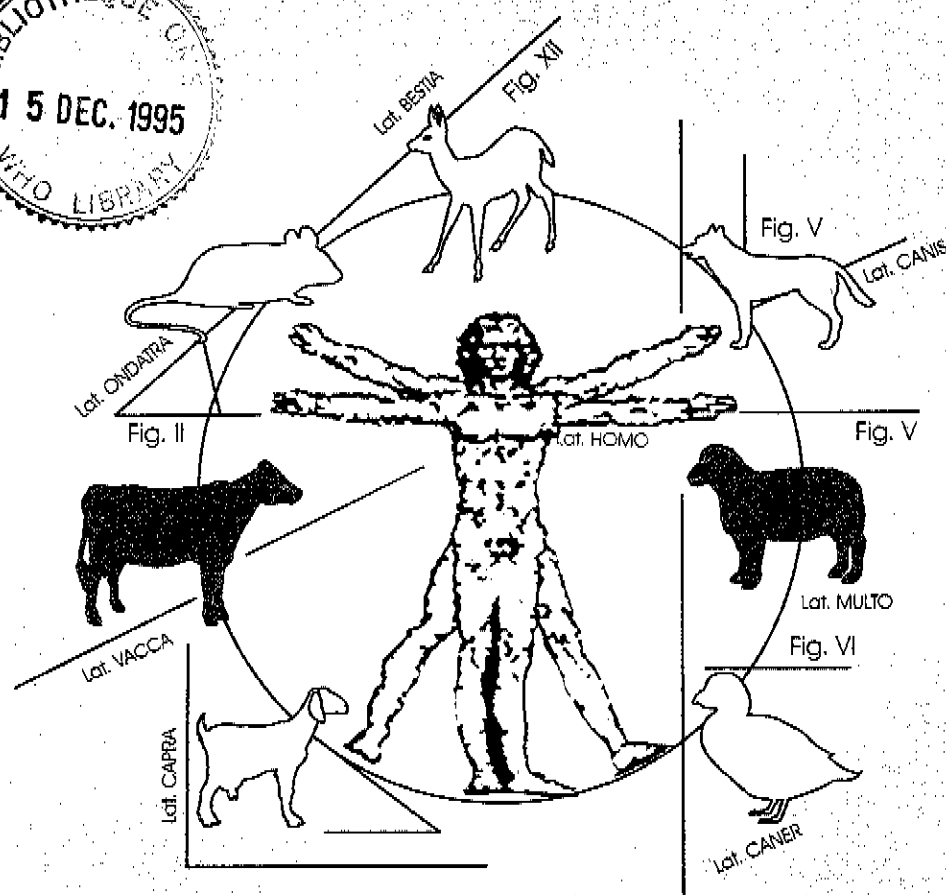
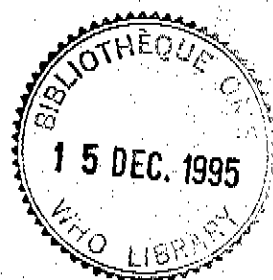


Report of a WHO consultation on public health issues related to human & animal transmissible spongiform encephalopathies

GENEVA, 17-19 MAY 1995



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VETERINARY PUBLIC HEALTH UNIT

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1. INTRODUCTION

A meeting on the transmissible spongiform encephalopathies (TSE) was held in Geneva, Switzerland, from 17-19 May 1995. This group of diseases includes bovine spongiform encephalopathy (BSE) which was first described in the United Kingdom but which has occurred subsequently in several other countries.

This meeting, following the two previous WHO consultations held in 1991 and 1993, reviewed the existing state of knowledge on spongiform encephalopathies, and evaluated pathways of transmission and associated hazards. This included the possible implications of the animal diseases, with special reference to BSE, arising from the use of animal tissues as animal feed, human food and in the preparation of medicinal and other products for human and animal use. During this Consultation recommendations were made to national health authorities on appropriate measures to minimize and manage any risk which BSE may pose to public health. Dr Gibbs was elected Chairman, Drs H. Windemann and M. Savey were nominated as Rapporteurs.

2. WORLDWIDE OCCURRENCE OF RECENTLY IDENTIFIED ANIMAL TSEs

2.1 *BSE epidemiology and control*

Between November 1986 to 12 May 1995, 148 200 cases were confirmed in 32 385 herds in Great Britain (GB). During the same period, 1 564 cases were confirmed in 1 074 herds in Northern Ireland (although the first case in the province was not confirmed until 29 November 1988).

At the time of this consultation, BSE had been reported in only 10 countries and territories outside the United Kingdom (UK).

In six of these (Canada, Denmark, Falkland Islands, Germany, Italy and Oman), the only cases (ranging from one to four per area) had been in cattle exported from the UK. In the other four countries, a limited number of cases (ranging from 12 to 143 per country) had occurred only in native-born cattle (France and Switzerland) or in native-born cattle and cattle imported from the UK (Republic of Ireland and Portugal) (see Table 1, page 25).

2.1.1 Country with a high incidence of BSE: UK

A number of descriptive epidemiologic features have remained unchanged during the course of the epidemic. These include a markedly greater incidence of dairy herds affected compared with beef suckler herds, which cumulatively are 53.3% and 14.7%, respectively. The number of suspect cases reported weekly has declined since 1993; during April and the first week of May 1995 it was approximately 50% of that reported during the same period in 1994. The peak incidence of BSE occurred at the end of 1992/beginning of 1993, which was equivalent to an annual incidence in adult cattle in GB of less than 1%. The subsequent reduction in incidence has been the result of the progressive reduction in incidence in each yearly age class commencing, in 1991, with a lower incidence in 2-year old animals, and with a reduction in incidence in animals less than 6-years old in 1994. A similar reduction in 6-year old animals is expected in 1995.

This indicates the effect of the ban on using ruminant protein for ruminant feeds which was introduced on 18 July 1988 in GB. However, it is likely that a quantity of contaminated feed manufactured before this date was used for some time thereafter for cattle feeding. Furthermore, the use of brain, spinal cord, tonsil, spleen, thymus and intestines - from duodenum to rectum inclusive - collectively known as the Specified Bovine Offals (SBOs) (see Sections 5.1.1 and 7.1) in feed for non-ruminant animals and poultry has only been prohibited as from September 1990. It is possible that feed containing meat and bone meal intended for pigs or poultry could have been mistakenly fed to cattle, or that feed

intended for ruminants could have accidentally been contaminated in mills or feed stores. However, these factors are diminishing in importance and additional measures are being taken to reinforce the effectiveness of the feed ban. The number of BSE affected animals born in each year after the ban is markedly declining as a result of the decreasing risk in exposure (see Figure 1, page 30).

2.1.2 Countries with lower incidence of BSE in native cattle

France, Ireland, Portugal and Switzerland have diagnosed BSE in native cattle, although at a significantly lower incidence than that observed in the UK. All four countries have taken appropriate actions to minimize the risk of further transmission, including (a) governmental action to make BSE a compulsorily notifiable disease, (b) complete destruction of all affected cattle, (c) a ban on the feeding to ruminants of protein products originating from ruminants, and (d) establishment of an active BSE surveillance programme. Epidemiologic investigations in these countries attribute the majority of cases in native cattle to consumption of meat and bone meal containing ruminant-derived protein and it is possible that some of this may have originated from the UK. However, in a few cases intensive investigations failed to associate them with the feeding of meat and bone meal. More detailed information for the above-mentioned countries is given in Annex 2 and Table 1.

2.1.3 Countries and territories with BSE in imported cattle only

All cases of BSE in Canada, Denmark, Falkland Islands, Germany, Italy and Oman have clearly been demonstrated to originate directly from the importation of live cattle from the UK. Measures including the complete destruction of all confirmed cases, were taken by each country. No cases of BSE have been observed in native cattle in any of these countries.

2.2 Other new animal TSEs

Before 1986, three naturally occurring spongiform encephalopathies (SEs) had been reported in animals (scrapie of sheep and goats, transmissible mink encephalopathy (TME) and chronic wasting disease in mule deer and elk (CWD)). In November 1986 the first cases of BSE were identified in GB. Between then and the end of 1991, domestic cats and five further species of Bovidae were reported to have succumbed to SE. Because of the temporal and geographic coincidence of these diseases with the emergence of BSE, and because three of them were shown later to be associated with transmissible agents having similar strain characteristics when experimentally injected into a panel of mice of specified genotypes, it is presumed that all share a common origin and that the probable vehicle of infection was feed.

Spongiform encephalopathy was reported for the first time in wild mountain sheep (*Ovis musimon*) in GB but is presumed to be more closely related to scrapie of sheep and goats than BSE (Wood *et al.*, 1992).

Since 1991, spongiform encephalopathy has been reported in one further Bovidae species in captivity in GB, a single case in a scimitar-horned oryx (Bradley, 1994), and in three species of captive wild Felidae: a puma, four cheetahs (two in GB, one each in Australia and the Republic of Ireland, both exported from GB) (Kirkwood and Cunningham, 1994), and an ocelot. Up to January 1995, Feline Spongiform Encephalopathy (FSE) had been confirmed in 61 domestic cats in the UK, and in one indigenous cat in Norway; and SE had been confirmed in one nyala, one gemsbok, one Arabian oryx, one scimitar horned oryx, five eland and six greater kudu - all in the UK.

3. CURRENT STATUS OF BSE RESEARCH

3.1 Experimental host range of BSE

BSE has been experimentally transmitted by parenteral challenge to cattle (Dawson *et al.*, 1994a), sheep and goats (Foster *et al.*, 1993),

pigs (Dawson *et al.*, 1990), marmosets (Baker *et al.*, 1993), mink (Robinson *et al.*, 1994) and mice (Fraser *et al.*, 1992; Bruce *et al.*, 1994).

Experimental oral transmission has been attempted in all these species except marmosets, and has been successful in sheep and goats (Foster *et al.*, 1993), mink (Robinson *et al.*, 1994), and mice (Middleton and Barlow, 1993). The minimum incubation periods were generally longer than when parenteral routes were used, even though the amount of infected material administered was much larger.

Some of the cattle challenged orally with 100 g infected brain from cattle with confirmed BSE have developed BSE, about three years after dosing (Wells *et al.*, 1994, G.A.H. Wells, 1995, personal communication). (see 3.3, p. 8)

Transmission has so far been unsuccessful following oral challenge of pigs with either infected brain from cattle with confirmed BSE (five years post-challenge), or sheep scrapie brains (more than one year post-challenge).

Unsuccessful attempts have been made to transmit BSE directly to hamsters and to chickens. The latter study, using oral or parenteral routes of infection, is not yet complete five years after challenge.

3.2 Tissue distribution of agent in confirmed cases of BSE

Studies of tissues from naturally infected cattle, clinically affected with BSE have detected infectivity only in brain and spinal cord using bioassays in susceptible mice.

Infectivity has not been detected in a large number of extraneural and other neural tissues following bioassay in susceptible mice (Table 3, page 27) (Fraser and Foster, 1994; MAFF¹,

¹ MAFF (1995). Bovine spongiform encephalopathy in Great Britain: a progress report. May 1995. Ministry of Agriculture, Fisheries and Food and Food, United Kingdom.

1995, H. Fraser, 1995, personal communication). Large quantities of some tissues, including udder/milk, spleen, placenta, supramammary, mesenteric and carcass lymph nodes from clinically-affected, confirmed cases of BSE were fed to susceptible mice, but no disease was transmitted even after sub-passage (Middleton and Barlow, 1993). In a further study using milk from naturally infected cattle at different stages of lactation, either fed to mice over a long period, or by a single parenteral inoculation, no disease or SE was transmitted (Taylor *et al.*, 1995)

Cattle have been challenged by the oronasal route with placenta from clinically affected cattle in advanced pregnancy, which were subsequently confirmed to have had BSE. The challenged cattle remain healthy after more than five years. Some cattle in this study were killed two years after challenge and showed no evidence of SE, and tissues from them did not transmit disease to mice.

The titre of infectivity in terminally affected cattle brain is up to $10^{5.3}$ mouse intracerebral, LD₅₀ per gram (Fraser *et al.*, 1992). This is of the same order of magnitude as is found in natural scrapie in goats and Suffolk sheep (Hadlow *et al.*, 1980, 1982; see Table 3, p. 27).

3.3 Tissue distribution of agent in cattle during the incubation period

Because asymptomatic naturally infected cattle cannot be identified, a study is being carried out to determine the temporal and spatial development of infectivity and pathology following oral exposure of calves to a single, large (100g) dose of affected cattle brain homogenate (Wells *et al.*, 1994). Challenged and control animals were killed at approximately four month intervals, commencing at six months of age (two months after dosing). Some 46 tissues were collected at each kill (identical tissues from different challenged animals were pooled), and each animal tissue was separately inoculated parenterally into susceptible mice which are monitored clinically throughout their lifespan, and brains are examined histopathologically

after death.

Cattle in kill numbers 1 to 8 have shown no clinical evidence of SE prior to slaughter, but by January 1995, clinical disease consistent with a statutory clinical diagnosis of BSE had been observed in some cattle. BSE has been confirmed by microscopic examination of the brains of these cattle.

No infectivity was found in any tissues of cattle killed at six months of age (two months after dosing) but infectivity was found in the distal ileum of challenged calves at kills 2, 3, 4 and 5 (10, 14, 18 and 22 months of age, 6, 10, 14, and 18 months after infection; G.A.H. Wells, personal communication, 1995). No infectivity has been found in any other tissues from kills 2 and 3, but examination of other tissues from kills 4 and 5, and from all subsequent kills is still incomplete (G.A.H. Wells, personal communication, 1995). This is reminiscent of results from studies in natural scrapie in Suffolk sheep (Hadlow *et al.*, 1982) where the distal ileum was among the first tissues to show infectivity (at 10 months of age).

3.4 Vertical and horizontal transmission studies

This aspect of the epidemiology of BSE is addressed in a long-term cohort study. No results will be available until the study is completed early in 1997. In the meantime a case-control study of animals born on or after 1 November 1988 has been conducted to determine the risks of maternal and horizontal transmission (Hoinville *et al.*, 1995). The results indicate that the offspring of animals which developed clinical BSE were not found significantly more often among the cases, but that there was a marginal statistically significant risk for animals born up to three days after a subsequently affected animal calved. It is difficult to reconcile the two findings, and probable that the latter does not indicate a causal association, particularly as there was no evidence that the risk was less if the animal was born in isolation or if the placenta was collected and removed. It is also pertinent that in other studies the placenta has shown no detectable infectivity when bioassayed in mice, or

following oro-nasal challenge in calves which are still being observed five years after the challenge.

3.5 Strain characteristics of TSE agents in mice

Transmissions of BSE from seven geographically and temporally unrelated cattle sources from the UK (Bruce *et al.*, 1994) and preliminary data from two contemporary sources in native-born cattle from Switzerland (Bruce, 1994) have yielded remarkably uniform disease characteristics (incubation period length and lesion profile) in each of four inbred lines of mice and an F₁ hybrid, with differing, but consistent, characteristics between each inbred line. In contrast, isolates from more than 20 previous and contemporary cases of natural sheep and goat scrapie are quite different (Bruce *et al.*, 1994).

Transmissions to mice of spongiform encephalopathy from three domestic cats with FSE, sheep, goats and a pig with experimental BSE, as well as a nyala and a greater kudu with naturally occurring SE, have given results similar to those from direct transmissions from BSE affected cattle (Bruce *et al.*, 1994). These results show that the BSE agent has strain characteristics different from known strains of scrapie.

3.6 BSE and scrapie agent decontamination

The infectivity in macerates of bovine brain infected with BSE, and of rodent brain infected with the 263K or ME7 strains of scrapie agent, was substantially reduced when subjected to porous load autoclaving at 134° - 138°C for up to 60 minutes. However, none of the autoclaving procedures totally inactivated any of these agents.

There was no detectable infectivity after homogenates of BSE-infected brain had been exposed for 120 minutes to solutions of sodium hypochlorite containing 16 500 ppm available chlorine. However, dichloroisocyanurate, giving the same levels of available chlorine, did not completely inactivate BSE agent (Taylor *et*

al., 1994). Homogenates of BSE-infected bovine brain and rodent brain infected with either 263K or ME7 strains of scrapie agent, were exposed for 120 minutes to 1M or 2M sodium hydroxide, but neither procedure completely inactivated any of the agents (Taylor *et al.*, 1994).

A study of the inactivation of BSE and scrapie infectivity in ruminant waste by various rendering processes in use in Member States of the European Union (EU) is in progress, co-funded by the EU. Animal waste was spiked with a pool of 850 brains from cattle with BSE; the infectivity in the pool was measured by bioassay in mice. The spiked material was subdivided and rendered in a pilot-scale plant by processes representative of those used in the EU and by employing different time/temperature combinations. The end-products of rendering, meat and bone meal (all samples) and tallow (some samples), were bioassayed. The titre in the brain pool was only high enough to detect an 80-fold loss in the titre of rendered material. Nevertheless, two processes reduced the titre in meat and bone meal less than 80-fold. All tallow samples tested showed no detectable infectivity. These results support the view that infected ruminant waste rendered via specific processes to produce meat and bone meal could be the source of infection for cattle.

The study is being repeated using brain from natural cases of scrapie in sheep. The results of the BSE study have not yet been published in full.

4. CREUTZFELDT-JAKOB DISEASE

Since the previous consultation at WHO on BSE and Creutzfeldt-Jakob disease (CJD), research has continued in the UK and elsewhere on the epidemiology of CJD and there have been other developments in the field, most noticeably in relation to molecular biology.

4.1 Incidence of Creutzfeldt-Jakob disease

Epidemiological surveillance of CJD has continued in the UK since 1990. The number of sporadic cases of CJD has risen from 26 in 1990 to 46 in 1994 and this is, in part, related to increased ascertainment in the elderly. Through the BIOMED1 programme of the EU, epidemiologic surveillance programmes in a number of European countries have been coordinated in order to share common methodologies, diagnostic criteria and case-control questionnaires.

In 1993 and 1994 the incidence of CJD in France, Germany, Italy, the Netherlands and the UK was remarkably similar; this also applies to other analyses including age-specific incidence, sex ratio and duration of illness. The incidence of CJD in a number of other countries worldwide is also remarkably consistent. In countries in which there have been serial surveys there has been an apparent rise in the incidence of CJD with time, almost certainly due to improved case ascertainment.

4.2 Risk factors for Creutzfeldt-Jakob disease

4.2.1 Iatrogenic cases

The major change in the epidemiology of CJD since 1990 has been an increase in the number of cases of iatrogenic CJD that have been identified. Worldwide there are 62 cases of CJD in human growth hormone recipients and 20 cases of CJD in recipients of human dura mater grafts. All iatrogenic cases have involved cross-contamination of human tissues sourced from or adjacent to the central nervous system. The results of bioassays of whole blood and blood fractions are inconsistent, but epidemiologic evidence indicates that blood transfusion is unlikely to be a risk factor for CJD.

4.2.2 Occupation

The occupational history in individuals dying of CJD has been studied since 1990 in the UK. A case-control study has provided no evidence of increased risk in relation to specific occupations. However, three dairy farmers who had BSE in their herds have been identified as suffering from Creutzfeldt-Jakob disease. Statistical analysis of relative risk is extraordinarily difficult because of the small number of patients, the small "at-risk" populations, and the different denominators that can be used in statistical analyses. Through the BIOMEDI programme it has been possible to compare the incidence of CJD in farmers in participating countries. These data indicate that the incidence of CJD in those ever employed in farming is similar in participating countries, regardless of their BSE status, and that the incidence of CJD in farmers is also comparable in participating countries.

4.2.3 Diet

Since 1990, a case-control study of dietary exposures has been carried out in the UK. In 1993, analysis showed an apparently increased risk of developing CJD in relation to the consumption of "puddings". However, this particular foodstuff was no longer an apparent risk factor in 1994, indicating the fragility of data based on small patient numbers when the apparent statistical significance is associated with wide confidence intervals. In the 1994 analysis, a number of foodstuffs were apparently associated with an increased risk of CJD, most notably venison with an odds ratio of 9.3, and veal with an odds ratio of 13.3, but there is good evidence that these findings are due to selective recall bias².

4.2.4 Molecular Biology

Approximately 12% of all cases of CJD are associated with mutations of the prion protein gene. In the majority of these cases, there is a positive family history of a neurodegenerative

² CJD Surveillance in the UK: 3rd Annual Report of the National CJD Surveillance Unit, Edinburgh: September 1994.

disorder and in many, there is a family history of Creutzfeldt-Jakob disease itself. Seventeen point or insertional mutations have now been identified and there are no familial aggregates of cases in which a mutation of the prion protein gene is not present.

Analysis of the common polymorphisms at codon 129 of the PrP gene has also demonstrated that approximately 80% of all cases of sporadic CJD are associated with methionine homozygosity while in iatrogenic cases, both valine and methionine homozygotes are over-represented in relation to the normal population.

4.3 Conclusion

Epidemiologic surveillance of CJD in the UK has shown no definite change that can be attributable to the occurrence of BSE. However, continuing epidemiologic surveillance is essential since a public health risk due to BSE cannot be excluded in view of the potentially prolonged incubation periods in these diseases.

5. RISK ASSESSMENT OF PUBLIC AND ANIMAL HEALTH CONSEQUENCES OF BSE

Prior to BSE, over 30 species in four mammalian Orders had been shown to be experimentally susceptible to one or other of the transmissible agents associated with scrapie, TME, kuru and CJD. This suggests that all mammals, including man, should be regarded as being potentially susceptible to BSE if sufficiently exposed. The same conclusion follows from the fact that susceptibility to TSEs depends on the PrP gene which appears to be present in all mammals. Therefore, the risks to other species from BSE have to be assessed quantitatively in terms of the effective exposure. Exactly the same principles of risk assessment should apply to both animals and humans, exposed via food, pharmaceuticals or cosmetics.

The effective exposure is a function of three

major factors: amount of agent, route of exposure and the species barrier.

5.1 Amount of the agent

Exposure depends on the amount of a given bovine tissue, the infectivity titre of the tissue and any reduction in titre achieved during manufacture or preparation.

5.1.1 Specified Bovine Offals (SBOs)

The bovine tissues which by analogy with natural scrapie, would be most likely to support significant multiplication of the BSE agent and therefore, the most relevant to the foodborne exposure of man, are brain, spinal cord, tonsil, spleen, thymus and intestines (the last because of the presence of Peyer's patches) (Report of the WHO Consultation on Public Health Issues Related to Animal and Human Spongiform Encephalopathies, Geneva, 12-13 November 1991 - WHO document WHO/CDS/VPH/92.104). Collectively these are known as the specified bovine offals (SBOs) and their use in human food was banned in the UK in 1989, and later in some other countries with BSE. It should be noted that the purpose of the SBO ban was to minimize exposure, not to eliminate all risks.

Logically, the SBOs should also include lymph nodes but it was considered that obvious lymph nodes and obvious nervous tissue would be excluded as part of normal working practices in abattoirs, and when individual cuts of meat are prepared.

To be effective, the SBOs ban had to be applied to all cattle regardless of whether or not they were infected. The only exception is calves younger than six months; they were exempted on the grounds that no infectivity had been found in natural scrapie in Suffolk sheep under 10 months old (see also 3.3 & 5.1.2). Furthermore, following the introduction of the ruminant protein feed ban in 1988, fewer and fewer cattle would have been exposed to foodborne infection.

5.1.2 Other tissues

The circumstances of greatest risk could be defined further in terms of the limited number of tissues in which TSE agents can be demonstrated to replicate to high titres e.g. brain and spinal cord. All other tissues, including milk and muscle, showed little or no detectable infectivity by experimental parenteral injection, and according to a number of studies mainly carried out in mice (see Section 3.2), none at all by alimentary exposure because of its lower efficiency of infection. There is no evidence that repeated exposures to limiting infectious doses can accumulate to yield a higher effective dose (Kimberlin, 1979; Kimberlin & Walker, 1989).

A growing body of data is now available on the infectivity titres in tissues from confirmed cases of BSE (see Table 3, pp. 27-29). So far, only brain and spinal cord have shown any detectable BSE infectivity. This does not necessarily mean that none is present in any of the other tissues because BSE (and scrapie) titres are likely to be underestimated when measured across a species barrier, in mice. An experiment to determine this is in progress. However, it is important to note that the risks of BSE infection of man and other species would also be across a species barrier.

There are several factors which suggest that detection of infectivity in distal ileum, but no other tissues, from cattle killed at six and 10 months following experimental oral challenge with a single dose of 100 g of a bovine brain pool (see Section 3.3) does not indicate that the risk from calves was previously underestimated.

- The study described is experimental and, of necessity, involved a very high dose of BSE which does not reflect the field situation with respect to level of exposure. The incubation period may be expected to be influenced by the dose (among other factors).
- Calves for veal are usually fed a milk-based diet (though not always).
- Furthermore, animals currently under six months of age were born at least six years

after the feed ban was introduced in the UK, and have, therefore, not been exposed to foodborne infection (except for possible rare accidents). Therefore, while the data indicate that infectivity could be present in calves six months after oral exposure to a high dose of infected brain, this is considered to present a very significantly lower threat of exposure of the human population compared to the threat from older animals.

5.2 Route of exposure

Comparisons of the relative efficiencies of different routes of infection are largely based on studies using the 139A strain of mouse-adapted scrapie bioassayed in mice, when there is no species barrier. It was found that the oral route of infection was about 100 000 times less efficient than the intracerebral (i.c.) route. A measure of the oral-i.c. difference across the bovine-mouse species barrier can now be obtained from the data published by Middleton and Barlow (see Kimberlin, 1994). The difference is again about 100 000-fold, possibly more.

A consistent finding throughout the BSE epidemic in the UK is the low average within-herd incidence of BSE cases. Because genetic variation in the PrP or other cattle genes is not a major factor in the occurrence of BSE, this important finding can be interpreted as a consequence of a low average exposure to BSE infection to which all cattle in affected herds are more or less equally susceptible.

The concept of low-dose exposure of cattle to BSE strengthens the main conclusion from many BSE risk assessments that UK-like epidemics are extremely unlikely to occur in other countries as a consequence of endemic scrapie (see Kimberlin & Wilesmith, 1994).

In light of the present and provisional data on horizontal and maternal transmission, the possibility that limited numbers of animals born after the feed ban are infected post- or perinatally cannot be excluded. However, maternal transmission alone, however often it occurred, would not prevent elimination of BSE in the UK, because the necessary 1 to 1 contact

rate could not be achieved.

5.3 Species barrier

The species barrier depends on at least two factors: (a) the PrP gene sequence of the species infected and the species exposed, and (b) the strain of agent. The species barrier can be a major obstacle to experimental transmissions between species but it is usually overcome by parenteral exposure to very high-titre material. Natural exposure, however, will often be insufficient (see Kimberlin and Wilesmith, 1994).

6. PREVENTION AND CONTROL OF BSE IN CATTLE

Activities of veterinary services designed to prevent and control BSE disease in cattle protect both animal and public health.

6.1 Control of BSE in affected countries

The participants in the Consultation wish to reiterate the basic measure for the control of the disease, where it is present, which consists of eliminating the exposure of cattle to TSE agents through feed. This has been achieved in countries where BSE occurs by means of a ban on the use of ruminant proteins (other than milk) to feed cattle.

6.2 Prevention of spread of BSE between countries

Detailed guidelines covering these aspects were proposed by the specialists advising the Office International des Epizooties (OIE) during a meeting held in Paris from 1-2 September 1994. Recommendations to national veterinary administrations importing live animals, fresh meat and meat products, including meat and bone meal, from areas with either a high or low incidence of BSE, or of unknown status, have been specified in the revised chapter 3.2.13. of the OIE International Zoo-Sanitary Code on Bovine Spongiform

Encephalopathy approved by the OIE International Committee during the 63rd General Session in May 1995. The revised chapter re-emphasizes the importance of surveillance and safe sourcing, and strengthens recommendations to reduce risks of importation of BSE into a country (for details see OIE chapter on BSE shown in Annex 3).

OIE has recommended that cattle sent from countries with a high or low incidence of BSE must not be the first generation progeny of BSE suspect or confirmed females. It is considered that calves which meet this and other conditions specified by OIE may be imported from countries with a high or low incidence of BSE without significant risk of introducing the BSE agent into other countries. It is important that the UK and other countries ensure that the various feed bans are properly implemented, and that certification is checked to ensure that cattle exported from a country with a high or low incidence are not the first generation progeny of BSE suspect or confirmed females.

6.3 Prevention of indigenous BSE

In BSE-free countries where the relevant risk factors are present (e.g. use of ruminant protein in ruminant feed, occurrence of scrapie, size of sheep population relative to that of cattle, etc.), consideration might be given to excluding from ruminant feed selected tissues which might contain high titres of the agent. These tissues are the SBOs described in sections, 2.1.1, and 5.1.1.

6.4 Assessment of country status

The BSE status of a country cannot be known unless there are sufficient surveillance data. In countries where the disease is not reported an assessment of the BSE risk factors may provide an estimate of potential risk of emergence of the disease.

7. MINIMIZING THE PUTATIVE RISKS OF BSE TO HUMANS

7.1 Putative foodborne transmission from cattle

Since none of the food processing technologies, such as heat treatment (cooking, pasteurization, sterilization, freezing, drying), chemical treatment (acidification, fermentation including pickling, and irradiation), are fully effective to inactivate the infectious agent, prevention of foodborne transmission must be based on sourcing from healthy animals and, in countries with a high incidence of disease, preventing the use of SBOs which in an incubating animal may contain infectivity (see Tables 2 and 3, pages 26 & 29). Infection is unlikely to be detected in any tissues of naturally infected calves which are incubating BSE before they are six months old. Any detectable infectivity in animals older than six months is expected to remain confined to the central nervous system and/or the lymphoreticular system. Therefore, in countries where there is a high incidence of BSE, the SBOs from cattle over six months old, and any protein material derived from them, should not be used in human food or animal feed, including that for pet animals and birds. The list of SBOs in Section 5.1.1 should be continuously reviewed in the light of new scientific knowledge.

"Intestines" excludes oesophagus and stomachs, because these organs, when prepared for food, do not contain significant quantities of lymphoid tissue. Casings derived from intestines (as defined above) are included because lymphatic tissue from Peyer's patches may be partially retained after processing.

Where BSE occurs with a high incidence, the SBO ban, in conjunction with the mandatory slaughter of suspect cases, with total destruction of carcasses, is considered to be sufficient for protecting the health of humans.

Examples of other measures taken by various countries include:

- the removal from meat (skeletal muscle) of obvious nervous and lymphatic tissue during the cutting process; and
- the application of rendering procedures which have been shown to achieve the parameters judged effective for that type of process for inactivation of TSE agents.

Tallow is one of the two end-products of rendering (the other being meat meal, or meat and bone meal). The higher grades of tallow are used in the manufacture of human food, animal feed (including pet food) and for the preparation of toilet soaps, cosmetics and detergents. The lower grades have various industrial uses. Because of the proteinaceous nature of the TSE agents, they would tend to remain with the cellular residues of meat and bone meal during the extraction process, rather than be extracted with the lipids of tallow. This is consistent with epidemiologic observations showing that the geographical distribution of BSE in the UK is related to the use of meat and bone meal, not tallow. Tallows are filtered at the rendering plant to remove protein and other impurities and then subjected before use to further rigorous processing. For these reasons, tallow does not appear to be a risk for human and animal health.

7.2 Putative occupational transmission

Descriptive and analytic epidemiologic studies have provided no evidence of a link between specific occupations and an increased risk of developing CJD (section 4). Concern has nevertheless been expressed that specific occupational groups may have been exposed to a greater risk than others because of the possibility of accidental inoculation of the BSE agent in cuts or lacerations. Guidelines equivalent to those applicable to other potential occupational groups such as abattoir workers and veterinarians, in countries where BSE occurs, to minimize the possibility of accidental exposure.

Specific recommendations include:

1. taking care to avoid injuries especially with instruments which have been in contact

- with specified bovine offals;
2. covering open cuts and grazes with waterproof dressings;
3. wearing suitable protective equipment and clothing; and
4. minimizing direct contact with specified bovine offals.

In the laboratory appropriate precautions must be taken when handling or processing infected material such as brain. The possibility that new laboratory techniques may lead to a greater theoretical risk than previous techniques should be continuously reviewed.

7.3 Putative risks from medicinal products

On the basis of current scientific knowledge about the agents causing BSE and other TSEs, the group stressed that the careful selection of source materials is still the best way to secure maximum safety of ingredients or reagents of bovine origin used in the manufacture of medicinal products. Therefore the epidemiologic and surveillance situation of BSE in countries and herds as well as the age of source animals should be taken into account by manufacturers of medicinal products wishing to procure raw material of bovine origin.

However, for risk assessment of medicinal products, additional factors should be taken into account besides those mentioned above. These are the type and source of tissues used in manufacturing, the manufacturing process (which may have a significant capacity for the inactivation/removal of the BSE agent), and additionally, the dose and route of administration of the final product.

In the light of new information in this area, the consultation recommends that factors affecting the safety of the product should be assessed, and expressed as far as possible in quantitative terms. The consultation decided to leave unchanged the classification of bovine tissues based on relative scrapie infectivity in tissues and body fluids from naturally infected sheep and goats with clinical scrapie (Tables 2 and 3, pp. 26-29) until more data on BSE infectivity titre in bovine tissues become available. For reference, the current data on

BSE titres appear in Table 3, pp. 27-29.

The national licensing authorities should consider the risk to benefit ratio of the medicinal product concerned.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

- The available data suggest that all mammals including humans should be regarded as being potentially susceptible to BSE if sufficiently exposed.
- BSE is being continuously monitored in all affected countries. It is declining in the UK.
- Current control policies are considered adequate to minimize the risk of exposure to BSE of all species including humans.
- Within the limits of sensitivity of the current mouse bioassay, the BSE agent, in contrast to the situation of scrapie in sheep, has so far been detected only in the central nervous system of naturally infected cattle.
- The epidemiologic evidence in Europe does not indicate a change in the incidence of CJD that can be attributed to BSE.

8.2 Recommendations

- After reviewing the most recent information on BSE titres in tissues which may be used in medicines or in their manufacture, and on the effectiveness of BSE inactivation procedures, it is recommended that WHO should arrange a meeting of specialists to formulate guidelines to minimize the risk of transmitting the agents causing spongiform encephalopathy via medicinal products and medical devices.
- Continuing research is encouraged to improve the diagnosis of BSE, to define the pathogenesis of the disease, to develop methods for inactivating the causal agent and to investigate the public health

significance of SE infections especially BSE in other species (including domestic cats).

- The development of surveillance guidelines should be encouraged to support the risk assessment of countries as sources of bovine material to be used in medicinal products and medical devices.

9. ACKNOWLEDGEMENTS

In its discussion and preparation of this report the group took into consideration (a) appendix XVII of the report of the meeting of the OIE International Animal Health Code Commission organized by OIE in Paris, 16-20 January 1995 (document 63SG/12/CS1, pp. 81-83); and its amended version endorsed by the 63rd OIE General Session on 16 May 1995; (b) draft "Note for guidance for minimizing the risk of transmitting agents causing spongiform encephalopathy via medicinal products" approved by the European Community's Committee for Proprietary Medicinal Products (Brussels, December 1991, Document No. III 3298/91); and (c) the following European decisions of the Commission:

- Decision 94/794 of 14 December 1994 concerning certain protection measures relating to bovine spongiform encephalopathy and repealing Decisions 89/469/EEC and 90/200/EEC;
- Decision 95/29/EC of 13 February 1995 amending Decision 94/382/EC on the approval of alternative heat treatment systems for processing animal waste of ruminant origin, with a view to the inactivation of spongiform encephalopathy agents.

ANNEX 1

LIST OF PARTICIPANTS

Dr S. Ammendrup, Head, Department for Exotic Diseases, The Danish Veterinary Service, Rolighedsvej 25, DK-1958 Frederiksberg C., Denmark

Mr R. Bradley, BSE Coordinator, Central Veterinary Laboratory, Ministry of Agriculture, Fisheries & Food, GB-New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

Dr H. Diringer, Robert Koch-Institut, Bundesinstituts für Infektionskrankheiten und nicht übertragbare Krankheiten, Nordufer, 20, D-13353 Berlin, Germany

Dr G. Ferri, Direzione Generale Servizi Veterinari, Ministero della Sanità, 22a P. G. Marconi 25, I-00144 Rome, Italy

Dr J. Gibbs, National Institute of Neurological and Communicative Disorders and Strokes, Bethesda, Maryland 20892, USA (**Chairman**)

Dr F. Guarda, Università degli Studi, Dipartimento di Patologia Animale, Via Nizza 52, I-10128 Turin, Italy

Dr B. Hörnlimann, Coordinateur BSE, Institut für Viruskrankheiten und Immunprophylaxe (IVI), CH-3147 Mittelhäusern, Switzerland

Dr W. D. Hueston, Epidemiologist and Program Leader, National Animal Health Monitoring System - NAHMS, USDA: APHIS: VS, 555 South Howes, Suite 200, Fort Collins, CO 80521, USA

Dr J. Kellar, Animal and Plant Health Directorate, Agriculture Canada, Nepean, Ontario K1A 0Y9, Canada

Dr R. H. Kimberlin, Scrapie and Related Diseases Advisory Service (SARDAS), 27 Laverockdale Park, Edinburgh EH13 0QE, United Kingdom

Dr M. Pocchiari, Section of Persistent and Slow Virus Infections, Laboratory of Virology, Istituto Superiore di Sanità, Viale Regina Elena 200, I-00161 Rome, Italy

Dr M. Ramos, Instituto de Protecção Agro-Alimentar, Lisbon, Portugal

Dr D. Riesner, Heinrich-Heine-Universität, Institut für Physikalische Biologie, D-40225 Düsseldorf, Germany

Dr P. J. Rogan, Senior Superintending Veterinary Inspector, Department of Agriculture, Food and Forestry, Kildare Street, Dublin 2, Ireland

Dr M. Savey, Direction Générale, Centre national d'Etudes vétérinaires et Alimentaires, 22 rue Pierre Curie, B.P. 19, F-94701 Maisons-Alfort Cedex, France (**Rapporteur**)

Dr W. K. Schwerdtfeger, Federal Ministry of Health, Referat 123, D-53131 Bonn, Germany

Mr J. Sloggem, Medicines Control Agency, Market Towers, 1 Nine Elms Lane, GB-London, SW8 5NQ, United Kingdom

Dr A. Somogyi, Director, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Postfach 33 00 13, D-14191 Berlin, Germany

Mr K. C. Taylor, Ministry of Agriculture, Fisheries and Food, Government Buildings, Hook Rise South, GB-Tolworth, Surbiton, Surrey KT6 7NF, United Kingdom

Dr J.M.R. Telo, Instituto de Protecção da Produção Agro-Alimentar, Lisbon, Portugal

Dr E. D. Weavers, Senior Research Officer, Department of Agriculture, Food and Forestry, Kildare Street, Dublin 2, Ireland

Mr J. W. Wilesmith, Head, Epidemiology Dept, Central Veterinary Laboratory, GB-New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

Dr R. G. Will, Consultant Neurologist, National Creutzfeld-Jakob Disease Surveillance Unit, Department of Clinical Neurosciences, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, United Kingdom

Dr H. Windemann, Head, Biochemistry Section, Office Intercantonal de Contrôle des Médicaments, Erlachstrasse 8, CH-3000 Berne 9, Switzerland (**Rapporteur**)

Other organizations

Dr P. Peters, Unit of Public Health Analysis, Policy and Programme Coordination, Development and Evaluation, Directorate of Public Health and Safety at Work, DGF/F/1, European Commission, TMO C4/67, rue Alcide de Gaspari, L-2920 Luxembourg

Mr B. A. Marchant, Administrateur Principal vétérinaire, Commission des Communautés européennes, Direction générale - Agriculture, Rue de la Loi, 200, B-1049 Brussels, Belgium

Mr E. Wijkstrom, World Trade Centre, Centre William Rappard, Rue de Lausanne 154, CH-1211 Geneva 21, Switzerland

Observers

Dr H. David, Ministry for the Environment, Regional Development and Agriculture of Northrhine-Westphalia, Schwannstrasse 3, D-40190 Düsseldorf, Germany

Secretariat

Dr F.-X. Meslin, Chief, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland (**Secretary**)

Dr O. Cosivi, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland

Dr S. Baillargaux, Health Legislation, World Health Organization, Geneva, Switzerland

Mr S. S. Fluss, Health Legislation, World Health Organization, Geneva, Switzerland

Dr E. Griffiths, Chief, Biologicals, Division of Drugs Management and Policies, World Health Organization, Geneva, Switzerland

Dr F. Käferstein, Chief, Food Safety, Division of Food and Nutrition, World Health Organization, Geneva, Switzerland

Dr S. Miyagawa, Food Safety Unit, Division of Food and Nutrition, World Health Organization, Geneva, Switzerland

Dr A. Padilla, Biologicals, Division of Drugs Management and Policies, World Health Organization, Geneva, Switzerland

Dr K. Stöhr, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland

ANNEX 2

Summaries of low BSE incidence country reportsFrance

From December 1990 to April 1995, 116 suspected clinical cases of BSE were investigated; 12 were found to be positive, and all were in native cattle belonging to 11 different farms. In addition, 603 possible rabies cases were investigated; none was positive for BSE. The cattle affected by BSE all belonged to a dairy breed and their ages ranged from 4.5 to 8 years. The role of meat and bone meal (MBM) as the origin of the disease could not be proven in every case.

Because of its sporadic occurrence (i.e. less than 1 case of BSE in 1 000 000 adult cattle/year), detection of the disease requires a dedicated surveillance network.

A ban on the importation of cattle and MBM from the UK was established in 1989. Information was provided to professionals and veterinarians (1989-1991). A ban on the incorporation of MBM into cattle feedstuffs was implemented in July 1990 and extended to all ruminants in January 1995. Epidemiological surveillance and diagnosis is coordinated by the CNEVA Bovine Disease Laboratory located in Lyon.

Ireland

BSE was first diagnosed in Ireland in 1989. In the period 1989-1995 a total of 106 cases have been confirmed. Eighty-two per cent of cases were found in dairy cows.

Twelve of the 106 cases of BSE were in animals imported from the United Kingdom. With the exception of four cases where herd records were deficient, all the remaining 94 cases were exposed to either (a) compound feedstuffs containing meat and bone meal imported from the United Kingdom, or (b) meat and bone meal imported from the United Kingdom and incorporated into compound feedstuffs in Ireland.

National control and surveillance measures were introduced in 1989 and 1990, including movement restrictions, destruction of confirmed cases, prohibition of importation and feeding of meat and bone meal. In 1990 a programme of voluntary depopulation of affected herds was introduced.

For an indigenous source of BSE agent to have existed in Ireland during the early part of the 1980s, scrapie would have had to be widespread and sheep by-products included in meat and bone meal at a significant level in relation to beef by-products. Neither situation existed during the period when exposure to the BSE agent is believed to have taken place.

Portugal

In Portugal, BSE has occurred in 22 dairy cattle (Friesian) in the north of the country. Among the BSE positives, six cows originated from the UK and 16 were born in Portugal. Cows from the UK were born during the years, 1984, 1986 and 1987, while native cows were born during the second semester of 1988 and first semester of 1989. Some meat and bone meal were imported from the UK during the above period.

The BSE positive cows born in Portugal probably became infected through the consumption of concentrates incorporating MBM contaminated by a scrapie-like agent.

Most cases were five years old. The incidence in each farm has been one case, with the exception of one farm where nine BSE cases were diagnosed.

National control and surveillance measures are being implemented, including the introduction of national and community legislation, training of staff for diagnosis, establishment of a committee for BSE, epidemiological surveys of cattle breeds and manufacturers of concentrated feedstuffs for animals, sequestration of farms with periodical visits, movement restrictions, etc.

Switzerland

Bovine spongiform encephalopathy (BSE) was first diagnosed in Switzerland on 2 November 1990. BSE notification in Switzerland and a "feed ban" were put into effect on 1 December 1990. The yearly BSE incidence rates per 100 000 cows, from 1990 to 20 April 1995 (total number of cases 143), were 0.13 (1990), 1.13 (1991), 1.92 (1992), 3.80 (1993) and 8.39 (1994). The cases have been scattered over most parts of the country.

Only domestic Swiss dairy cows have been affected with BSE. There is no evidence for vertical transmission of infection since no case has occurred in an offspring of a BSE-affected cow. There seems to be no breed predisposition. The type and frequency of clinical signs resemble those in the UK. The 143 cases have occurred on 139 farms, i.e. most cases occurred as single cases on a farm. Only one farmer had three cases, and two farmers had two cases. There seems to be no evidence for horizontal infection. All affected dairy cattle were born between 1984 and 1991 (the exact birth date of five animals is not known). The average age of affected animals has been increasing since 1993.

ANNEX 3

Chapter 3.2.13. of the OIE International Zoo-Sanitary Code on Bovine Spongiform Encephalopathy

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 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 authorize 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.

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, *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 protein product derived from tissues listed below originating from ruminants over six months of age to cattle 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 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) *ante mortem* inspection is carried out on all bovines over 18 months of age;
- 3) affected cattle are slaughtered and completely destroyed.

Article 3.2.13.6.

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) 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 3.2.13.7.

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 than milk or milk products) in feed.

Article 3.2.13.8.

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

for fresh meat (bone-in or deboned) and meat products from cattle, excluding tissues listed in Article 3.2.13.4.

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) 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.

Article 3.2.13.9.

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., 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;
- 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.

Meat and bone-meal containing any ruminant protein which originated from countries with a high or low incidence of BSE 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.

Bovine 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.

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.

TABLE 1: Total BSE cases and cases occurring after the food ban by country and territory

COUNTRY	Ruminant Food Ban Date	Cases Born After Ban (BABs)	TOTAL CASES	FARMS	DATE
Great Britain	18.7.88	18 730	148 200	32 385	12.05.95
Northern Ireland	11.1.89	104	1 564	1 074	"
Switzerland	1.12.90	2	143	139	15.4.95
Republic of Ireland	August 1990	1	106	106	May 1995
Portugal	June 1994	-	22	14	5.5.95
France	27.7.90	-	12	11	20.4.95
Canada	-	-	1	1	19.1.95
Germany	July 1994*	-	4	4	May 1995
Italy	July 1994*	-	2	1	19.1.95
Sultanate of Oman	-	-	2		"
Denmark	June 1990	-	1	1	"
Falkland Islands	-	-	1	1	"

* EU-wide feed ban introduced (94/381/EC)

TABLE 2: Relative scrapie infectivity titres in tissues and body fluids from naturally infected Suffolk sheep and goats with clinical scrapie

Category I	High infectivity - BRAIN, SPINAL CORD -
Category II	Medium infectivity - SPLEEN, TONSIL, LYMPH NODES - - ILEUM, PROXIMAL COLON -
Category III	
(a)	Some infectivity - SCIATIC NERVE, PITUITARY, ADRENAL - - DISTAL COLON, NASAL MUCOSA -
(b)	Minimal infectivity - CEREBROSPINAL FLUID, THYMUS, BONE MARROW - - LIVER, LUNG, PANCREAS -
Category IV	No detectable infectivity - SKELETAL MUSCLE, HEART - - MAMMARY GLAND, COLOSTRUM, MILK - - BLOOD CLOT, SERUM, FAECES - - KIDNEY, THYROID, SALIVARY GLAND, SALIVA - - OVARY, UTERUS, TESTIS, SEMINAL VESICLE -

The above is based on the data published by Hadlow W. J., *et al*, 1980, 1982 which were obtained by bioassays of infectivity using mice injected intracerebrally. This table was produced by the WHO Consultation on Public Health Issues Related to Animal and Human Spongiform Encephalopathies, Geneva, 12-14 November 1991: Memorandum from a WHO meeting on public health issues related to animal and human spongiform encephalopathies, *Bulletin of the World Health Organization*, 70 (2): 183-190 (1992).

TABLE 3: Infectivity titres (bioassayed in mice) in tissues from up to 9 Suffolk sheep (34-57 months old) and up to 3 goats (38-49 months old) at the clinical stage of natural scrapie, compared to the titres in tissues from 1 or more confirmed cases of BSE

Tissues	Titre (mean \pm SEM of (n) samples) ^a		Titre ^a
	Scrapie sheep	Scrapie goats	BSE cattle
<u>Category I</u>			
Brain	5.6 \pm 0.2 (51)	6.5 \pm 0.2 (18)	5.3
Spinal cord	5.4 \pm 0.3 (9)	6.1 \pm 0.2 (6)	+ve
<u>Category II</u>			
Ileum	4.7 \pm 0.1 (9)	4.6 \pm 0.3 (3)	<2.0
Lymph nodes	4.2 \pm 0.1 (45)	4.8 \pm 0.1 (3)	<2.0
Proximal colon	4.5 \pm 0.2 (9)	4.7 \pm 0.2 (3)	<2.0
Spleen	4.5 \pm 0.3 (9)	4.5 \pm 0.1 (3)	<2.0
Tonsil	4.2 \pm 0.4 (9)	5.1 \pm 0.1 (3)	<2.0

Tissues	Titre (mean \pm SEM of (n) samples) ^a		Titre ^b
	Scrapie sheep	Scrapie goats	BSE cattle
Category III			
Sciatic nerve	3.1 \pm 0.3 (9)	3.6 \pm 0.3 (3)	<2.0
Distal colon	<2.7 \pm 0.2 (9)	3.3 \pm 0.5 (3)	<2.0
Thymus	2.2 \pm 0.2 (9)	<2.3 \pm 0.2 (3)	??
Bone marrow	<2.0 \pm 0.1 (9)	<2.0 (3)	<2.0
Liver	<2.0 \pm 0.1 (9)	-	<2.0
Lung	<2.0 (9)	<2.1 \pm 0.1 (2)	<2.0
Pancreas	<2.1 \pm 0.1 (9)	-	<2.0
Category IV			
Blood clot	<1.0 (9)	<1.0 (3)	<1.0
Heart muscle	<2.0 (9)	-	<2.0
Kidney	<2.0 (9)	<2.0 (3)	<2.0
Mammary gland	<2.0 (7)	<2.0 (3)	<2.0

Tissues	Titre (mean \pm SEM of (n) samples) ^a		Titre ^a
	Scrapie sheep	Scrapie goats	BSE cattle
Category IV			
Milk	-	<1.0 (3)	??
Serum	-	<1.0 (3)	<1.0
Skeletal muscle	<2.0 (9)	<2.0 (1)	<2.0
Testis	<2.0 (1)	-	<2.0

^a Titres are expressed as arithmetic means (\pm SEM) of log₁₀ mouse i.c. LD₅₀/g or ml of tissue.

+ v indicates titre > 2.0 log₁₀ i.c. LD₅₀/g

- indicates not determined.

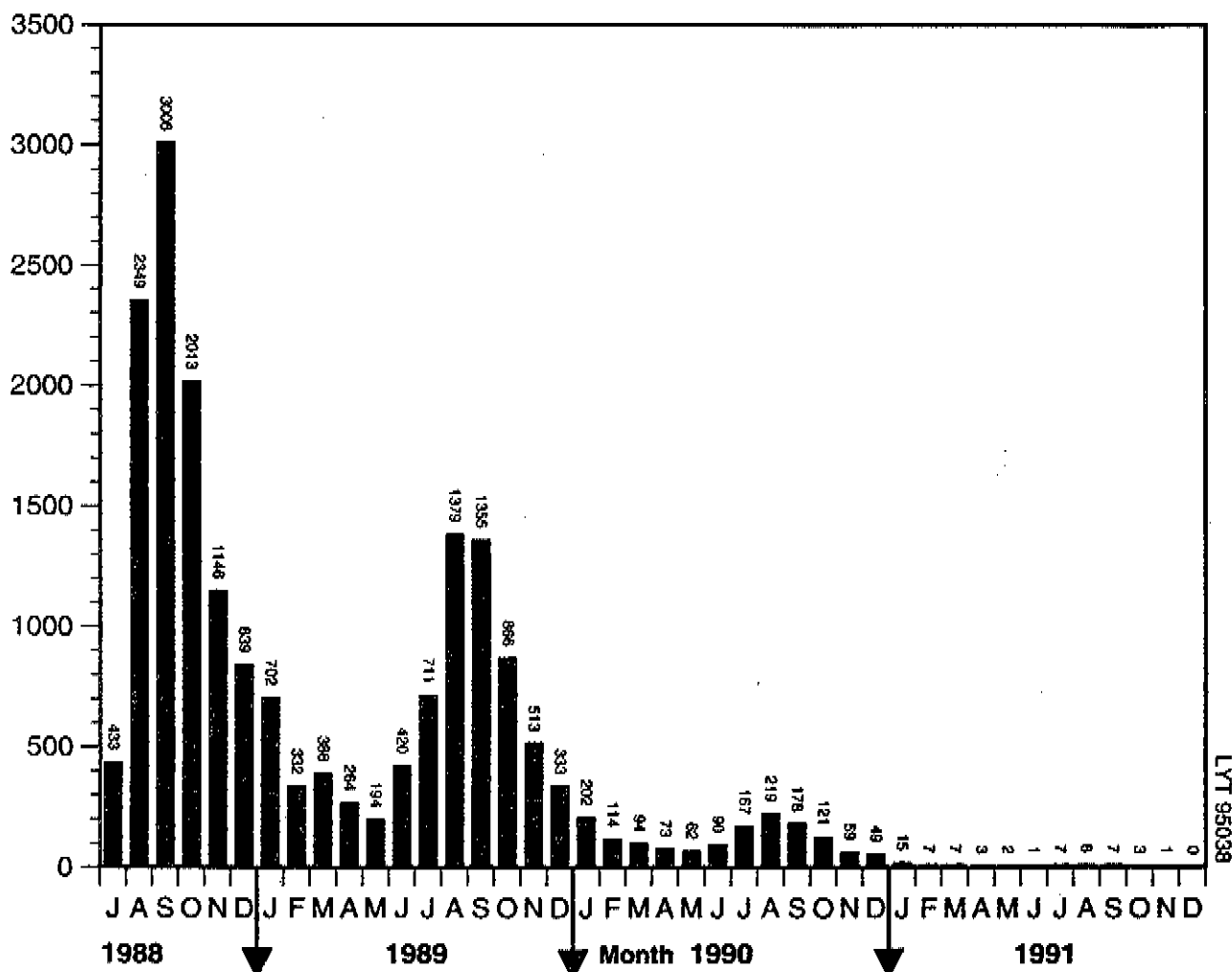
?? indicates data not yet available.

The table is taken from Kimberlin (1994) and compiled from the following sources: sheep scrapie, Hadlow *et al.*, (1982); BSE, Fraser *et al.*, (1992); Fraser and Foster (1994).

The classification of tissues is according to the CPMP Guidelines referred to in the Acknowledgements.

NOTE: The values shown are maxima based on the limits of detectability of the bio-assay in mice (calculated for 30 μ l of inoculum injected intracerebrally).

FIGURE 1: BSE in suspects born after 18 July 1988 - confirmed cases by date of birth



Total to May 1995 = 18730 of which 1988=9786; 1989=7455; 1990=1428; 1991=61

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