a low incidence of BSE, or of unknown status, *Veterinary Administrations* should require:

for bovine *embryos/ova*

the presentation of an *international animal health certificate* attesting that:

1) the disease is compulsorily notifiable;

2) affected cattle are slaughtered and completely destroyed;

3) suspect heifers or cows close to calving are isolated;

4) embryos/ova for export are derived from females which:
   a) are not affected with BSE;
   b) are not the daughters of BSE affected females; and
   c) were not suspected of being so affected at the time of embryo collection.

Article 3.2.13.[9].10

When importing from countries with a high incidence of BSE, *Veterinary Administrations* should require:

for bovine *embryos/ova*

the presentation of an *international animal health certificate* attesting, in addition to the requirements set forth in Article 3.2.13.[6].9, that:

[1] the feeding of protein products derived from tissues listed in Article 3.2.13.4. originating from ruminants over six months of age to cattle has been banned;

1) the feeding of *meat-and-bone meal* derived from ruminants to ruminants has been banned;

2) embryos/ova for export are derived from females which were born after the introduction of the ban referred to in paragraph 1) above.
Article 3.2.13.[10][11]

Meat-and-bone-meal containing any ruminant protein which originates from countries with a high or low incidence of BSE, or of unknown status, should not be traded between countries for use in ruminant feed.

For use in other species, meat and bone-meal should have been processed in plants which are approved by the Veterinary Administration following validation that each plant can achieve the parameters judged effective for that type of process for inactivation of transmissible spongiform encephalopathy agents.

Article 3.2.13.[11][12]

Bovine brains, eyes, spinal cord, tonsils, thymus, spleen and intestines, [tissues listed in Article 3.2.13.4.] and protein products derived from them from cattle over six months of age originating from countries with a high incidence of BSE should not be traded between countries for use in human food or animal feed.

Article 3.2.13.[12][13]

Careful selection of source materials is the best way to ensure maximum safety of ingredients or reagents of bovine origin used in the manufacture of medicinal products.

Countries wishing to import bovine materials for such purposes should therefore consider the following factors:

1) the BSE status of the country and herd(s) where the animals have been kept, as determined under the provisions of Article 3.2.13.1.;

2) the age of the donor animals;

3) the tissues required and whether or not they will be pooled samples or derived from a single animal.

Additional factors may be considered in assessing the risk from BSE, i.e.:

1) precautions to avoid contamination during collection of tissues;

2) the process to which the material will be subjected during manufacture;

3) the amount of material to be administered;

4) the route of administration.
Appendix V

SUPPORTING DOCUMENT
FOR THE OIE INTERNATIONAL HEALTH CODE CHAPTER 3.2.13.
ON BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)
(Updated May 1996)

Introduction

1. An ad hoc group of experts was convened to consider the implications of BSE and produced a report (59 SG/13/CS 4B) in September 1990 which was adopted by the 59th General Session. The 59th General Session resolved in addition that a draft chapter on BSE should be prepared for the OIE International Animal Health Code.

2. A Supporting Document was prepared in 1991 to take account of developments since 59 SG/13/CS 4B was prepared, and to provide supporting information relevant to the BSE chapter (3.2.13) of the OIE International Animal Health Code, which was adopted by the 60th General Session in May 1992. The Ad hoc Group again reviewed chapter 3.2.13 on 1-2 September 1994, and asked that the Supporting Document be updated. This updated document supported the amendment of chapter 3.2.13 adopted by the 63rd General Session in May 1995.

The following is a further update of the Supporting Document by the Ad hoc Group on BSE which met in 2-3 May 1996 based on new scientific information since the last revision in December 1994. It is prepared for a proposed further amendment of Code Chapter 3.2.13.

Development of the epidemic

3. BSE was first recognised in Great Britain in November 1986 (71), although evidence collected later indicated that the first clinical cases had occurred in April 1985 (79). Initial epidemiological studies indicated an extended common source epidemic in which all affected animals were index cases (79). Epidemiological studies considered a wide range of possible causative factors which might provide a common link between cases (use of therapeutic and agricultural chemicals, and biological products; introduction via imported animals; semen or animal products; transmission of the agent directly or indirectly on farms which also had sheep). The results of these studies, together with the disease's similarity with scrapie, identified the exposure of cattle to the agent of a transmissible spongiform encephalopathy (TSE) through feed containing ruminant-derived protein in the form of meat-and-bone meal as the likely source of the disease (76,79). The role of meat-and-bone meal is strongly supported by the results of a case-control study (77) and the decline in the incidence of the epidemic in Great Britain (GB) following the introduction of the ban on feeding ruminant protein to ruminant animals in July 1988 (40,51). The ability of the BSE agent to survive certain commercial rendering processes has also been demonstrated (22,69) and this also supports the meat-and-bone meal hypothesis. Two possible hypotheses as to the origin of this agent were consistent with the epidemiological findings: either that it was an increase in exposure of cattle to the agent of scrapie itself, or that it was an increase in exposure of cattle to a cattle-adapted strain of scrapie agent (76).

4. Computer simulations indicate that exposure to the agent sufficient to give rise to clinical disease began abruptly in 1981/1982 (76,79). The key factor appears to be timing of certain changes in rendering practice in the United Kingdom (UK) which were coincident with this increase in exposure, although other risk factors are also clearly relevant, notably the relationship between numbers of cattle and sheep (on which will depend the proportion of meat-and-bone meal represented by sheep material) and the prevalence of scrapie (76,79).
5. Epidemiological studies indicate that the majority of BSE infected animals have been exposed in calfhood (79). It is predominantly animals in dairy herds that are affected (79). At 29 March 1996, 59.3% of dairy herds and 15.3% of beef suckler herds in GB had had one or more cases of BSE. Over 80% of the cases in beef suckler herds occurred in cows that originated from (and, presumably, became infected in) dairy herds. The age of peak incidence in the absence of control measures is four to five years. In 1988 it was reported that the incubation period varies from 2.5 to at least eight years (79) and possibly for the lifespan of cattle (10). The youngest case recorded to December 1994 was a single animal 20 months old at the time of clinical onset. The rate at which cases have been reported increased between 1986 and 1992, but then fell as control measures took effect. A marked increase in the incidence of clinical disease which occurred in 1989 (78) can be explained by the recycling into the cattle population through meat-and-bone meal of material from cattle which themselves were infected (49,75). Nevertheless, the incidence of BSE cases nationally in GB has been low (10 cases per thousand adult cattle per year at its peak and about 3 cases per thousand in 1995) because of the generally low effective exposure of cattle to infection in feed (49).

Further epidemiological studies have confirmed the hypothesis of a common source epidemic having arisen because of exposure through feed (76). As a result of the ruminant feed ban in the UK there has been a decline in the incidence of BSE. This was seen first in the youngest cattle and progressed and was maintained through each age class in turn up to and including the six year old age class, ie cattle between 6 and 7 years old (51). Because some infected feed was still in the supply chain and was fed to cattle after the ban was introduced, and because ruminant protein was sometimes included by accident in cattle feed, over 25,000 cattle Born After the date of the Ban (BAB) have developed BSE. The majority of these cases were born in the 1988/1989 calving season (40,51), (77% of British Dairy cattle calve in the period July to December) and there were greatly reduced numbers of reports and confirmations of disease in cattle born in each subsequent season (40, 51). At 29 March 1996 there were 26,293 confirmed BAB cases, but none that were born after June 1993. 3,330 cases were in cattle born in 1990, 1,098 in cattle born in 1991, 60 born in 1992 and only 1 born in June 1993.

All other countries with indigenous cases of BSE, and some others, introduced feed bans from 1990 onwards. In June 1994 a ban on the feeding of mammalian-derived protein to ruminant animals was introduced throughout the European Union (21). The word 'mammalian' was used instead of 'ruminant' because most meat-and-bone meal is derived from mixed ruminant and non-ruminant species. However, there is provision to allow the continued use of porcine-derived meat-and-bone meal if a Member State can demonstrate that it will include no ruminant protein (21). This can be achieved if ruminant and non-ruminant waste is processed separately in dedicated plants.

A case control study of animals born after 30 October 1988 was conducted to estimate the risk of infection from maternal transmission and from horizontal transmission. This study (41, 51) found no evidence that maternal transmission or horizontal transmission could be responsible for the majority of BAB cases, and thus the most likely source of infection was feed. Further studies are in progress.

A number of other studies on, or related to, maternal transmission have been completed or are in progress. These include experimental and epidemiological studies. No detectable infectivity has been found in susceptible mice fed placenta from affected confirmed cases of BSE (4,6,51,54), nor in placenta, placental fluids, ovary or uterine caruncle following mouse inoculation (7,9,51), nor so far (now over 6 years 3 months post challenge) in cattle oro-nasally exposed to infected placenta (7,9). Viable and non-viable embryos from clinically affected cows confirmed to have BSE and washed in accordance with International Embryo Transfer Society protocols (and uterine flushing fluids) have shown no detectable infectivity following inoculation of susceptible mice. Recipient cows and offspring derived from similar viable embryos remain healthy following embryo transfer, but this study will not be complete until 2001 (8). The oldest cattle derived from these embryos are now over four years old.
The observed incidence of BSE in the offspring of confirmed cases is no greater than would be expected if feed were the only source of infection (7,9,10). In a cohort study, 316 offspring of BSE confirmed cows (cases) and 316 offspring from cows over six years old and without BSE from the same farm and age cohort (controls) are being observed under controlled conditions over a seven year period. The purpose of the study is to determine whether maternal transmission occurs, and the incidence if it does. To April 1996 only 47 cattle have succumbed to BSE, but most, if not all of these may have been exposed to infection via feed. 422 additional cattle in this study have been killed so far without showing signs of BSE. The study is being conducted blind and results will not be available until 1997 when the last animals will have been killed, all brains will have been examined and data analysed.

The conclusion from all these studies is that although the possibility that maternal transmission occurs cannot be excluded, it can only be occurring, if at all, so infrequently as to be undetectable using these methods (51). The same applies to horizontal transmission which, based on the evidence from sheep scrapie, would depend to some extent on the occurrence of maternal transmission. Even if maternal transmission were to occur, the disease would still die out if cattle are no longer exposed to the agent through feed, because the necessary contact rate of at least 1:1 would not be maintained (75).

6. **Important conclusions can be drawn from the information set out in paragraphs 3-5 above:**

   6.1 there is very powerful evidence that infected feed is the cause of the disease (41,49,76,79);

   6.2 there is evidence that other methods of exposure are not likely to be important in the spread of disease. The evidence currently available therefore suggests:

   - that it is unlikely, although not impossible, that any animal not exposed to contaminated feed would develop the disease;

   - that the risk of an animal developing disease depends upon exposure, not upon the past or present BSE status of the herd to which the animal belongs; and

   - that the calf of a confirmed case is not significantly more likely to develop disease than the calf of a cow which has not succumbed (41,51).

   Therefore each confirmed case of BSE should be recorded and reported as an outbreak, even when more than one case occurs on the same premises.

   6.3 Because of the long incubation period of the disease, it is possible for infection to be recycled in animal feed before a significant number of clinical cases have occurred (49,75); and

   6.4 even if the source of infection is cut off, new cases can be expected to emerge for several years (i.e. until the maximum incubation period is reached), after which the incidence of disease is likely to fall markedly.
Appendix V (contd)

Geographical Incidence

7. The numbers of confirmed cases and incidences of BSE in different countries with indigenous cases by date of clinical onset in each year are described in TABLES 1 and 2 respectively.

**TABLE 1**

Cases of BSE in different countries by year of service of restriction order

<table>
<thead>
<tr>
<th>Country</th>
<th>Adult &gt;2 yrs cattle population (thousands) 1993</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK (Northern Ireland)</td>
<td>4,399*** (542)</td>
<td>442 (0)</td>
</tr>
<tr>
<td>Rep. of Ireland</td>
<td>3,615</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>953</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>12,100</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>1,345</td>
<td></td>
</tr>
</tbody>
</table>

* GB cases (within the UK total) prior to BSE being made notifiable in June 1988 are given by year of clinical onset of disease, whereas cases throughout the UK since BSE was made notifiable are given by year of service of restrictions.

** Provisional figures (to 31 March 1996 for UK, 19 April 1996 for Switzerland, 30 April 1996 for France and Portugal).

*** Breeding cattle only.

**TABLE 2**

Annual incidence of BSE in different countries (by year of service of restriction order) expressed as a percentage of the adult cattle population over two years of age

<table>
<thead>
<tr>
<th>Country</th>
<th>Adult &gt;2 yrs cattle population (Thousands ) 1993</th>
<th>Annual % incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK (Northern Ireland)</td>
<td>4,399** (542)</td>
<td>0.010 (0.001)</td>
</tr>
<tr>
<td>Rep. of Ireland</td>
<td>3,615</td>
<td>0.0004</td>
</tr>
<tr>
<td>Switzerland</td>
<td>953</td>
<td>0.0002</td>
</tr>
<tr>
<td>France</td>
<td>12,100</td>
<td>0.00004</td>
</tr>
<tr>
<td>Portugal</td>
<td>1,345</td>
<td></td>
</tr>
</tbody>
</table>

* Provisional figures

** Breeding cattle only
(Annual percentage incidence figures have been calculated using 1993 adult cattle population figures)
Appendix V (contd)

Cases of BSE have been reported in imported cattle only in the Sultanate of Oman (19) in the Falkland Islands, Canada, Denmark, Germany and Italy. Some, or all, of the cases reported in France, the Republic of Ireland, Portugal and Switzerland have occurred in native-born cattle. The numbers in these other countries are small, and the UK is at present the only country where the incidence of BSE is regarded as high. It is possible that cases may occur in other countries without being recognised or reported. Unless continuous surveillance is carried out the BSE status of a country must be regarded as "unknown".

8. Within the UK, incidence is higher in the south of England than elsewhere (76,78,79). This is due in part to the geographical distribution of rendering plants that had ceased to use processes, such as solvent extraction and the re-processing of greaves, which either destroy infectivity or significantly reduce the titre. In addition, there are likely to have been regional variations in the amount of infected material entering different rendering plants, and in the use of meat-and-bone meal in concentrated feeds or in protein supplements. Incidence is substantially lower in Northern Ireland (25).

Nature of the disease

9. In addition to the strong evidence that BSE arises from exposure of cattle to the agent causing scrapie or to a cattle-adapted form of it, there are a number of very close similarities between BSE and scrapie (71,74), as well as some significant differences (18). BSE resembles scrapie in its long incubation period, symptomatology, age incidence and progression. The general nature of the pathological lesions is very similar to that found in scrapie, and indeed the other spongiform encephalopathies in other species. As with the related disorders, there are characteristic spongiform changes in particular areas of the brain which are visible by light microscopy (71,72,74). Detergent-treated extracts of affected bovine brain yield scrapie associated fibrils (SAF) visible by electron microscopy (71). These fibrils contain a modified host-coded protein (PrP) which is the bovine homologue of the fibril protein obtained from the brains of sheep with scrapie (42). There is no epidemiological evidence for the direct spread of infection between cattle, suggesting that BSE is not highly contagious. Certain laboratory strains of mice injected intracerebrally and intraperitoneally with BSE-affected brain from nine UK and two Swiss cases have developed spongiform encephalopathy. These sources gave remarkably similar incubation periods and pathological characteristics on primary passage (cow to mice) in a standard panel of mouse strains, suggesting that the same major strain of agent was present in each source from different geographical locations and over different time periods (17,18,31 M. Bruce, personal communication). Bioassay in susceptible laboratory species such as mice is, at present, the only practical way of detecting and measuring BSE infectivity in a large number of samples. The transmission results for BSE to mice differed from those of 20 transmissions from sheep with natural scrapie in the 1970s and also from three transmissions of natural scrapie collected since 1986 (17,18). This suggests that the agent strains in cattle (BSE) and sheep (scrapie) from these transmissions differ, but this is still consistent with the hypothesis that BSE was originally derived from sheep scrapie (18). As with scrapie, the transmission of BSE to mice has also been accomplished by feeding affected brain (4,54). BSE has also been transmitted to cattle by combined intracerebral and intravenous injection of affected brain (23) and also by the oral route following dosing with affected brain (73).

10. It can therefore be concluded that:

10.1 BSE is indisputably a member of the group of TSEs, of which scrapie is the prototype;

10.2 hypotheses about the way in which BSE agent will behave biologically in cattle can be based on what is known about scrapie, even though the BSE agent does not resemble scrapie agent in every respect. However, now that results of studies on the pathogenesis of BSE in cattle are coming forward (70,73 and see para 11.4.3) it will be possible to substitute fact for hypothesis.
Appendix V (contd)

Transmission of BSE

11. The best understood naturally occurring diseases in the same category as BSE and scrapie are set out in the table at Annex 1. Experimental transmissibility is a key factor in the definition of these diseases. The mechanisms by which transmission occurs naturally vary with the disease, but conclusions about the risks of transmission to both animals and man can still be drawn from an understanding of the relevant factors.

11.1 Route of transmission

The oral route has been shown experimentally to transmit BSE (4,6,30,51,54,61), scrapie (57,58), TME (52), kuru (33), Creutzfeldt-Jakob disease (CJD) (33) (in the case of kuru and CJD with brain passed first through primates), and it plays a role in the natural transmission of all these diseases except CJD. However, compared to parenteral exposure, ingestion is a relatively inefficient route of transmission in all the TSE’s that have been studied experimentally. For mouse scrapie, the oral dose required is about a hundred thousand times greater than the intracerebral dose (46,47). In studies of BSE (54), the amount of affected cattle brain required to produce the disease in mice was calculated to be 200,000 fold greater by the oral route than by intracerebral injection (44).

11.2 Species barrier

There is no known causal association between scrapie in sheep or goats and spongiform encephalopathies in man. The incidence of CJD in the world (see also Annex 1) is independent of the presence of sheep, natural scrapie (or any other transmissible spongiform encephalopathy) and the use of sheep products in human food. Individuals with CJD show no particular occupational or other exceptional exposure to sheep or sheep products (12,27). Likewise individuals with CJD show no particular, consistent statistical association with consumption of beef or beef products. CJD has been reported in three dairy farmers and a beef farmer in Great Britain who had cases of BSE in their herds (27, Dr R.G. Will, personal communication). The type of disease in each case was typical of sporadic CJD and no mechanism of cross contamination has been identified (27). Specifically, analysis of the relative risk in farmers in Europe has revealed a similar relative risk in France, Germany and Italy to that for farmers in the UK (27). These other countries have either had no cases of BSE in native born cattle or only a very small number of cases. If there is a real increased risk of sporadic CJD occurring in farmers, that risk does not seem at present to be due to exposure to cattle with BSE. Analysis of occupation at diagnosis in patients with sporadic CJD in the UK (27) shows a wide range in apparent risk of CJD in relation to occupation, including an increased apparent risk in occupations with no obvious increased risk in relation to BSE, including vicars and professional drivers. Contrarywise there is a low risk for people such as abattoir workers, butchers and veterinarians who would be more likely to be exposed to central nervous tissue from cattle and sheep.

CJD has been observed in a lifelong vegetarian (53), and has an average incidence in the UK that is similar to that in European countries where BSE occurs at a very low incidence or is absent altogether (1,27). Spongiform encephalopathies transmit more readily to animals of the same species than to other species. BSE has been transmitted by experimental parenteral inoculation to mice (31), sheep and goats (30), pigs (24), marmosets (3) and mink (61). Such experimental cross-species transmissions usually require high doses. With repeated experimental passage in the new host, the incubation period usually becomes shorter (45). Experiments have confirmed that BSE behaves in the same way. The species which have succumbed to spongiform encephalopathy following oral challenge with brain from cattle with confirmed BSE are mice, sheep, goats, cattle and mink. Pigs and chickens have not succumbed to oral challenge, and chickens and hamsters have not succumbed to parenteral challenge.
11.3 **Dose**

Transmission of spongiform encephalopathies is dependent on the size of the infective dose.

11.4 **Tissue distribution of the agent**

The points covered in 11.1 - 11.3 above are relevant to any possible risk to animals of the same species, of different species, and to man, from exposure to the BSE agent. Clearly, there would be little or no risk of infection as a result of oral exposure to tissues which contain no detectable infectivity as determined experimentally using the most efficient routes of inoculation. There is a large literature on the amounts of infectivity in different tissues of animals infected experimentally or naturally with one or other of the TSE agents. Although the general patterns are similar, the amounts of infectivity in tissues vary with the different diseases. Data obtained from studies of natural scrapie in sheep (35,38) and goats (36) are particularly valuable in making "worst-case" predictions of the potential risks due to BSE from various bovine tissues. The pattern seen with natural scrapie, based on extensive studies summarised in Annexes 2a and 2b, is described in the next paragraph.

11.4.1 **Development of natural scrapie in sheep and goats**

No agent was detected in any tissue from lambs of up to eight months of age (35,38). At 10-14 months of age low infectivity was present in the large masses of lymphoreticular tissue in the intestines (Peyer's patches), lymph nodes associated with the gastrointestinal tract and elsewhere, spleen and tonsil (35,38). The titres in these tissues increased subsequently and, before clinical signs appeared, infectivity was detected in the spinal cord, medulla and some other areas of the brain (35,38). By the time animals showed clinical disease, levels of infectivity in the central nervous system, including the spinal cord, had risen above those in the lymphoreticular system (35,36,38).

11.4.2 **Non-infectious tissues in natural scrapie in sheep and goats**

Attempts have been made to detect scrapie infectivity in a wide range of other tissues. Those which have been shown **not** to harbour detectable infection include:

- skeletal muscle (*ie* carcase meat) (35,36,38)
- heart (35,38)
- kidney (35,36)
- colostrum (35) and milk (36)
- mammary gland (35,36,38)
- uterus (35,36,38)
- ovary (35,36,38)
- seminal vesicle, and testis (36)
- blood clot (35,36,38)
- saliva/salivary gland (35,36)
- skin (62).
Appendix V (contd)

TABLE 3
Tissues from clinically affected cattle with no detectable infectivity by parenteral inoculation of mice grouped by anatomical system (7,9,51).

<table>
<thead>
<tr>
<th>Tissues from clinically affected cattle</th>
<th>Tissues from clinically affected cattle</th>
<th>Tissues from clinically affected cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal fluid</td>
<td>Spleen</td>
<td>Oesophagus</td>
</tr>
<tr>
<td>Cauda equina (of spinal cord)</td>
<td>Tonsil</td>
<td>Reticulum</td>
</tr>
<tr>
<td>Peripheral nerves:</td>
<td>Lymph nodes:</td>
<td>Rumen (pilliar)</td>
</tr>
<tr>
<td>N. sciaticus (proximal)</td>
<td>Prefemoral</td>
<td>Rumen (oesophageal groove)</td>
</tr>
<tr>
<td>N. tibialis</td>
<td>Mesenteric</td>
<td>Omasum</td>
</tr>
<tr>
<td>N. splanchnic</td>
<td>Retropharyngeal</td>
<td>Abomasum</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>Proximal small intestine</td>
</tr>
<tr>
<td>Tonsil</td>
<td></td>
<td>Distal small intestine</td>
</tr>
<tr>
<td>Lymph nodes:</td>
<td></td>
<td>Proximal colon</td>
</tr>
<tr>
<td>Prefemoral</td>
<td></td>
<td>Distal colon</td>
</tr>
<tr>
<td>Mesenteric</td>
<td></td>
<td>Rectum</td>
</tr>
<tr>
<td>Retropharyngeal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clotted blood</td>
<td>Testis</td>
<td>Ovary</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>Prostate</td>
<td>Uterine caruncle (pregnant cow)</td>
</tr>
<tr>
<td>Foetal calf blood</td>
<td>Seminal vesicle</td>
<td>Placental cotyledon</td>
</tr>
<tr>
<td>Serum</td>
<td>Epididymis</td>
<td>Placental fluids:</td>
</tr>
<tr>
<td></td>
<td>Semen</td>
<td>Amniotic fluid</td>
</tr>
<tr>
<td></td>
<td>Semen</td>
<td>Allantoic fluid</td>
</tr>
<tr>
<td></td>
<td>Semen</td>
<td>Embryos</td>
</tr>
<tr>
<td>M. longissimus</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>semitendinosus</td>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>M. diaphragma</td>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td>M. masseter</td>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Trachea</td>
<td></td>
</tr>
</tbody>
</table>

Studies in some of these tissues from different source cattle and others not so far reported are still in progress.

Data courtesy of Dr H. Fraser and Dr D.M. Taylor.

11.4.3 Tissue distribution of agent in BSE

Transmission experiments in mice have been undertaken with a wide range of tissues from confirmed cases of BSE. So far, BSE infection has been transmitted, by feeding (4,54) or by injection (17,18,31,51,68), only by brain, cervical and terminal spinal cord and retina (7,9,51). Experiments with mice that were fed milk and mammary gland; placenta; lymph nodes or spleen (4,54) have failed to transmit the disease within the natural lifespan of the animals, or even to establish subclinical BSE infection of the lymphoreticular system. Furthermore, mice exposed parenterally to the tissues listed in TABLE 3 did not succumb to disease within their natural lifespan (7,9,51). A more recent experiment using milk derived from cattle with BSE in early, mid and late lactation and either inoculated or fed to susceptible mice has revealed no evidence of infectivity (68). There is a cow to mouse species barrier in these studies. However, all calves receive colostrum and beef calves are suckled for up to six months of age. Since there is no epidemiological evidence that maternal transmission occurs in BSE (the way that transmission from milk would be exhibited if it occurred), it can be concluded that bovine milk does not contain any infectivity.
Because bioassay in mice is less sensitive than bioassay in cattle, a comparative bioassay is in progress in which pooled brains from five confirmed cases of BSE have been titrated in cattle and mice. Though incomplete, provisional results show that the titre measured is 100-1000 fold higher when assayed in cattle than in mice. However, when pooled spleens and pooled lymph nodes from these same cattle were assayed, no detectable infectivity was found in mice and the inoculated cattle are still healthy more than 37 months post-challenge; this is more than double the incubation period in cattle inoculated with brain.

If there is any infectivity in bovine spleen/lymph node, it must be about 100,000 fold less than in brain, and any infectivity in other tissue (including meat) will be even lower.

The pathogenesis of experimental BSE in cattle, following oral challenge of calves at four months of age with a single, very large dose of 100g of pooled brain from confirmed cases, is in progress. The objectives are to determine the temporal and spatial development of infectivity and pathology following oral dosing. In calves killed at six months of age (two months after dosing) no infectivity was found in any tissue following bioassay of 46 tissues from the challenged or control calves. However, at 10, 14, 18 and 22 months of age (6, 10, 14 and 18 months after dosing) infectivity was detected in the distal ileum of challenged calves (70, G.A.H. Wells, personal communication). This part of the small intestine contains Peyer's patches consisting of lymphoreticular tissue and is also amongst the first tissues in which infectivity is detected in natural scrapie (35,38).

Bovine skin and bone are used to produce gelatin and collagen. These tissues contain no detectable infectivity when bioassayed in mice, but skulls or vertebrae (excluding tail vertebrae) which may contain residual central nervous tissues should not be used. Provided processes for the production of gelatin are used which have been experimentally shown to reduce infectivity by at least 5 log$_{10}$ LD$_{50}$/g (e.g. those used by the Gelatin Manufacturers of Europe (GME)), gelatin can be regarded as a safe commodity.

The process must include:

- in respect of bones:

  - pressure washing (degreasing)
  - acid demineralisation
  - either acid or prolonged alkaline treatment
  - filtration, and
  - sterilisation at ≥ 138°C for a minimum of 4 seconds.

- in respect of skins

  - prolonged treatment with saturated milk of lime or sodium hydroxide
  - neutralisation
  - filtration, and
  - sterilisation as above.

Collagen produced by a similar process can also be considered to be a safe product.
11.5 Maternal transmission

As indicated above (paragraphs 5 and 6.2) field and experimental experience with live cattle suggests that transmission from cow to calf is not likely to be a factor in the development of BSE (41, 51, 75). This is in marked contrast to scrapie, but is consistent with the situation with natural transmissible mink encephalopathy (TME) (37) and kuru, which are both "dead-end" diseases. The data summarised in 11.4.3 suggests the tissue distribution of BSE infectivity resembles that in TME in which maternal transmission does not occur (37), being concentrated in the central nervous system (CNS) of clinical cases at titres some 100,000 times higher than in any non-CNS tissue (annex 2a). In natural sheep scrapie the lymphoreticular system contains high levels of infectivity (35, 38), placenta from affected sheep can experimentally transmit disease following oral exposure of sheep and goats (57, 58) and on present evidence the transmission of scrapie by embryo transfer cannot be ruled out. In BSE no detectable infectivity has been found in any male or female reproductive tissue, or in placenta or embryos (collected by IETS protocols) by bioassay in susceptible mice. Furthermore, uterine flushings have been similarly tested and have shown no detectable infectivity. There is a cow to mouse species barrier in these studies. However, cattle have been challenged oro-nasally with placenta derived from clinically affected, confirmed cases of BSE and no disease has yet resulted more than 75 months after challenge. Embryos collected from a large number of clinically affected confirmed cases of BSE by IETS protocols have been transferred into 347 recipient heifers imported from New Zealand and kept in quarantine. No BSE has resulted in any recipient cow or offspring, the oldest of which is now over 4 years of age. The study will not be complete until 2001.

12. The conclusions emerging from these considerations are:

12.1 The risk of infection with BSE arises only from exposure to certain tissues of infected animals; or products prepared from those tissues.

12.2 The mouse can be concluded to be an acceptable test animal to identify infectivity in BSE-affected animals.

12.3 In naturally infected cattle exhibiting clinical signs of BSE and confirmed to have the disease post mortem, infectivity, detected by bioassay in susceptible mice, has been found only in the brain, cervical and terminal spinal cord and the retina and no infectivity was detected in 50 other tissues.

12.4 Detectable infectivity following high dose, experimental, oral challenge with brain from confirmed BSE cases has been found in the distal ileum of calves of ten 14, 18 and 22 months of age, (6, 10, 14 and 18 months after dosing) (51, 70, 73, G.A.H. Wells, personal communication);

12.5 Conversely, there is a range of tissues from cattle in which no detectable infectivity is expected to occur at any time, even in clinically affected animals. These tissues include:

- Carcase meat
- Milk
- Hides
- Skins
- Semen
- Embryos washed in accordance with the protocols of the International Embryo Transfer Society.

12.6 Tissue infectivity studies so far support the hypothesis that maternal transmission is not likely to be a significant factor in the transmission of BSE, and hence that animals not fed infected meat-and-bone meal are unlikely to be incubating the disease (7, 9, 41, 51).
Qualities of the agent

13. Infection with these agents does not provoke a detectable immunological reaction, so there is at present no practical means of detecting infection in healthy animals. The agents causing scrapie, and by inference BSE, are exceptionally resistant to heat (63), ultraviolet and ionising radiation, and chemical disinfection (63,64,66). Scrapie and BSE agents appear to respond similarly to physical and chemical inactivation (65). Wet heat (48) inactivates more effectively than dry heat (15,16,63,64). The method used in hospitals and laboratories in the UK to inactivate the CJD agent is based on studies of the scrapie agent. It involves porous load autoclaving at 134°C-138°C for 18 minutes at 30 psi (= 207 hPa) (hold temperature and time) (26). There is now some experimental evidence that temperatures at the lower end of this range may permit some residual infectivity to remain (66,67). Sodium hypochlorite providing two percent (20,000 ppm) available chlorine acting for one hour at 20°C is completely effective (16,48,64,65,66). A useful alternative disinfectant is 1 N (four percent) or 2N (eight per cent) sodium hydroxide acting for one hour at 20°C (63,64,65,66) though either treatment may allow some residual infectivity to remain. The results of a complex experimental study of commercial rendering processes used in the European Union (EU) to produce meat-and-bone meal have revealed two processes that do not inactivate the BSE agent. Infectivity was detected in meat-and-bone meal, but not tallow, derived by these processes from animal waste, to which brains from cattle with confirmed BSE had been added. The use of both processes has now been banned when processing ruminant waste in Member States of the European Union (22). Further studies using brains from sheep with scrapie are virtually complete. Only the batch pressure natural fat process using minimal (133°C, 3 bar for 20 minutes) or higher values produced meat-and-bone meal with no detectable infectivity. However, tallow was free of detectable infectivity before filtration even from a process which failed to inactivate the scrapie agent in meat-and-bone meal. Grade 2 tallow derived from carcases, passed for human consumption, can thus be considered safe for consumption for man and animals, provided it is filtered before use. The EU Scientific Veterinary Committee has recommended that all ruminant protein waste is treated at 133°C, 3 bar for 20 minutes or by systems providing the same degree of security.

Tallow is not used in pharmaceutical products, but tallow derivatives are used. Provided these are produced by hydrolysis of tallow from non-SBO material at temperatures ≥250°C at 50 bar pressure for at least 3 hours followed by distillation and filtration, or by processes which give the same degree of assurance, they can be considered as safe for any purpose.

Precautions

14. The action which should be taken by countries with regard to BSE should be based on the conclusions set out above. The main risk factors to be considered are:

- the use of ruminant protein (excluding certain derived products such as gelatin and tallow produced by approved methods and milk) in ruminant feed
- systems of commercial rendering and other methods of animal waste disposal
- the existence of measures to avoid or reduce exposure to potentially infected material
- the incidence of scrapie
- the size of the sheep population, absolutely and relative to that of cattle
- routes of exposure.

Action should be designed to distinguish between the risks to animal and public health, and falls into four categories: (Paras 14.1-14.4).
14.1 Surveillance

Because of the long incubation period of the disease and the absence of any detectable serological or other tests for infection, animals could be incubating the disease, even in countries which have now taken precautions (6) such as those outlined below. **It is therefore important to:**

14.1.1 **make suspicion of BSE notifiable;**

14.1.2 **ensure farmers, veterinarians and the national Veterinary Services are aware of the clinical signs of BSE; and**

14.1.3 **ensure pathologists have the knowledge, experience and techniques to confirm the disease to a common standard (Protocols have been produced by the Scientific Veterinary Committee of the European Commission (28)) and consider BSE as a differential diagnosis in all nervous diseases in cattle, including rabies.**

14.2 Control of the disease where it is present

The carcases of suspect cases should be destroyed by incineration. In the absence of clear evidence about the treatment needed to remove or inactivate the BSE agent, the key requirement is to eliminate the possibility that cattle might be exposed to the agent through their feed. On the basis that feed is the only significant route by which infection is transmitted this should be enough to ensure the disappearance of the disease. This requirement may be implemented by means of a ban on feeding ruminant protein to ruminant animals although milk protein and other products such as dicalcium phosphate derived from defatted bones are not perceived to be a risk and may be excluded from any ban. Such a measure would protect deer and other ruminant species such as those in zoos and wildlife parks from exposure to infection through food. In addition, the existence of such a ban in an exporting country would mean that trade restrictions on exports of ruminant species born after the introduction of the ban were not justified on account of BSE. If ruminant waste cannot be effectively separated from other animal waste before and during processing, the feeding of mammalian protein to ruminant animals should be banned, as has been done in EU Member States (21). The effectiveness of the ruminant feed ban can be reinforced by requiring that certain minimum standards are used to process ruminant animal waste, to produce meat-and-bone meal devoid of detectable BSE or scrapie infectivity. The standards adopted by Member States of the EU, as an interim measure, could be followed (22). Observance of a ban can be monitored by sampling and testing feed and feed ingredients, using an ELISA test developed in the UK to detect protein from mammalian, including ruminant, species in feed (2). Although transmission from cow to calf is not likely to be a significant factor, measures should be taken to isolate any suspect cases which are giving birth, to dispose of placenta safely and to cleanse and disinfect the isolation accommodation in order to minimise the risk of transmission.

14.3 Avoiding the occurrence of BSE in a country where disease is absent

Because of the long incubation period of the disease, the agent may be present in those countries where the relevant risk factors exist, without having caused a sufficient number of clinical cases to be recognised. The recycling of undetected infection through cattle feed could already be taking place in such countries. **Therefore, in countries where BSE has not been recognised, whenever possible:**

14.3.1 **studies should be undertaken to determine the extent to which the risk factors are present e.g. the occurrence and incidence of scrapie, the method of disposal or processing of ruminant wastes and the origin, use of and inclusion rate of ruminant protein in rations fed to ruminants;**
14.3.2 Consideration might also be given to excluding from ruminant rations those tissues which, in sheep affected with scrapie or cattle infected with BSE, are most likely to contain high titres of the agent. (See 14.4 below). In a number of countries this has been implemented by means of a mammalian or ruminant protein ban similar to that described in paragraph 14.2 above.

14.3.3 Minimum standards for processing of ruminant waste to produce meat-and-bone meal for feed purposes could be considered.

14.4 Eliminating any risk for man, or any other species

Any risk for man or animal species can be avoided by reducing exposure to the infectious agent to a level below which infection capable of causing disease will not occur.

Preventive measures should take account of evidence about the tissue distribution of infectivity (see 11.4 above), and potential routes of infection (see 11.1 above).

14.4.1 Only animals exposed to infection through feed are likely to present a risk of carrying infection themselves. Infection is unlikely to be detectable in any part of an animal which is incubating BSE before it is six months old (35,38). Any detectable infectivity in older animals is likely to be confined to the CNS or lymphoreticular system. Restrictions to minimise any theoretical risk to man or other non-ruminant species should therefore apply to the following tissues (the "specified offals") from animals over six months old, and protein material derived from them:

- brain, spinal cord, eyes, tonsil, thymus, spleen and intestines (from duodenum to rectum inclusive).

14.4.2 Such restrictions should be considered where there is a high incidence of disease, or where the risk factors suggest that a substantial number of cases might arise. Additional reassurance could be provided by removing and destroying obvious nervous and lymphatic tissue exposed during the meat cutting process. Clearly, more such tissue can be removed in the preparation of de-boned meat; the removal of the tissues from bone-in meat traded internationally would be a matter for the importing country.

14.4.3 Because of the greater efficiency of transmission by parenteral exposure (see 10.1) than via the alimentary tract (44,46,47), any risk that exists will be greater, dose for dose, with materials which might be inoculated, intentionally or accidentally. This factor needs to be taken into account in reaching decisions about sourcing bovine material for the manufacture of pharmaceutical products for veterinary and human medical use, and in offering advice on hygienic practices to workers in sectors where inoculation might occur.

14.4.4 In any country where a case of BSE is suspected, the animal should be compulsorily slaughtered and the brain examined in accordance with the diagnostic techniques set out in the Manual. The carcase of confirmed BSE cases should be totally destroyed, so that no part can enter any food or feed chain.

15. Definitions and explanations

15.1 In respect of the Code, meat-and-bone meal is defined as the protein product when waste animal tissues are treated by heat (rendered) and include any intermediate protein product.

15.2 In regard to Article 3.2,13.5 of the Code, the UK introduced new controls prohibiting the use mammalian meat-and-bone meal in any farm animal feed or use as an agricultural fertiliser in April 1996. The new controls were fully enforced by 30 April 1996.
REFERENCES


2. ANSFIELD M. (1994). - Production of a sensitive immunoassay for detection of ruminant proteins in rendered animal material treated to >130°C. Food and Agricultural Immunology, 6, 419-433.


Appendix V (contd)


Appendix V (contd)


### ANNEX I

**Naturally-occurring transmissible spongiform encephalopathies (SE) (7,9)**

<table>
<thead>
<tr>
<th>Host</th>
<th>Disease</th>
<th>Reported distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>Kuru</td>
<td>Papua New Guinea</td>
</tr>
<tr>
<td></td>
<td>CJD</td>
<td>Worldwide</td>
</tr>
<tr>
<td></td>
<td>GSS</td>
<td>Familial, worldwide, but extremely rare</td>
</tr>
<tr>
<td>Sheep/Goats</td>
<td>Scrapie</td>
<td>Widely distributed</td>
</tr>
<tr>
<td>Moufflon</td>
<td>Scrapie</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Mule deer</td>
<td>CWD</td>
<td>North America</td>
</tr>
<tr>
<td>Elk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmed mink</td>
<td>TME</td>
<td>North America, mainland Europe</td>
</tr>
<tr>
<td>Cattle</td>
<td>BSE†</td>
<td>UK, Republic of Ireland (RoI), France, Portugal, Switzerland</td>
</tr>
<tr>
<td>Nyalá</td>
<td>SE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Gemsbok⁺</td>
<td>SE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Arabian oryx⁺</td>
<td>SE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Greater kudu</td>
<td>SE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Ankole⁺</td>
<td>SE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Eland⁺</td>
<td>SE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Scimitar-horned oryx⁺</td>
<td>SE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Cat</td>
<td>FSE</td>
<td>British Isles, Norway</td>
</tr>
<tr>
<td>Puma⁺</td>
<td>FSE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Cheetah⁺</td>
<td>FSE</td>
<td>Australia*, Great Britain, RoI*</td>
</tr>
<tr>
<td>Ocelot⁺</td>
<td>FSE</td>
<td>Great Britain</td>
</tr>
<tr>
<td>Tiger⁺</td>
<td>FSE</td>
<td>Great Britain</td>
</tr>
</tbody>
</table>

+ = transmission not attempted  * = Presumptively exposed in Great Britain

CJD = Creutzfeldt-Jakob Disease. (Incidence: sporadic c. 85%; familial c. 14%; iatrogenic <1%)
GSS = Gerstmann-Sträussler (Scheinker) Syndrome
CWD = Chronic Wasting Disease
TME = Transmissible Mink Encephalopathy
BSE† = Bovine Spongiform Encephalopathy (countries with cases in native-born cattle only)
FSE = Feline Spongiform Encephalopathy
Creutzfeldt-Jakob Disease

Creutzfeldt-Jakob Disease (CJD) occurs worldwide at an incidence of about one case per 2 million per annum (14). However, there have been rare instances of geographical clustering of cases where the annual incidence has been 30 times or more of this norm (14). Such clusters have been found in Israel (56) and Slovakia (55). They are clearly related to the presence of point mutations at codon 200 of the \(PrP\) gene (34). Up to about 15% of all CJD cases are familial and some of these are associated with a variety of other mutations in the \(PrP\) gene. The great majority of cases (>85%) occur sporadically. A tiny proportion of these cases are iatrogenic. These include cases which result from accidental use of CJD-contaminated surgical instruments or electrodes, corneal transplant, dura mater transplants (all presenting as relatively short incubation dementia) (13) and human cadaver, pituitary-derived growth hormone or gonadotrophin (all presenting with relatively long (9-30 years) incubation and cerebellar signs) (13,14). Epidemiological studies in many countries (including France (14,20) and the UK (39), have shown that sheep are not a reservoir of infection causing CJD, and there is no evidence that CJD has an origin in any animal disease. The incidence of CJD in 1993 is very similar in France, Germany, Italy the Netherlands and the UK, and contrasts with the marked variation in incidence of BSE in these countries and suggests that the emergence of BSE has not resulted in a change in the occurrence of CJD in 1993 (1,27,80). Any hypothetical risk from bovine tissues used in pharmaceutical preparations, and sourced from countries, regions or herds where BSE exists, is eliminated by following the guidelines given in the Code (also see paragraph 14.4.3 of this document).

Two cases of sporadic CJD in teenagers in the UK were reported during 1995 (5,11). A further eight cases, confirmed by necropsy or biopsy, have occurred in patients under 42 years of age and been reported in 1996 (81). All these cases have had a previously unrecognised and consistent disease pattern. In particular, the pathology in the brain of the eight necropsy cases showing prominent \(PrP\) plaques extensively distributed throughout the cerebrum and cerebellum surrounded by a zone of spongiform change, appears not to have been seen in any of the 175 other cases of sporadic CJD that were investigated. The young age, long average clinical course, absence of EEG findings typical of CJD and clinical onset from 1994 onwards in these cases suggests a common cause. The consistency of the neuropathology might indicate that a common strain of agent is involved. The strain type is being investigated. Since the publication referred to above, a further single case has been identified and confirmed in Lyon, France. None of the cases shows any exceptional, occupational or dietary risk though eight are homozygous for methionine at codon 129 of the \(PrP\) gene which is known to be polymorphic. None of the UK cases have a known \(PrP\) gene mutation. No direct link has been made between these cases of CJD and BSE, although this remains a possible explanation for them.

Feline spongiform encephalopathy

Feline spongiform encephalopathy (FSE) was first reported in a domestic cat in Great Britain in May 1990 (82). To the end of December 1994, 58 cases have been reported in the UK and one, in an indigenous cat, in Norway. The clinical and pathological features in five of these have been described (83) and the disease reviewed (60). The presence of SAFs and \(PrP^{Sc}\) (59) and transmission of disease to mice following parenteral inoculation of brain tissue from an affected cat (18,32) has confirmed FSE is a member of the naturally occurring TSE group of diseases and it is therefore listed in the table above. Furthermore, the temporal occurrence (shortly after the emergence of BSE) and the similarity of the biological characteristics in mice at first passage to those of the BSE agent suggest an origin from cattle. Exposure is presumed to have been via feed.

Spongiform encephalopathy in ungulates

In addition to the diseases listed above, naturally occurring spongiform encephalopathies have been identified in seven species of ungulates that were kept in zoos or wildlife parks in Great Britain (7,9,29,43,50, D. Matthews, personal communication). These animals were fed the same type of concentrated feeds that caused BSE, but the apparently shorter incubation periods in the zoo animals suggest that they were more susceptible to infection than cattle. Experimental transmission of disease from the formalin-fixed brain tissue of a nyala and greater kudu to mice has been achieved and, as in cats, the biological characteristics at first passage are remarkably similar to those resulting from challenge of mice with BSE agent, thus suggesting a common source of infection (18). All seven species of these ungulates belong to the Family BOVIDAE and are more closely related phylogenetically to cattle, sheep and goats (all Family BOVIDAE), than they are to deer (Family CERVIDAE).
### ANNEX 2A

**Infectivity titres (bio-assayed in mice) in tissues from up to nine Suffolk sheep (34-57 months old) and up to three goats (38-49 months old), at the clinical stage of natural scrapie, compared to the titres in tissues from one or more confirmed cases of BSE**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Titre (mean ± SEM of (n) sample)</th>
<th>Titre&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scrapie sheep</td>
<td>Scrapie goats</td>
</tr>
<tr>
<td><strong>Category I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>5.6 ± 0.2 (51)</td>
<td>6.5 ± 0.2 (18)</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>5.4 ± 0.3 (9)</td>
<td>6.1 ± 0.2 (6)</td>
</tr>
<tr>
<td><strong>Category II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>4.7 ± 0.1 (9)</td>
<td>4.6 ± 0.3 (3)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>4.2 ± 0.1 (45)</td>
<td>4.8 ± 0.1 (3)</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>4.5 ± 0.2 (9)</td>
<td>4.7 ± 0.2 (3)</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.5 ± 0.3 (9)</td>
<td>4.5 ± 0.1 (3)</td>
</tr>
<tr>
<td>Tonsil</td>
<td>4.2 ± 0.4 (9)</td>
<td>5.1 ± 0.1 (3)</td>
</tr>
<tr>
<td><strong>Category III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>3.1 ± 0.3 (9)</td>
<td>3.6 ± 0.3 (3)</td>
</tr>
<tr>
<td>Distal colon</td>
<td>&lt; 2.7 ± 0.2 (9)</td>
<td>3.3 ± 0.5 (3)</td>
</tr>
<tr>
<td>Thymus</td>
<td>2.2 ± 0.2 (9)</td>
<td>&lt; 2.3 ± 0.2 (3)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>&lt; 2.0 ± 0.1 (9)</td>
<td>&lt; 2.0 (3)</td>
</tr>
<tr>
<td>Liver</td>
<td>&lt; 2.0 ± 0.1 (9)</td>
<td>...</td>
</tr>
<tr>
<td>Lung</td>
<td>&lt; 2.0 (9)</td>
<td>&lt; 2.1 ± 0.1 (2)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>&lt; 2.1 ± 0.1 (9)</td>
<td>...</td>
</tr>
<tr>
<td><strong>Category IV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood clot</td>
<td>&lt; 1.0 (9)</td>
<td>&lt; 1.0 (3)</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>&lt; 2.0 (9)</td>
<td>...</td>
</tr>
<tr>
<td>Kidney</td>
<td>&lt; 2.0 (9)</td>
<td>&lt; 2.0 (3)</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>&lt; 2.0 (7)</td>
<td>&lt; 2.0 (3)</td>
</tr>
<tr>
<td>Milk</td>
<td>...</td>
<td>&lt; 1.0 (3)</td>
</tr>
<tr>
<td>Serum</td>
<td>...</td>
<td>&lt; 1.0 (3)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>&lt; 2.0 (9)</td>
<td>&lt; 2.0 (1)</td>
</tr>
<tr>
<td>Testis</td>
<td>&lt; 2.0 (1)</td>
<td>...</td>
</tr>
</tbody>
</table>

The data are taken from the following sources: sheep scrapie, Hadlow et al (1982); goat scrapie, Hadlow et al (1980); BSE, Fraser et al (1992); Fraser & Foster, these proceedings.

The classification of tissues is according to the Guidelines of the European Community's Committee for Proprietary Medicinal Products (CPMP) (EC, 1991).

<sup>a</sup> Titres are expressed as arithmetic means of log<sub>10</sub> mouse i.c. LD<sub>50</sub>/g or ml of tissue (+ve = > 2.0).

Note: None of the bovine tissues in Categories II and III and no tissues in Category IV had any detectable infectivity. The values shown are maxima based on the limits of detectability of the bioassay in mice (calculated for 30µl of inoculum injected intracerebrally).

SEM: standard error of the mean.

Table from Reference (44) with permission.
### ANNEX 2B

Scrapie infectivity titres in category I and II tissues from preclinically infected sheep compared to clinical cases

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Titres (Mean ±SEM of ( n ) samples)*</th>
<th>Pre-clinical</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-8 months( a )</td>
<td>10-14 months( b )</td>
</tr>
<tr>
<td><strong>Category I</strong> (Central nervous system - CNS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- cerebral cortex</td>
<td></td>
<td>...</td>
<td>&lt;2.0( l )</td>
</tr>
<tr>
<td>- diencephalon</td>
<td></td>
<td>...</td>
<td>2.2( l )</td>
</tr>
<tr>
<td>- midbrain</td>
<td></td>
<td>...</td>
<td>&lt;2.0( l )</td>
</tr>
<tr>
<td>- medulla</td>
<td></td>
<td>&lt;2.0( 8 )</td>
<td>3.8( l )</td>
</tr>
<tr>
<td>Cervical cord</td>
<td></td>
<td>...</td>
<td>&lt;2.0( l )</td>
</tr>
<tr>
<td><strong>Averages</strong></td>
<td></td>
<td>&lt;2.0</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td><strong>Category II</strong> (Lymphoreticular system - LRS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- BM</td>
<td></td>
<td>...</td>
<td>&lt;2.0( 8 )</td>
</tr>
<tr>
<td>- MP</td>
<td>&lt;2.0( 16 )</td>
<td>3.3 ± 0.4( 8 )</td>
<td>5.2( l )</td>
</tr>
<tr>
<td>- PreS</td>
<td>&lt;2.0( 16 )</td>
<td>&lt;2.0( 8 )</td>
<td>4.5( l )</td>
</tr>
<tr>
<td>- PreF</td>
<td>&lt;2.0( 6 )</td>
<td>&lt;2.4 ± 0.2( 3 )</td>
<td>3.2( l )</td>
</tr>
<tr>
<td>- RP</td>
<td>&lt;2.0( 16 )</td>
<td>3.8 ± 0.4( 3 )</td>
<td>5.2( l )</td>
</tr>
<tr>
<td>Ileum</td>
<td>&lt;2.0( 16 )</td>
<td>&lt;3.1 ± 0.5( 3 )</td>
<td>5.3( l )</td>
</tr>
<tr>
<td>Spleen</td>
<td>&lt;2.0( 16 )</td>
<td>3.0 ± 0.4( 3 )</td>
<td>4.3( l )</td>
</tr>
<tr>
<td>Tonsil</td>
<td>&lt;2.0( 10 )</td>
<td>&lt;2.6 ± 0.3( 3 )</td>
<td>4.7( l )</td>
</tr>
<tr>
<td><strong>Averages</strong></td>
<td></td>
<td>&lt;2.0</td>
<td>&lt;2.8</td>
</tr>
</tbody>
</table>

* All titres are expressed as log\(_{10}\)i.c. LD\(_{50}\)/g or ml of tissue

SEM: standard error of the mean.

a) None of the tissues from lambs aged eight months or less had any detectable infectivity. The values shown represent maxima based on the limits of the sensitivity of the bio-assay in mice.

b) Infectivity was detected in only eight out of fifteen sheep exposed to scrapie.

c) Infectivity was detected in only one out of three sheep exposed to scrapie.

The above is a re-analysis of the data published in ref. 35.