Bovine spongiform encephalopathy: an update *

Summary: A specialist group of the Office International des Epizooties met in May 1996 to prepare updated information on bovine spongiform encephalopathy (BSE): in particular on the development of the epidemic, geographical incidence, nature of the disease, transmission, precautions and control measures. A revised Chapter 3.2.13. of the International Animal Health Code dealing with BSE, and an outline of the spongiform encephalopathies, are appended, along with a comprehensive bibliography.


INTRODUCTION

1. An Ad hoc Group of experts was convened to consider the implications of bovine spongiform encephalopathy (BSE) and produced a report (59) in September 1990 which was adopted by the 59th General Session of the Office International des Epizooties (OIE). 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 the report 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 on 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. (see Appendix I).

DEVELOPMENT OF THE EPIDEMIC

3. BSE was first recognised in Great Britain in November 1986 (74), although evidence collected later indicated that the first clinical cases had occurred in April

* This document, prepared in May 1996 by the Ad hoc Group of the OIE on Bovine Spongiform Encephalopathy, was entitled: 'Supporting Document for the OIE International Animal Health Code Chapter 3.2.13. on bovine spongiform encephalopathy (updated May 1996)'.

1985 (80). Initial epidemiological studies indicated an extended common source epidemic in which all affected animals were index cases (80). 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 similarity of the disease to 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 (80, 81). The role of meat-and-bone meal is strongly supported by the results of a case-control study (82) 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 (42, 56). The ability of the BSE agent to survive certain commercial rendering processes has also been demonstrated (27, 73) 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 (81).

4. Computer simulations indicate that exposure to the agent sufficient to give rise to clinical disease began abruptly in 1981/1982 (80, 81). 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 (80, 81).

5. Epidemiological studies indicate that the majority of BSE-infected animals have been exposed in calfhood (80). It is predominantly animals in dairy herds that are affected (80). At 29 March 1996, 59.3% of dairy herds and 15.3% of beef suckler herds in Great Britain 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 (80) 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 (83) can be explained by the recycling into the cattle population through meat-and-bone meal of material from cattle which themselves were infected (51, 79). Nevertheless, the incidence of BSE cases nationally in Great Britain 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 (51).

Further epidemiological studies have confirmed the hypothesis of a common source epidemic having arisen because of exposure through feed (81). 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, i.e. cattle between 6 and 7 years old (56). 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 (42, 56) – 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 (42, 56). 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 (26). 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 (26). 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 (43, 56) 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, 55, 56), nor in placenta, placental fluids, ovary or uterine caruncle following mouse inoculation (7, 9, 56), 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 (IETS) 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. An additional 422 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 (56). 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 (79).

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
(43, 51, 80, 81);

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 (43, 56).

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 (51, 79); 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.

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 I
and II respectively.

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 (80,
81, 83). 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 (23).
### Cases of bovine spongiform encephalopathy (BSE) in different countries
(by year of restriction of suspected clinical cases)

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<tbody>
<tr>
<td>United Kingdom (Northern Ireland)</td>
<td>4,399 *** (542)</td>
<td>442 (0)</td>
<td>2,473 (4)</td>
<td>7,166 (29)</td>
<td>14,294 (113)</td>
<td>25,202 (170)</td>
<td>37,055 (375)</td>
<td>34,830 (460)</td>
<td>24,288 (344)</td>
<td>14,136 (173)</td>
<td>1,230 (30)</td>
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<tr>
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<td>14</td>
<td>17</td>
<td>18</td>
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<td></td>
</tr>
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<td>15</td>
<td>29</td>
<td>64</td>
<td>68</td>
<td>25</td>
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<tr>
<td>Portugal</td>
<td>1,345</td>
<td>12</td>
<td>14</td>
<td>4</td>
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* Cases in Great Britain (within the United Kingdom total) prior to BSE being made notifiable in June 1988 are given by year of clinical onset of disease, whereas cases throughout the United Kingdom since BSE was made notifiable are given by year of restriction of suspected clinical cases.

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

*** Breeding cattle only.
### TABLE II

Annual incidence of bovine spongiform encephalopathy in different countries expressed as a percentage of the adult cattle population over two years of age (by year of restriction of suspected clinical cases)

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<tbody>
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<td>0.06</td>
<td>0.16</td>
<td>0.33</td>
<td>0.57</td>
<td>0.84</td>
<td>0.79</td>
<td>0.54</td>
<td>0.32</td>
</tr>
<tr>
<td>(Northern Ireland)</td>
<td>(542)</td>
<td>(0.001)</td>
<td>(0.005)</td>
<td>(0.02)</td>
<td>(0.03)</td>
<td>(0.07)</td>
<td>(0.08)</td>
<td>(0.06)</td>
<td>(0.03)</td>
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<tr>
<td>Republic of Ireland</td>
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<td>0.0004</td>
<td>0.0005</td>
<td>0.0005</td>
<td>0.0004</td>
<td>0.0005</td>
<td>0.0004</td>
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<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>953</td>
<td>-</td>
<td>0.0002</td>
<td>0.0008</td>
<td>0.0016</td>
<td>0.003</td>
<td>0.007</td>
<td>0.007</td>
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<td>France</td>
<td>12,100</td>
<td>0.00004</td>
<td>-</td>
<td>0.00004</td>
<td>0.00001</td>
<td>0.00003</td>
<td>0.0002</td>
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<td></td>
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</tr>
<tr>
<td>Portugal</td>
<td>1,345</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0009</td>
<td>0.001</td>
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</tbody>
</table>

* Provisional figures  
** Breeding cattle only  

(Annual percentage incidence figures have been calculated using 1993 adult cattle population figures)
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 (74, 75), 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 (74, 75, 77). Detergent-treated extracts of affected bovine brain yield scrapie-associated fibrils (SAF) visible by electron microscopy (74). 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 (44). 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, 55). BSE has also been transmitted to cattle by combined intracerebral and intravenous injection of affected brain (21) and also by the oral route following dosing with affected brain (78).

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 (76, 78; and see paragraph 11.4.3.) it will be possible to substitute fact for hypothesis.

TRANSMISSION OF BOVINE Spongiform Encephalopathy

11. The best understood naturally occurring diseases in the same category as BSE and scrapie are set out in the table at Appendix II. 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.

II.1. Route of transmission

The oral route has been shown experimentally to transmit BSE (4, 6, 30, 55, 56, 65), scrapie (61, 62), transmissible mink encephalopathy (TME) (53), kuru (35), Creutzfeldt-Jakob disease (CJD) (35) (in the case of kuru and CJD, with brain passaged 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 transmissible spongiform encephalopathies that have been studied experimentally. For mouse scrapie, the oral dose required is about a hundred thousand times greater than the intracerebral dose (47, 50). In studies of BSE (55), 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 (46).

II.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 Appendix II) 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, 25). 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 (25; 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 (25). 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 (25). 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 (25) 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. In contrast, 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 (54), 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, 25). 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 (22), marmosets (3) and mink (65). Such experimental cross-species transmissions usually require high doses. With repeated experimental passage in the new host, the incubation
period usually becomes shorter (49). 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 paragraphs 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 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 (37, 39) and goats (38) 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 Appendices IIIa and IIIb, 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 (37, 39). 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 gastro-intestinal tract and elsewhere, spleen and tonsil (37, 39). 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 (37, 39). 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 (37, 38, 39).

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 (i.e. carcass meat) (37, 38, 39)
- heart (37, 39)
- kidney (38, 39)
- colostrum (39) and milk (38)
- mammary gland (37, 38, 39)
- uterus (37, 38, 39)
- ovary (37, 38, 39)
- seminal vesicle and testis (38)
- blood clot (37, 38, 39)
- saliva/salivary gland (38, 39)
- skin (66).

11.4.3. Tissue distribution of agent in bovine spongiform encephalopathy

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, 55) or by injection (17, 18, 31, 56, 72), only by brain, cervical and terminal spinal cord and retina (7, 9, 56). Experiments with mice that were fed milk and mammary gland, placenta, lymph nodes or spleen (4, 55) 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 III did not succumb to disease within their natural lifespan (33, 56). 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 (72). 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 100-1,000 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 100 g 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 (76; 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 (37, 39).

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
TABLE III

**Tissues from clinically affected cattle with no detectable infectivity by parenteral inoculation of mice, grouped by anatomical system**

(33, 56, 72)

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<thead>
<tr>
<th>Nervous tissues</th>
<th>Alimentary tract</th>
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<tbody>
<tr>
<td>Cerebrospinal fluid</td>
<td>Oesophagus</td>
</tr>
<tr>
<td>Cauda equina (of spinal cord)</td>
<td>Reticulum</td>
</tr>
<tr>
<td>Peripheral nerves</td>
<td>Rumen (pillar)</td>
</tr>
<tr>
<td>- sciaticus (proximal)</td>
<td>Rumen (oesophageal groove)</td>
</tr>
<tr>
<td>- tibialis</td>
<td>Omasum</td>
</tr>
<tr>
<td>- splanchnic</td>
<td>Abomasum</td>
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<tr>
<td></td>
<td>Proximal small intestine</td>
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<tr>
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<td>Rectum</td>
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<table>
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<td>- buffy coat</td>
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<td>- clotted</td>
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<tr>
<td>- prefemoral</td>
<td>- foetal calf</td>
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<td>- mesenteric</td>
<td>- serum</td>
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<td>- retropharyngeal</td>
<td>Bone marrow</td>
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<td>Fat (midrum)</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
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<tr>
<td></td>
<td>Liver</td>
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<tr>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>- semitendinosus</td>
</tr>
<tr>
<td></td>
<td>- diaphragma</td>
</tr>
<tr>
<td></td>
<td>- longissimus</td>
</tr>
<tr>
<td></td>
<td>- masseter</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
</tr>
<tr>
<td></td>
<td>Trachea</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reproductive tissues</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td></td>
</tr>
<tr>
<td>Epididymis</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td></td>
</tr>
<tr>
<td>Semen</td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
</tr>
<tr>
<td>Uterine caruncle (pregnant cow)</td>
<td></td>
</tr>
<tr>
<td>Placental cotyledon</td>
<td></td>
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<tr>
<td>Placental fluids</td>
<td></td>
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<tr>
<td>- amniotic fluid</td>
<td></td>
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<tr>
<td>- allantoic fluid</td>
<td></td>
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<tr>
<td>Embryos</td>
<td></td>
</tr>
<tr>
<td>Udder</td>
<td></td>
</tr>
<tr>
<td>Milk</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 H. Fraser and D.M. Taylor.
(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 $5 \log_{10} \text{LD}_{50}/g$ (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 $\geq 138^\circ \text{C}$ 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 (43, 56, 79). This is in marked contrast to scrapie, but is consistent with the situation with natural TME (40) and kuru, which are both 'dead-end' diseases. The data summarised in 11.4.3. suggest that the tissue distribution of BSE infectivity resembles that in TME, in which maternal transmission does not occur (40), being concentrated in the central nervous system (CNS) of clinical cases at titres some 100,000 times higher than in any non-CNS tissue (Appendix IIIa). In natural sheep scrapie the lymphoreticular system contains high levels of infectivity (37, 39), placenta from affected sheep can experimentally transmit disease following oral exposure of sheep and goats (61, 62) 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. No infectivity was detected in fifty 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 10, 14, 18 and 22 months of age (6, 10, 14 and 18 months after dosing) (56, 76, 78; 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:
- carcass meat
- milk
- hides
- skins
- semen
- embryos washed in accordance with the protocols of the IETS.

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, 43, 56).

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 (67), ultraviolet and ionising radiation, and chemical disinfection (67, 68, 71). Scrapie and BSE agents appear to respond similarly to physical and chemical inactivation (69). Wet heat (48) inactivates more effectively than dry heat (14, 16, 67, 68). 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) (24). There is now some experimental evidence that temperatures at the lower end of this range may permit some residual infectivity to remain (70, 71). Sodium hypochlorite providing 2% (20,000 parts per million) available chloride acting for one hour at 20°C is completely effective (14, 48, 68, 69, 71). A useful alternative disinfectant is 1N (4%) or 2N (8%) sodium hydroxide acting for one hour at 20°C (67, 68, 69, 71) 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 EU (27). 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 carcasses,
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-specified bovine offals 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 (paragraphs 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 that farmers, veterinarians and the national Veterinary Services are aware of the clinical signs of BSE; and
14.1.3. ensure that 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 carcasses 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. Considering feed as 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 (26). 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 (27). Observance of a ban can be monitored by sampling and testing feed and feed ingredients, using an enzyme-linked immunosorbent assay (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 bovine spongiform encephalopathy 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 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 (37, 39). 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 task 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 (46, 47, 50), 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 OIE Manual of Standards for Diagnostic Tests and Vaccines (60). The carcasses 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 OIE International Animal Health Code (see Appendix I), 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 of mammalian meat-and-bone meal in any farm animal feed or in use as an agricultural fertiliser in April 1996. The new controls were fully enforced by 30 April 1996.

* *
* *
Chapter 3.2.13. of the Office International des Epizooties (OIE) 
*International Animal Health Code, as approved by the 
International Committee on 24 May 1996*

**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. 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 (under study). 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 number of investigations and confirmed cases.

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

**ARTICLE 3.2.13.2.**

Countries may be considered free of BSE if:

1) there has been no clinical case of BSE, the disease is notifiable, and an effective and continuous surveillance and monitoring system is practised; or

2) all cases of BSE have been clearly demonstrated to originate directly from the importation of live cattle from countries where BSE has been reported, provided that the disease is made notifiable and suspect animals are slaughtered, investigated and, if disease is confirmed, completely destroyed and an effective and continuous surveillance and monitoring system is practised.

**ARTICLE 3.2.13.3.**

*Veterinary Administrations* can authorise without restriction the import or transit through their territory, directly or indirectly, of milk, milk products, hides and skins originating from healthy animals from countries where BSE has been reported. There is also no scientific evidence of a risk associated with the trade in semen from healthy animals. By-products, such as gelatin, collagen and tallow, are considered to be safe if produced by processes (under study) which inactivate any residual BSE infectivity.
ARTICLE 3.2.13.4.

When importing from countries with a low incidence of BSE, 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) an effective and continuous surveillance and monitoring system is practised in accordance with Article 3.2.13.1.;
5) the feeding of meat-and-bone meal derived from ruminants to ruminants has been banned and effectively enforced;
6) 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 calves 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:
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 effectively enforced; 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; and
3) have never been fed ruminant meat-and-bone meal.

ARTICLE 3.2.13.6.

When importing from countries with a low incidence of BSE, 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) affected cattle are slaughtered and completely destroyed;
3) ante-mortem inspection is carried out on all bovines;
4) an effective and continuous surveillance and monitoring system is practised in accordance with Article 3.2.13.1.;
5) the meat products do not contain brain, eyes, spinal cord or distal ileum from cattle over six months of age which were born before the date on which the feed ban referred to in paragraph 5 of Article 3.2.13.4. was effectively enforced.

ARTICLE 3.2.13.7.

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

for fresh bone-in meat from cattle

the presentation of an international sanitary certificate attesting, in addition to the requirements set forth in Article 3.2.13.6., that:
1) the tissues listed in Article 3.2.13.12. are removed from all cattle at slaughter and destroyed;
2) the cattle from which the meat originates:
   a) were born after the date on which a ban on the use of ruminant meat-and-bone meal in feed for ruminants has been effectively enforced; or
   b) were born and had only been kept in herds in which no case of BSE had been recorded; and
   c) have never been fed ruminant meat-and-bone meal.

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) affected cattle are slaughtered and completely destroyed;
3) ante-mortem inspection is carried out on all bovines;
4) an effective and continuous surveillance and monitoring system is practised in accordance with Article 3.2.13.1.;
5) the tissues listed in Article 3.2.13.12. are removed from all cattle at slaughter and destroyed;
6) nervous and lymphatic tissues exposed during the cutting process have been removed and destroyed.
ARTICLE 3.2.13.9.

When importing from countries with a low incidence of BSE, 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) an effective and continuous surveillance and monitoring system is practised in accordance with Article 3.2.13.1.;
5) the feeding of meat-and-bone meal derived from ruminants to ruminants has been banned and effectively enforced;
6) 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 3.2.13.10.

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 that embryos/ova for export are derived from females which comply with the conditions in Article 3.2.13.5. and paragraph 6 of Article 3.2.13.9.

ARTICLE 3.2.13.11.

Meat-and-bone meal containing any ruminant protein which originates from countries with a high incidence of BSE should not be traded between countries.

Meat-and-bone meal containing any ruminant protein which originates from countries with a low incidence of BSE should not be traded between countries for use in ruminant feed. For other uses, it should have been processed in plants which are approved and regularly controlled by the Veterinary Administration following validation that each plant can achieve the parameters judged effective for that type of process for inactivation of animal transmissible spongiform encephalopathy agents (under study).

ARTICLE 3.2.13.12.

Bovine brains, eyes, spinal cord, tonsils, thymus, spleen and distal ileum (tissues under study) 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.
Bovine brains, eyes, spinal cord and distal ileum (tissues under study) and protein products derived from them from cattle over six months of age which originate from countries with a low incidence of BSE and were born before the date on which the feed ban referred to in paragraph 5 of Article 3.2.13.4. was effectively enforced, should not be traded between countries, unless they comply with the provisions of Article 3.2.13.11.

ARTICLE 3.2.13.13.

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. and Article 3.2.13.2.;
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 II

**Naturally occurring transmissible spongiform encephalopathies**

*Distribution of naturally occurring transmissible spongiform encephalopathies and affected hosts*

*(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>World-wide</td>
</tr>
<tr>
<td></td>
<td>GSS</td>
<td>Familial, world-wide, 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, elk</td>
<td>CWD</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>United Kingdom, Republic of Ireland, France, Portugal, Switzerland</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nyalal</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, Republic of Ireland **</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 = 85%; familial = 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)

SE: Spongiform encephalopathy

FSE: Feline spongiform encephalopathy
Creutzfeldt-Jakob disease

Creutzfeldt-Jakob disease (CJD) occurs world-wide at an incidence of about one case per 2 million per annum (15). However, there have been rare instances of geographical clustering of cases where the annual incidence has been 30 times this norm or more (15). Such clusters have been found in Israel (58) and Slovakia (57). They are clearly related to the presence of point mutations at codon 200 of the PrP gene (36). 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 transplants, dura mater transplants (all of which present themselves as relatively short incubation dementia) (13) and human cadaver, pituitary-derived growth hormone or gonadotrophin (which present themselves with relatively long [9-30 years] incubation and cerebellar signs) (13, 15). Epidemiological studies in many countries, including France (15, 20) and the UK (41), 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 was very similar in France, Germany, Italy, the Netherlands and the UK, and contrasts with the marked variation in incidence of bovine spongiform encephalopathy (BSE) in these countries. This suggests that the emergence of BSE has not resulted in a change in the occurrence of CJD in 1993 (1, 25, 84). 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 Office International des Epizooties (OIE) International Animal Health 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 (85). 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 electroencephalogram findings typical of CJD and clinical onset from 1994 onwards in these cases suggest 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 Lyons, 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 has 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 (86). 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 (87) and the disease
reviewed (64). The presence of scrapie-associated fibrils (SAFs) and $PrP^{Sc}$ (63) and transmission of disease to mice following parenteral inoculation of brain tissue from an affected cat (18, 34) have confirmed that FSE is a member of the naturally occurring TSE group of diseases. 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, 45, 52; 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).
Appendix IIIa

Infectivity titres (bioassayed 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 bovine spongiform encephalopathy (BSE) (32, 33, 38, 39, 72)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>titre (mean ± SEM of [n] samples)</th>
<th>Titre&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scrapie-affected sheep</td>
<td>Scrapie-affected goats</td>
</tr>
<tr>
<td>Category I</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>Category II</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>Category III</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 ± 0.1 (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>Category IV</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>

SEM: standard error of the mean
...

<sup>a</sup> Tities are expressed as arithmetic means of log<sub>10</sub> mouse intracerebral LD<sub>50</sub>/g or ml of tissue (+ve = ≥ 2.0)

The classification of tissues is according to the 1991 Guidelines of the European Community Committee for Proprietary Medicinal Products

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 30 µl of inoculum injected intracerebrally)

Data courtesy of R.H. Kimberlin (46)
Appendix IIIb

Scrapie infectivity titres in Category I and II tissues from pre-clinically infected sheep and from clinical cases

(39)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Pre-clinical</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-8 months$^b$</td>
<td>10-14 months$^c$</td>
</tr>
<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cerebral cortex</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>diencephalon</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>midbrain</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>medulla</td>
<td>...</td>
<td>&lt; 2.0 (8)</td>
</tr>
<tr>
<td>Cervical cord</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Averages</td>
<td>&lt; 2.0</td>
<td>&lt; 2.4</td>
</tr>
<tr>
<td><strong>Category II (lymphoreticular system)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bronchio-mediastinal</td>
<td>...</td>
<td>&lt; 2.0 (8)</td>
</tr>
<tr>
<td>mesenteric</td>
<td>&lt; 2.0 (16)</td>
<td>3.3 ± 0.4(8)</td>
</tr>
<tr>
<td>prescapular</td>
<td>&lt; 2.0 (16)</td>
<td>&lt; 2.0 (8)</td>
</tr>
<tr>
<td>prefemoral</td>
<td>&lt; 2.0 (6)</td>
<td>&lt; 2.4 ± 0.2 (3)</td>
</tr>
<tr>
<td>retropharyngeal</td>
<td>&lt; 2.0 (16)</td>
<td>3.8 ± 0.4 (3)</td>
</tr>
<tr>
<td>ileum</td>
<td>&lt; 2.0 (16)</td>
<td>&lt; 3.1 ± 0.5 (3)</td>
</tr>
<tr>
<td>spleen</td>
<td>&lt; 2.0 (16)</td>
<td>3.0 ± 0.4 (3)</td>
</tr>
<tr>
<td>tonsil</td>
<td>&lt; 2.0 (10)</td>
<td>&lt; 2.6 ± 0.3 (3)</td>
</tr>
<tr>
<td>Averages</td>
<td>&lt; 2.0</td>
<td>&lt; 2.8</td>
</tr>
</tbody>
</table>

SEM: standard error of the mean

...: not available

$^a$ All titres are expressed as arithmetic means of log$_{10}$ mouse intracerebral LD$_{50}$/g or ml of tissue

$^b$ None of the tissues from lambs aged eight months or less had any detectable infectivity. The values shown are maxima based on the limits of the detectability of the bioassay in mice (calculated for 30 µl of inoculum injected intracerebrally)

$^c$ Infectivity was detected in only eight out of fifteen sheep exposed to scrapie

$^d$ Infectivity was detected in only one out of three sheep exposed to scrapie

Data courtesy of R.H. Kimberlin

* *
REFERENCES


