SUBJECT TO EDITORIAL CHANGES

Overview of the BSE risk assessments of the European Commission's Scientific Steering Committee (SSC) and its TSE/BSE *ad hoc* Group

Adopted between September 1997 and April 2003

Prepared under the scientific secretariat of

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FOREWORD

By Professor Dr. Gérard Pascal, 1997-2003 chairman of the SSC

Scarce are the members of the Scientific Steering Committee who were, before its creation in 1997, involved in the evaluation of the risk related to the exposure to TSE agents and in particular of BSE. Before the crisis of 1996, only the Scientific Veterinary Committee had really been interested in this issue. However, I seem to recall that while chairing the Scientific Committee for Food (SCF), we had discovered the gravity of the matter, at the time of the risk assessment related to the presence of some bovine tissues in infant and baby food. An opinion given by the SCF in 1996 underlined our concerns but we were far from imagining the turn of events.

Immediately after the announcement of a possible transmission of BSE to humans by the Ministry of Health of the United Kingdom, in March 1996, the crisis taking place within the Commission led to the creation of the Multidisciplinary Scientific Committee (MDSC) on BSE located near the Secretariat General of the Commission. I had the opportunity to take part from July 1996 to October 1997 in the meetings chaired by Professor Fritz Kemper. I was the second "ingenuous" of the group, with our other colleagues being among the best European specialists in prion diseases. It was for me an enriching experience and undoubtedly useful for my further commitment to the SSC. Prof. Kemper and I learned much through the interaction with our colleagues. I, for my part, perceived that from a scientific concern with so many unknowns and uncertainties, it was necessary to stand back from the specialists' opinions dealing with specific aspects.

The Commission realised a work of visionary proportions when it created, in autumn 1997, within the Health and Consumer Protection Directorate General, eight specialised scientific committees and a scientific steering committee specifically charged with the matters related to TSE/BSE. It was wise to create from the start a TSE/BSE *ad hoc* group within the frame of the SSC. The tradition of organising scientific committees quickly resulted in setting up additional working groups. From the beginning the work structure consisted thus of a multidisciplinary committee mainly with non-TSE specialists, adopting opinions based on the analyses by specialised groups. I am convinced of the efficiency of such an organisation to implement rigorous scientific analysis and at the same time, in case of uncertainty, to express a senior experts' judgement.

The SSC provided, during its two mandates since 1997, most useful opinions for the risk managers, even though sometimes certain Member-States did sometimes voice their

protest. The events, however, often proved us right; I think in particular of the geographical BSE risk assessments (GBR). Others opinions diverged from those emitted by national committees. One should not be surprised in situations of scientific uncertainties where, in addition to recognised facts, it is advisable to take into account the plausibility of assumptions, plausibility which may be interpreted in different ways.

I want to express my gratitude to all the members of the SSC since its creation. Even though we experienced difficult moments, I was always happy to chair a group of this quality, consisting of scientists having multidisciplinary skills, a great experience in fields as different as those from human food and animal feed, animal welfare, veterinary sciences, cosmetics, medicinal products or ecotoxicology. Coming from diverse scientific and intellectual backgrounds we made the effort to listen to each other in order to better understand our points of view. Overall, we quickly showed a large mutual respect which made it possible for the group to be united and to express a great solidarity. Each one knew, among the SSC, how to show independence of thought, without ever defending the national positions beyond what decency allowed.

We have also shown, I believe, humility in front of many unknown factors, to answer the questions posed by the Commission. Our attitude was pragmatic, the stones being added slowly one after one in order to gradually build a scientifically founded process. We were able to listen to the specialists while preserving our judgement capacity. We have come a long way since our first opinion of December 1997 on the specified risk materials. It is thanks to members' competencies, experience and judgement capacity in the two consecutive SSC. It is also thanks to the huge work of secretaries of the Committee.

Paul Vossen and Joachim Kreysa were the first scientific secretaries of the SSC and its TSE/BSE *ad hoc* Group. They followed step by step the evolution of our reflection, when needed underlined the inconsistencies of some of our opinions, provided us the factual elements necessary for our task and knew how to translate ideas not always put forward with clarity on delicate subjects.

The SSC vice-chairmen, *ad hoc* group chairmen, working group leaders and all the members of these structures played a role in a set of opinions which have to be available to everyone in a compilation that underlines their overall consistency.

They are thanked all.

Gérard Pascal

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SCOPE

At the end of the eighties-early nineties of the previous century Bovine Spongiform Encephalopathy (BSE) rapidly evolved into a new issue of major public concern for which no ready at hand solutions were available. It is a quite difficult challenge to manage risk on a day-to-day basis in an area that almost is entirely composed of unknowns and uncertainties. On one hand uncertainties about the cause of the disease, its transmission and epidemiology and the absence of any diagnostic test or cure justify that this risk be addressed with the highest precaution to avoid that the disease would eventually evolve into a pan-European and possibly a pandemic threat. On the other hand, the precautions taken need to be as much as possible proportional to the real threat and avoid whenever possible unnecessary major societal and economic disturbances.

Between 1997 and early 2003, the European Commission relied on the Scientific Steering Committee and its TSE/BSE *ad hoc* Group for scientific advice and risks assessments related to Transmissible Spongiform Encephalopathies (TSE) in general and Bovine Spongiform Encephalopathy (BSE) in particular. This report in the first place intends to provide all interested parties with an exploitable account of 6 years of BSE risk assessments. It is therefore expected to contribute to continuity in BSE risk assessment at the EU level now that the SSC and the TSE/BSE *ad hoc* group have completed their mandate. The report will also provide risk managers and other interested people with an understandable introduction to BSE and to all detailed SSC opinions adopted since 1997.

After a general introduction on TSEs in humans and animals, the report in a first part presents the remit and functioning of the European Commission's Scientific Steering Committee (SSC) and its TSE/BSE *ad hoc* Group and their careful step-wise approach in BSE risk assessment. The first part then provides a synthetic overview of BSE-related reports and opinions prepared since 1997, clarifies the most relevant criteria for BSE risk assessment, and shows how these were used and converted into a consistent approach for BSE risk assessment.

Executive summaries of the SSC's main opinions and reports work on a number of specific issues are therefore provided in Part II. They cover issues related to TSE in human and animals, BSE risk reduction strategies, the safety of ruminant-derived products and quantitative risk assessment.

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In the period of September 1997 to March 2003, about 200 scientists and experts have contributed, in the framework of the European Commission's Scientific Steering Committee and its TSE/BSE *ad hoc* Group and specialised working groups, to the collection of data and information and the preparation of scientific reports and opinions related to BSE risks. A list of all scientists who contributed since 1997 is provided further on. Their work and contributions are gratefully acknowledged.

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– Industrial associations active in fields related to the use, recycling or disposal of animal products, by-products or waste;

– Scientific advisory bodies in Member States and Third Countries, whose excellent work was occasionally challenged by the SSC but in far most of the cases provided essential evidence and views;

– Other administrations and competent authorities in EU Member States and Third Countries;

– Consumer associations.

PART I

EXECUTIVE SUMMARY OF BSE RISK ASSESSMENTS

I. INTRODUCTION: TSEs IN HUMANS AND ANIMALS

BY H. BUDKA, G.A.H. WELLS AND H.A. KRETZSCHMAR

Transmissible spongiform encephalopathies (TSEs), also designated as prion diseases, subacute spongiform encephalopathies, transmissible degenerative encephalopathies, slow virus infections, unconventional slow virus diseases or transmissible cerebral amyloidoses, are rare, progressive and invariably fatal neurodegenerative disorders that occur in humans (**Table 1**) and animals (**Table 2**). Almost all TSEs are transmissible within the host species and to some other species and are characterised by a non-inflammatory CNS disease with characteristic microscopic (spongiform) changes. They may occur in sporadic, acquired and inherited forms [1] (**Table 1**). The origin for some TSEs is known, but infection sources and mode of transmission within the species are not always identifiable (**Table 2**). Medical and scientific experience with these disorders varies to great extent; scrapie has been known for more than 250 years, Creutzfeldt-Jakob disease (CJD) for at least 80 years, whereas experience of the more recently identified diseases; familial and sporadic fatal insomnia (FFI, SFI), chronic wasting disease (CWD), Bovine Spongiform Encephalopathy (BSE) and Variant Creutzfeldt-Jakob disease (vCJD), is more limited.

Research into these enigmatic diseases has emerged as one of the hot spots in modern biomedicine. The reasons are twofold, one scientific and the other socio-economic. First, these diseases are on the interface of heredity and infectivity, a unique situation and a provocative new paradigm in biomedicine. Second, the emergence of the epidemic of BSE in the UK and the identification of its counterpart in humans, vCJD, has caused significant public health concern and global publicity about the transmission risk to other species including man. As the subsequent reviews in the Overview cover mainly animal diseases, this introduction outlines the general characteristics of TSEs, and provides details of the human forms.

Table 1: Human transmissible spongiform encephalopathies and their origin

Disease	Origin
Creutzfeldt-Jakob disease (CJD):	
• Sporadic (idiopathic) CJD	**Unknown**, probably spontaneous conformation change of PrP^c or somatic mutation
• Familial CJD	**Genetic** (*PRNP* mutations or insertions)
• Iatrogenic CJD	**Infectious** [dural or corneal transplants, hormone treatment with preparations from cadaveric pituitary gland, intracerebral electrodes or neurosurgical instruments]
• Variant CJD (v-CJD)	**Infectious** (presumed food-borne exposure to the BSE agent)
Gerstmann-Sträussler-Scheinker disease (GSS)	**Genetic** (*PRNP* mutations, classically P102L)
Familial fatal insomnia (FFI)	**Genetic** (*PRNP* 178 mutation, 129M)
Sporadic fatal insomnia (SFI) (same phenotype as FFI)	**Unknown** (probable causation as in sporadic CJD)
Kuru	**Infectious** (ritual cannibalism by Fore people in Papua-New Guinea)

Table 2: Transmissible spongiform encephalopathies in animals: natural host range and assumed transmission modes within the host species

Disease	Natural host	Main mode of transmission
Scrapie	Sheep, goats	Horizontal
Transmissible Mink encephalopathy (TME)	Mink	Contaminated feed (scrapie?)
Chronic wasting disease*	Mule and white-tailed deer, Rocky Mountain elk	Horizontal
Bovine spongiform encephalopathy (BSE)	Bovines	Contaminated feed
Feline spongiform encephalopathy (FSE)	Felines	Contaminated feed (BSE)
Exotic ungulate encephalopathy	Zoo ungulates	Contaminated feed (BSE)

* As CWD has relatively recently become a possible concern in North America, the SSC has produced a monograph on this disease. An executive summary is attached in an Annex I.

General characteristics of TSEs

Elegant disease modelling has demonstrated a normal cell protein, the prion protein (PrP^C), as prerequisite for disease manifestation [2]. Although mice in which the PrP^C was "knocked-out" did not feature any particular disease phenotype, some experimental data indicate a role for PrP^C in circadian rhythm regulation [3], synaptic transmission [4], ion currents [5], nerve fibre organisation [6], copper ion trafficking [7], nucleic acid-chaperoning [8], antioxidant [9] and anti-apoptotic processes [10]. Although it is predominantly expressed in neural tissue, including neurons [11] and glial cells [12], other organs (e.g. uterus, placenta, thymus, heart, lung, muscle, gastrointestinal tract) also contain considerable amounts [13]. Upregulation of the prion protein seems to be important in inflammatory conditions of muscle [14], skin [15] and liver [16], as well as in neurodegenerative disorders including Alzheimer and prion diseases [17].

A conformationally abnormal, protease-resistant isoform (PrP^{res} or PrP^{Sc}, the latter term derived from scrapie) accumulates in the CNS in the whole group of TSEs or prion

disorders and has become the most important diagnostic marker. Routine detection of PrP^{Sc} for diagnostic purposes uses methods such as immunocytochemistry, immunoblotting or ELISA assays performed on diseased tissue samples from patients obtained at autopsy, or from slaughtered animals as is done with current EU-wide testing of cattle for BSE. PrP^{Sc} exists in a predominantly beta-pleated form in contrast to the alpha-helix dominant PrP^{C} [18]. Substantial evidence supports the notion that PrP^{Sc} itself is the infectious agent [18], albeit others argue for a viral or other microbial agent as the pathogen involved in either the transmission of PrP^{Sc} or causation of the PrP^{C}-PrP^{Sc} change [19,20]. The PrP^{C} to PrP^{Sc} conversion is considered by many to be the basis for propagation of infectivity in an auto-catalytic refolding process. While PrP^{Sc} is usually a good predictor of infectivity, failure to show its presence does not necessarily indicate absence of infectivity [21]. PrP^{Sc} and TSE infectivity are not only protease-resistant, but resistant to a wide range of physicochemical influences as well, thus necessitating very aggressive and unusual procedures for prion-specific decontamination [22].

Infectivity is not uniformly distributed in an individual or animal affected with a TSE. Two distinct groups can be distinguished: in the first, infectivity has been detected in a distribution mainly limited to the central nervous system (brain, spinal cord, parts of the eye and some ganglia close to the CNS). This pattern of distribution of infectivity is typical of sporadic and iatrogenic CJD, genetic human TSEs and BSE of cattle. In the second, infectivity involves also peripheral tissues, in particular the lymphoid system and this pattern is a feature of scrapie, BSE in sheep, CWD and vCJD. In all TSEs, however, most infectivity resides in the CNS during clinical disease or late in the incubation period. This differential distribution of infectivity according to species and disease phenotype is one important factor when considering risks for transmission.

Specific mutations and insertions in the PrP-encoding gene *PRNP* associate with familial TSEs that constitute 5-15% of human TSEs. So far 38 genetic aberrations have been described [23]. *PRNP* codon 129 is important as a genetic susceptibility factor in sporadic [24] and iatrogenic CJD [25] as well as determining clinico-pathological phenotypes in all human TSEs [26,27]. Distinct Western blot patterns in disease subtypes in combination with the codon 129 constellation have become the basis of a molecular classification of human TSEs [27,28]. While genetic aberrations are well recognised in human TSEs, much less is known about molecular genetics of animal TSEs. A notable exception is distinct PrP^{C} *Prnp* polymorphisms that associate with susceptibility or resistance to scrapie in sheep. However, the PrP gene has been identified and sequenced in many species across a broad phylogenetic spectrum, from mammals to turtles and fish.

Although TSEs are transmissible by definition, it is not that easy to pass on the infectious agent to other individuals or animals under natural conditions. Important determinants of the efficacy of transmission include the type of TSE agent (e.g. the BSE agent has been shown to be much more promiscuous in experimental transmissions than scrapie strains), the infective dose, the infection route (the laboratory method of inoculating directly into the brain of recipient animals is much more effective than other routes, including the oral route which is most relevant to natural transmission) and the genetic background that is also part of the "species barrier" that impedes TSE transmission between species. To confirm and measure TSE infectivity, bioassays are conducted, usually in small rodents such as mice or hamsters. It is important that recipient species provide a model in which the variables controlling disease phenotype are constant. Thus inbred or congenic mice have been widely used in TSE bioassay studies. Because of such variables, the long incubation periods involved and the high maintenance costs, the use of larger host animal species is seldom practical. However, for assay of BSE infectivity, cattle have proved an effective model and obviate the species barrier. The cattle-to-mouse transmission is about 500 fold less efficient than cattle-to-cattle intraspecies transmission.

The exact cause of nerve cell death in TSE*s* is unknown. Oxidative stress [29,30], and apoptosis [31,32] contribute to the cell death process. As recently summarised [33], the neural pathogenesis involves either the neurotoxic effect of PrP^{Sc} or loss of function of PrP^{C}. Toxic intermediates or alternative pathogenic forms of PrP, like the unusual transmembrane form (indicated as ^{Ctm}PrP) might also have a role. Neuronal loss, which seems to be selective [34], is accompanied by astrogliosis and microgliosis and cytokine production, but typical inflammatory responses and cellular infiltration are lacking [35,36].

The immune system has a pivotal role in the pathogenesis of disease after extraneural inoculation, as best shown in experimental scrapie, and must also be considered in acquired forms of human TSEs. Briefly, the route of prion infection in experimental scrapie involves the intestinal epithelium, Peyer's patches, possibly blood constituents, and in particular follicular dendritic cells of lymphoid organs [37-39]. The complement system as well as B-cells also have a role in peripheral prion pathogenesis [40,41]. The link between the lymphoreticular system and the CNS seems to be certain components of the autonomic nervous system. After alimentary infection, spread of agent may occur from the intestine to the spinal cord via sympathetic pathways [42] and/or via parasympathetic pathways to the brain stem along the vagus nerve [43]. However, different prion strains may have distinctive pathogenetic pathways in relation to species and host genotype. In human TSEs, mobile cells like dendritic and monocyte/macrophage lineage cells in vessel

walls may be involved in transport of disease-associated prion protein and possibly also of infectivity [44].

Human TSEs

Creutzfeldt-Jakob disease (CJD) incidence has been shown to oscillate around an average annual value of 1 to 1.5 cases per million. The most frequent form, sporadic CJD, is of unknown origin (thus some prefer the term "idiopathic CJD"), although most researchers believe that a spontaneous refolding of PrP^{C} into PrP^{Sc} underlies its development. An epidemiological case control study was unable to identify any specific risk factors for sporadic CJD [45]. Meaningful studies on such rare diseases require appropriate case ascertainment. This must be achieved by using standardised definitions which may be based on clinical criteria and/or, if sufficient autopsy data are available, on neuropathological criteria. In addition, molecular genetic data have an important role. Both clinical (**Table 3**) and neuropathological (**Table 4**) case definitions have been formulated and have proved useful for surveillance studies. Transmission of sporadic CJD to other humans by invasive medical procedures, documented as iatrogenic CJD in about 400 patients [46] (**Table 1**), must be prevented by appropriate control measures in hospitals [47]. Unfortunately, human TSEs run a relentlessly progressive course that can not yet be effectively perturbed by any applied therapy, including the recently highly publicised use of quinacrine.

Most human TSEs are characterised by progressive cognitive decline accompanied by various neurological signs and symptoms. The terms CJD, GSS and FFI represent historical designations for diseases presenting with distinct clinical symptoms and neuropathological features. In general, CJD features prominent cognitive decline. GSS has usually a predominantly ataxic phenotype and longer duration. SFI and FFI feature sleep impairment (although sometimes recognisable only by polysomnography in the laboratory) accompanied by vegetative and neurological signs and symptoms. Sporadic CJD usually occurs at a relatively advanced age (median 64 years) with a comparatively rapid course (median 4 months). However, sporadic CJD does not have a uniform clinicopathological presentation and based on molecular markers such as the *PRNP* genotype at codon 129 and the PrP^{Sc} glycotype as seen on Western blot, classification into several distinct subtypes has been proposed with some of the subtypes typically showing a clinical course of 15 months [27,28].

Definite diagnosis of CJD and other human TSEs requires neuropathologic examination of the brain at autopsy or, in selected cases with potentially treatable alternative diagnoses, by biopsy. Alternatively, additional methodology such as demonstration of PrP^{Sc} on Western blots and/or preparation of scrapie-associated fibrils (SAF) has been used. Neuropathological confirmation is of paramount importance given the steadily growing spectrum of clinical and pathological phenotypes. The many historically described CJD variants, to which a variety of different names were ascribed, have been shown to be within this spectrum. The considerable variation may be influenced by length of the disease, by the *PRNP* genotype, and by not yet fully elucidated factors including strains of the infectious agent.

An immunoblotting CSF test for protein 14-3-3 has emerged as an important tool for a laboratory-supported diagnosis of CJD [48]. The EEG is still paramount in suspecting CJD, and magnetic resonance imaging is likely to become equally important. The following clinical diagnostic criteria have been successfully utilised by the EU Surveillance Group of Creutzfeldt-Jakob Disease in Europe (Project Leader: R.G. Will, Edinburgh) (**Table 3**). This Group's website provides a wealth of epidemiological data on human TSEs in various countries (http://www.eurocjd.ed.ac.uk/). When compared with autopsy confirmation in cases of a progressive dementing illness, the criteria have a sensitivity of 97% and a specificity of 65% [48]. The most important differential diagnoses comprise Alzheimer's disease, Lewy body dementia, vascular disorders, and rare conditions like Hashimoto encephalopathy [49].

Table 3: Clinical diagnostic criteria for CJD surveillance purposes [50]

Sporadic CJD ***Definite***	Diagnosed by standard neuropathological techniques; and/or immunocytochemically and/or western blot confirmed protease resistant PRP and/or presence of scrapie associated fibrils.
Sporadic CJD ***Probable*** (in the absence of an alternative diagnosis from routine investigation). And	Progressive dementia; and at least two out of four of the following four clinical features: • Myoclonus • Visual or cerebellar disturbance • Pyramidal/extrapyramidal dysfunction • Akinetic mutism • a typical EEG during an illness of any duration and/or • a positive 14-3-3 CSF assay and a clinical duration to death < 2 years
Sporadic CJD ***Possible*** And	Progressive dementia; and at least two out of four of the following four clinical features: • myoclonus • visual or cerebellar disturbance • pyramidal/extrapyramidal dysfunction • akinetic mutism • no EEG or atypical EEG; and • duration < 2 years
Iatrogenic CJD	Progressive cerebellar syndrome in a recipient of human cadaveric-derived pituitary hormone; or sporadic CJD with a recognised exposure risk, e.g. antecedent neurosurgery with dura mater graft.
Familial CJD	NB. For the purpose of surveillance this includes GSS disease and FFI. Definite or probable CJD plus definite or probable CJD in a first degree relative; and/or neuropsyhiatric disorder plus disease-specific *PRNP* mutation.

In a consensus report [51], guidelines for appropriate tissue handling, performance of the autopsy and decontamination in suspected cases of CJD and other human TSEs were

described. It is important to note that, following these guidelines, the autopsy on suspected cases of human TSEs can be performed in a way which is both safe and practical. Thus autopsies for neuropathological diagnosis should be performed as frequently as possible. In countries where an autopsy is not normally conducted, e.g. for religious reasons, an alternative might be to perform a brain "biopsy" post mortem, e.g. by needle insertion through a small burr hole in the skull or via the orbit. This might yield some tissue that can be used for neuropathological examinations including immunocytochemistry for PrP, and/or Western blotting for PrP. In another consensus report [52], neuropathological diagnostic criteria for CJD and other human TSEs were given and updated to include also new variant CJD, as listed here (**Table 4**):

Table 4: Neuropathological diagnostic criteria for human TSEs[50]

1. **Creutzfeldt-Jakob disease (CJD)**

   1.1. **Sporadic**, **iatrogenic** (recognised risk) or **familial** (same disease in 1st degree relative or disease-associated *PRNP* mutation):

   Spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical grey matter; and/or

   Encephalopathy with prion protein (PrP) immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivacuolar types).

   1.2. **Variant CJD.** Spongiform encephalopathy with abundant PrP deposition, in particular multiple fibrillary PrP plaques surrounded by a halo of spongiform vacuoles ("florid" plaques, "daisy-like" plaques) and other PrP plaques, and amorphous pericellular and perivascular PrP deposits especially prominent in the cerebellar molecular layer.

2. **Gerstmann-Sträussler-Scheinker disease (GSS)** (in family with dominantly inherited progressive ataxia and/or dementia and one of a variety of PRNP mutations): Encephalo(myelo)pathy with multicentric PrP plaques.

3. **Familial fatal insomnia (FFI)** (in member of a family with $PRNP^{178}$ mutation): Thalamic degeneration, variably spongiform change in cerebrum.

4. **Kuru**: Spongiform encephalopathy with cerebellar atrophy and presence of Kuru plaques.

Without PrP data, the crucial microscopical feature is the *spongiform change* accompanied by neuronal loss and gliosis. This spongiform change is characterised by diffuse or focally clustered small round or oval vacuoles in the neuropil predominantly of the deep cortical layers, cerebellar cortex or subcortical grey matter, which might become confluent. More recently, immunocytochemistry for PrP has been added to classical histological techniques and has rapidly evolved into a most useful diagnostic tool that is also widely used to diagnose animal TSEs. However, it should be used for diagnostic purposes only by an appropriately experienced laboratory. In CJD, immunoreactivity for PrP is seen mainly in four patterns which frequently overlap: plaque, diffuse synaptic, perineuronal and patchy / perivacuolar types.

Variant CJD (vCJD)

vCJD was identified in the UK in 1996, based on clinicopathological characteristics of 10 cases [53]. As of 13 March 2003, cases number 134 in the UK (including one originally attributed to Hong Kong), 6 in France, and one each in Italy, Ireland, USA and Canada. For the patients of the latter three countries, exposure to BSE occurred most likely in the UK. There is now some statistical evidence that the UK vCJD epidemic is no longer increasing at the rate seen previously; it may have reached or be reaching a plateau and is therefore no longer compatible with exponential growth [54].

There is very strong evidence that the origin of vCJD is from BSE [55]. Typing of different TSEs has been performed on $PrPr^{Sc}$ Western blots that show "signature" $PrPr^{Sc}$ patterns [56], and by experimental inoculation of inbred [57] or transgenic [56,58,59] mice; the incubation time, the neuropathological profile and death rates can be used as markers for comparison of distinct TSE strains. For vCJD, these markers differ from those of sporadic, familial and iatrogenic CJD, but are identical with those of natural and experimental BSE. Moreover, BSE transmitted to primates mimics the clinical and pathological features of vCJD [60,61]. The conclusion is that vCJD and BSE are due to the same form of TSE agent, so BSE has transmitted to humans.

Peculiar clinical features of vCJD include:

- Mostly young age, including teenage cases (mean age at death 29; however older persons have been affected, the oldest recorded vCJD patient being 74);

- psychiatric presentation at onset, with later development of cerebellar ataxia and only late cognitive impairment;
- long disease duration (median 13 months) as compared with sporadic CJD;
- no typical EEG change and rarely positive 14-3-3 CSF protein; and
- frequent occurrence of a hyperintense signal in the posterior thalamus in magnetic resonance imaging (MRI) [62].

For diagnosis of vCJD, autopsy (or exceptionally brain biopsy) with neuropathological confirmation is mandatory. However, growing experience has allowed clinical diagnostic criteria to be developed (**Table 5**). The abundant presence in brain of "florid" plaques appears to be the most distinctive neuropathological feature.

One important feature of difference between vCJD and other human TSEs concerns the distribution of PrP^{Sc} and infectivity. Whereas in the latter they are confined to the central nervous system and its adjacent tissues, they are much more widespread in vCJD, including the possibility that blood may also harbour infectivity [64,65]. This poses an important challenge for control of infection in hospitals, particularly in order to eliminate secondary vCJD transmission by blood and blood products. As lymphoid tissues contain prominent PrP^{Sc} and infectivity, biopsy examination of the tonsil has been used to support a vCJD diagnosis [66], and anonymous mass screening of surgical specimens [67] conducted to obtain information on the prevalence of the vCJD in the British population.

vCJD has so far been observed only in persons with a particular genetic background (methionine/methionine homozygosity at the polymorphic *PRNP* codon 129). This is also the most common genotype in patients with sporadic and iatrogenic CJD, whereas it is only half as common in the normal population. It is not known whether other genotypes are resistant to infection or might be affected after a prolonged incubation time, as has been observed in iatrogenicCJD.

Experimental data on PrP^{Sc}glycotyping in particular mice were interpreted to suggest that more than one BSE-derived prion strain might infect humans; it is therefore possible that some patients with a phenotype consistent with sporadic CJD may have a disease arising from BSE exposure [59]. This is interesting with regard to Switzerland, a BSE-affected country, since the incidence of apparently sporadic CJD increased there by two-fold in 2001, and figures from 2002 indicate that it continues to rise [68]. Nevertheless, apparently

sporadic CJD does not seem to increase in the UK, where exposure of the population to the BSE agent was highest.

Table 5: Diagnostic criteria for variant Creutzfeldt-Jakob disease [63]

I	A)	Progressive neuropsychiatric disorder
	B)	Duration of illness > 6 months
	C)	Routine investigations do not suggest an alternative diagnosis
	D)	No history of potential iatrogenic exposure
II	A)	Early psychiatric symptoms *
	B)	Persistent painful sensory symptoms **
	C)	Ataxia
	D)	Myoclonus or chorea or dystonia
	E)	Dementia
III	A)	EEG does not show the typical appearance of sporadic CJD *** (or no EEG performed)
	B)	Bilateral pulvinar high signal on MRI scan
IV	A)	Positive tonsil biopsy
Definite:		IA (progressive neuropsychiatric disorder) and
		Neuropathological confirmation of vCJD ****

Probable: I and 4/5 OF II and III A and III B

Or I and IV A

* depression, anxiety, apathy, withdrawal, delusions.

** this includes both frank pain and/ or unpleasant dysaesthesia

*** generalised triphasic periodic complexes at approximately one per second

**** spongiform change and extensive PrP deposition with florid plaques, throughout the cerebrum and cerebellum.

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II. THE SCIENTIFIC STEERING COMMITTEE AND ITS STEPWISE APPROACH IN TSE RISK ASSESSMENTS

1. The Scientific Steering Committee's (SSC) mandate expired in mid-2003. It was one of the nine scientific committees that since mid-1997 has formed the core of the current scientific advisory system of the European Commission (EC) with regard to consumer protection and public health. Eight sectorial committees cover the specific areas of: human food, animal feed, animal health and welfare, veterinary measures relating to public health, plants, cosmetic and non-food products, medicinal products and medical devices, toxicology, ecotoxicology and the environment. The ninth Committee, the SSC, provided to the Commission advice on multi- and interdisciplinary matters not covered by the mandate of the 8 sectorial committees and promotes co-operation between them on subjects requiring complementary experiences and competencies.

   The number of members per committee varied between 16 and 19. Members were selected via international calls for expression of interest published in 1997 and 2000. In total more than 1500 applications were received. Some members are from countries that are not EU Member States. The SSC is composed of 16 members; it included the 8 chairpersons of the 8 other committees that have sectorial competencies, plus 8 senior scientists with a multi-disciplinary experience in health- and consumer protection related fields, in risk assessment and in the preparation of scientific advice for decision makers.

   A condition for membership, in addition to excellence, was that members only represent themselves, not their institute or country. To guarantee their independence, Committee members had to make a declaration of possible vested interests at the beginning of each meeting and a general written declaration at the beginning of each calendar year. If an incompatibility or conflict of interest arose for a member, he or she may - at the discretion of the Committee as a whole - be requested either not to participate at all in the discussions or to contribute only to the scientific debate but not to the elaboration of the conclusions.

   Opinions are made publicly available via the Internet and upon request. In this way opinions are not only widely available but also open for permanent scientific

scrutiny and criticism. Experience has shown that this is an efficient mechanism and on several occasions it has resulted in opinions being revised following the submission of comments or additional data by individuals, research institutions or industry.

2. Because of their highly multi-disciplinary nature, **TSE-related questions are addressed by the SSC**. Issues relating to TSEs require expertise from a wide variety of scientific disciplines such as veterinary sciences, human medicine, epidemiology, microbiology, biochemistry, animal nutrition, human nutrition, toxicology, animal waste processing, and environmental sciences. To guarantee its multi- and interdisciplinarity in TSE-related matters, the SSC usually follows a 3-stage approach:

   During the **first stage** fundamental aspects were addressed, usually by a special working group established according to the issue of interest[1]. Since 1997 more than 150 specialists have participated in these working groups. The fields addressed so far are:

   – TSE infectivity distribution in tissues and its variations with age, species (cattle, sheep, goats), genotype and agent strain;

   – The TSE-infectivity clearance capacity of production processes[2], as well as related aspects such as intra-species recycling and disposal of animal waste;

   – Sourcing of (safe) animals

   – The Geographical BSE risk assessment.

   – Evaluation of rapid TSE tests; surveillance protocols;

   – Epidemiology (including also aspects such as active surveillance and culling);

   – Human exposure Risk;

   – Other fundamental science issues (for example prion chemistry and physics, strains and strain-typing, vertical transmission, etc.);

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1 For certain issues, mostly of a non-multidisciplinary nature, there is no need to install special working groups; a single rapporteur then prepares a report. This report is then discussed by the TSE/BSE ad hoc Group (see: stage 2)

2 For example, gelatine, tallow, dicalcium phosphate, hydrolysed proteins, hides, meat-and-bone meal and organic fertilisers.

In a **second stage,** the TSE/BSE *ad hoc* Group, which is a specialised permanent working group reporting to the SSC, discusses in detail the scientific report prepared by a working group or rapporteur, and prepares draft conclusions for the Scientific Steering Committee. If major questions arise with respect to the report, it may be sent back to the working group.

In **a last stage,** the SSC discusses in detail both the report of the *ad hoc* Group and the detailed scientific report from the working group and adopts the final opinion that eventually is used by the risk managers as the basis for decision making. The SSC may agree with the conclusions proposed by the TSE/BSE *ad hoc* Group and adopt the conclusions as they are. It is, however, not obliged to do so and may come to different conclusions.

3. **Interaction with risk managers at the level of Commission Services.**

In the course of the existence of the SSC, the origins of indications of a BSE-related scientific issue emerging with a potential (immediate) public health impact have been multiple:

- Internal to Commission services and mastered: for example, the outcome of a consultation of a scientific committee or panel on a specific question (including warnings by a scientific committee not directly related to an opinion) or the outcome of the SSC's intended regular exercise on emerging scientific issues;
- External and mastered: for example, a warning or information or an opinion from a Member State, a Third Country or an international organisation; a documented request/expression of concern by an individual, a Member of parliament, a consumer association; resolutions/recommendations from a reputed scientific congress; or, scientific findings published in a reputed international scientific journal after peer-review;
- External and not mastered: rumours; declarations in the press by individual scientists; public perception of an emerging risk (be it scientifically justified or not).

Whatever the origin of a possible concern, the decision to eventually consult the SSC and ask for an opinion, was always made by Commission Services.

The SSC's risk assessments have been strictly separated from risk management. However, the process from "identification of a possible reason for concern", through

the preparation of a scientific opinion by the risk assessors (the SSC, the TSE/BSE *ad hoc* Group, the working groups) to "the decision (not) to take action" by risk managers (the legislator, the political decision maker) has been highly interactive. This interaction has been participatory (deliberative) and at all stages of the risk assessment: when defining the mandate, when clarifying it and providing background information, when refusing or adjusting the formulation of a question, when providing/asking for additional information, etc.

4. **The interaction between the SSC and the European Commission's Research Directorate General.**

In order that its opinions are timely and proactive, the SSC and its working groups take account of recently published, as well as pre-publication research results. These results are in part derived from the Research Directorate General's Programme of TSE funded research. The Research Directorate General also contributes information resulting from contact with other research funders and stakeholders.

In addition, following the mandate of the Research Council of 16 November 2000, the Commission established an Expert Group consisting of national representatives and scientists. Members of the Commission's Scientific Steering Committee and its TSE/BSE *ad hoc* Group participate in the Expert Group, which guarantees that their recommendations are taken into account. The Group has analysed ongoing research activities both in Member States and at the EU level, identifying areas that could benefit from improved co-ordination, collaboration and structuring as well as new research areas.

It can in this context be noted that the research recommendations regularly made in the scientific opinions of the SSC mostly find a follow-up, either in dedicated projects (e.g. on the development and evaluation of rapid tests), as themes in the framework programmes or sometimes in additional calls for proposals.

III. THE SCIENTIFIC PRINCIPLES AND CRITERIA USED BY THE SSC AS BASES FOR ITS OPINIONS ON BSE RISK

[The cross-references refer to the scientific opinions listed in Annex II]

1. Assessing and reducing the risk of exposure to BSE in ruminant derived products can be divided into four parts: 1. Are the source animals likely to be infected? 2. Which are the tissues likely to be infected? 3. Will the production processes remove or destroy infectivity, or can they be modified to do so? 4. Does the end use change the risk estimate?

   Many scientific unknowns remain and in most cases there is insufficient data available to carry out comprehensive quantitative BSE-risk assessments. The unknowns include the exact nature of the infective agent, the minimum infective dose, the exact distribution of infectivity in tissues relative to incubation period and the magnitude of a possible species barrier for BSE between bovines and humans. Also, tests for the detection of pre-clinical BSE infectivity on live animals are not yet available for operational, wide-scale use. Nevertheless, the implicit logic through the SSC opinions has been that currently available scientific knowledge permits the elaboration of sound qualitative assessments to provide sufficient grounds for an appropriate risk management strategy.

   The *scientific evidence* on which the SSC opinions are based and used in judging the safety of a product can be grouped around the following **key evidences**. These may be updated in the future should new scientific evidence become available.

   The SSC has started the development of a method for the quantitative assessment of the residual BSE risk in ruminant-derived products. It did, however, not complete this exercise within its mandate.

2. **BSE in cattle**. Cattle are affected by a fatal neurological disorder belonging to a disease group called the transmissible spongiform encephalopathies (TSEs) which has been defined as Bovine Spongiform Encephalopathy (BSE). There is strong scientific evidence that humans may also become infected by the BSE agent after consumption of cattle products containing BSE infectivity. The disease in humans is called variant Creutzfeldt-Jakob Disease (vCJD).

3. **Scrapie and possible other naturally occurring TSEs in sheep and goats [30, 31, 32, 33, 34, 35, 36, 37,38].**

   The disease of scrapie has been recognised for more than 200 years but has not been shown to contribute to the epidemiology of human TSEs. Sheep have been exposed in the past to the same proprietary feed stuffs as cattle and therefore possibly to BSE contaminated meat-and-bone meal (MBM). BSE has been transmitted experimentally to TSE-susceptible sheep with clinical and pathological features closely similar to those of scrapie. However, to date, BSE has not been found in domestic flocks of small ruminants, nor is there other evidence that BSE is present in small ruminants under field conditions, or, indeed any indications pointing to an increased likelihood of such being the case. One should nonetheless keep in mind that the number of animals investigated for such occurrence is relatively small.

   Note: Potential differences between scrapie in sheep and goats.

   Ideally separate risk assessments should be carried out for scrapie in sheep and in goats. However, very limited data are available for goats. Therefore the SSC considers that conclusions for sheep are currently considered to be a reasonable and best possible approximation for goats.

4. The **minimum amounts** of BSE infectivity **needed to infect** another individual are not known for either humans or cattle **[3, 5]** nor any other species. For cattle it is lower than 1g of infected brain material.

5. The SSC therefore considers that the risk of human exposure to BSE infectivity should be reduced as far as is practical and that this can be achieved practically by a combined action on all parameters that have a possible impact on the level of BSE infectivity in a cattle-derived product (and in small ruminants products in case BSE is detected under natural conditions).

Relevant issues which have to be considered in this context are:

	See section:
– The geographical source of an animal or derived product	6
– The host susceptibility of the animal to BSE	7
– practices (including aspects such as feed contamination, cross-contamination, culling and disposal of risk materials)	8
– Vertical transmission risk (including aspects such as offspring cull and dam survival)	9
– Potential alternative mechanisms of transmission of TSE	10
– The type of animals: risk animals versus animals being fit for human consumption (including the application of rapid BSE tests)	11
– The management of Specified Risk Materials and the age of the animal	12
– The processing of raw material into derived products	13
– The intended end-use of a product (human, animal, technical, etc.) and the number and lengths of exposures	14

These are further discussed in the remaining sections hereafter.

6. The **geographical source of an animal or derived product [118→129]**

From a public health point of view, the ultimate goal is to identify animals that do not present a BSE risk. The achievement of this objective is subject to a number of considerations.

6.1. There are no operational pre-clinical tests available that can be applied to live cattle. The incubation period of the disease is long (mean about 5 years) implying that infectivity may be present in certain tissues well ahead of the appearance of clinical signs. Careful sourcing of animals is therefore an essential step to minimise or exclude the risk that they are BSE-infected. If animals and animal derived materials come from countries other than those for which BSE is highly unlikely, compensatory measures to reduce BSE risk should be taken, such as the exclusion from consumption of certain risk materials and/or the submission of the material to physical processing conditions with a proven capacity to reduce the infectivity level.

The correct assessment of the **geographical BSE risk** (**GBR**) is, therefore, essential. The SSC has developed a methodology for the assessment of the geographical BSE risk already applied to many countries and regions with results which have been confirmed so far several times. This assessment is commonly referred to as the "GBR-exercise" (see **Part II.B**); it has so far been applied to 63 countries that have submitted a dossier to the Commission to allow their GBR level to be assessed. The evaluation of 28 additional dossiers is ongoing.

The Geographical BSE-Risk (GBR) is a qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE, pre-clinically as well as clinically, at a given point in time, in a country. Where presence is confirmed, the GBR gives an indication of the level of infection as specified hereafter.

GBR level	**Presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a region or country**
I	Highly unlikely
II	Unlikely but not excluded
III	Likely but note confirmed or confirmed, at a lower level
IV	Confirmed, at a higher level

The GBR level of the countries that have been assessed so far by the Scientific Steering Committee is listed in Part II.

6.2. A "***Closed-Herd***" is defined **[84]** as a cattle-herd that is closed with regard to those factors which could introduce the BSE agent into the herd. Animals from a closed herd are therefore equally safe as animals from a GBR I country. (Note: Following the terminology in the medical sector, and because the term "closed herd" is differently used in the veterinary field, the term "**negligible BSE-risk herd**" may be preferred.)

6.3. Currently **rapid diagnostic tests** developed for the *post mortem diagnosis of clinical BSE* in screening programmes, if applied systematically on sound statistically significant numbers of animals in BSE screening programmes, contribute to the determination of the geographical BSE risk: they provide a tool for active surveillance that allows the detection of the first BSE cases at an earlier stage in the course of an epidemic or, more reliably excludes their presence. **[85, 86, 87, 88].**

6.4 Note: The Scientific Steering Committee on 7-8 November 2002 adopted a pre-emptive opinion on the geographical BSE risk for sheep and goats (GBR-S): adaptation of the cattle GBR methodology to small ruminants, in case BSE in small ruminants would become probable or evident under field conditions. **[118]**

The Scientific Steering Committee has been considering the risk of BSE in sheep since it first began the assessment of the risk relating to TSEs following the finding of the probable link between BSE in cattle and the development of vCJD in the UK in 1996. The relevance of this subject stems from the experimental evidence that some strains of sheep and goats developed BSE upon experimental ingestion of MBM made from BSE infected cattle material. On the other hand, there is no evidence that BSE is present in small ruminants under field conditions. The opinion of 7-8 November 2002 completes the series of other pre-emptive opinions related to this subject. Six other recent SSC opinions **[11, 12, 30, 31, 32, 33]** address the distribution of TSE infectivity in small ruminant tissues. Should BSE in small ruminants become probable or evident under field conditions, they propose a possible strategy to investigate the possible presence of BSE in sheep and explore possible approaches for safe sourcing of small ruminants based on genotyping, breeding, rapid TSE testing, flock certification and elimination of specified risk materials. Implementation would, however, be subject to a number of practical difficulties.

7. Host susceptibility to BSE

It has been demonstrated in experimental models of TSE diseases that the combination of strain of TSE infectious agent and the genotype of the host PrP gene play a major role in determining relative incubation periods between model systems.

Together strain of agent and PrP genotype also affect the targeting of infection to different organs and to different parts of the brain. The size of the dose required to infect the host is also affected by these two factors.

The use of the words "susceptible" and "resistant" in what follows requires careful definition. They should be seen as relative terms in a continuum of susceptibility, not as absolute statements. By "more susceptible" it is implied that animals can be infected by a relatively small amount of infectivity and by a relatively inefficient route (e.g. the oral route). By contrast "more resistant" implies that a larger dose of infective material is required to infect the animal and possibly by an efficient route (e.g. Intracerebral injection). Although it is often the case that more susceptible models have relatively short incubation periods, susceptibility and resistance should not be confused with length of incubation period, since in some cases highly susceptible animals can have long incubation periods.

7.1. As far as ***bovines*** are concerned, there is no evidence of genetic differences in susceptibility to BSE. Therefore, all cattle breeds and individuals must be considered to be susceptible to BSE.

As far as the *genetic susceptibility of **sheep** to BSE* is concerned **[30, 32, 35],** sheep PrP genotypes and their effect on incubation period and pathogenesis are very complex and poorly understood. The available knowledge is based on a few experiments carried out on small numbers of animals involving only a very small proportion of sheep breeds. The results obtained indicate variation such that it is difficult, at present, to make specific conclusions, or to make generalisation on host susceptibility to BSE in sheep.

Until demonstrated otherwise in several models of sheep TSEs it must be assumed, as a reasonable worst case, that after infection, there is a rapid rise in the amount of infectivity in lymphoid and other peripheral organs of both susceptible and semi-resistant sheep genotypes but that resistant sheep may harbour less infectivity early in the incubation period.

7.2. As available information on *BSE susceptibility and genotype in **goats*** is very limited, it is reasonable to assume, for the time being, that all goats are susceptible to TSE by the oral route under certain conditions. Further research on goat genetic susceptibility is required.

8. **Feeding practices [2]**

8.1. Contaminated feed is the main source of infection for **bovine animals**. It may be expected that healthy animals exposed to the same suspected feed source of infection as a confirmed case of BSE, are at greater risk than animals from a herd in which BSE is not present and exposure is not thought to have occurred. **[83, 84, 120, 121]**

8.2. For **sheep**, should BSE be diagnosed in sheep populations under field conditions, the routes of transmission may not to be limited to infected feed. If BSE in sheep behaves similarly to sheep scrapie it may also transmit via vertical and horizontal pathways (e.g. via the environment or by contact). **[33, 118]**

8.3. In practice, this means that an appropriate **culling [76, 77, 83]** strategy is needed of animals that may have been exposed to the same source of infection (e.g. feed) as a confirmed BSE case. It is additionally obvious that ruminants, tissues or by-products posing a BSE risk should never be **recycled** (e.g. in the form of meat-and-bone meal (MBM) as a protein source for animal feed) but **disposed [103, 104]** of. **Cross-contamination [56, 67]** of cattle feed with MBM must also be avoided.

9. **Vertical transmission risk [2, 6, 7]**

9.1. Offspring of sheep with scrapie and possibly other ruminants with a TSE have a higher probability of eventually developing the TSE. The exact mechanisms are not well known: it cannot be entirely excluded that *in utero* direct transmission may occur in sheep with scrapie but also other mechanisms are possible (e.g. exposure to the sheep placenta after parturition).

9.2. For *BSE in **bovines***, the evidence points toward minimal involvement of any form of vertical/maternal transmission of BSE in propagating the epidemic. The results of a single epidemiological study were consistent with an enhanced risk of up to approximately 10% of BSE in offspring born to dams

within 6 months[3] of onset of clinical signs of BSE, with much lower and rapidly decreasing rates up to 24 months prior to the onset of clinical signs in the dam. Enhanced genetic susceptibility cannot be excluded as the basis of these data but this is at present only speculative. What precedes reflects an area of uncertainty, as the average value of about 10% is based on *statistical* grounds, not on experimental evidence of maternal transmission. In this context the SSC wishes to refer to the opinion of September 1997 of the former Multidisciplinary Scientific Committee (MDSC) on Maternal Transmission, in which the wording "maternal risk enhancement" is used. The latter wording is considered to better reflect the uncertainty and may cover mechanisms other than direct maternal transmission.

9.3. For *scrapie in **sheep***, there is field evidence for vertical risk enhancement, although no quantified expression of the risk is available. **[30, 33]**

9.4. **Offspring culling** and (for bovines) **dam survival** without the occurrence of BSE for at least six months after calving, will increase the confidence that the offspring have not been infected. **[6, 7, 112, 113]**

On the basis of these data the UK Spongiform Encephalopathy Advisory Committee (SEAC) concluded that there is some evidence of direct maternal transmission at a low level may occur but they cannot rule out variation in genetic susceptibility to feed-borne infection as an additional factor. It is thus still unclear if maternal transmission of BSE in cattle in the traditional sense occurs or not, and if it does, which mechanism is involved. The analysis of the 34 BARB BSE cases between 1 August 1996 and 31 December 2002 **[105]** does however provide little field evidence for maternal transmission in this population. From this analysis appears also that maternal transmission does not contribute to the maintenance of the epidemic.

10. The existence of a **third route /mechanism of transmission of TSE [2]**, in addition to feed and vertical risk enhancement, such as via environmental pathways has been

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[3] In the SSC opinion of 18-19 March 1998 on vertical transmission, the figure of 12 months is given. This has subsequently been revised downwards to 6 months in the SSC opinion of 7-8 December 2000 on: Monitoring Some Important aspects of the evolution of the Epidemic of BSE in Great-Britain.

strongly suspected for scrapie in sheep. For BSE in cattle, there is no evidence of such routes or mechanisms, although theoretically they cannot be excluded *a priori.*[4].

11. **Risk in relation to health status of animals** The rapid TSE testing programmes that started in January 2001 (for cattle) and April 2002 (for small ruminants) on an EU-wide scale clearly shows the incidence of TSEs is significantly higher in fallen stock and other categories of risk animals. In Part II the BSE in cattle and TSE in small ruminant statistics as per 31 December 2002 are provided. The risk of exposure of humans to possible BSE infectivity is thus significantly reduced if the raw materials are only obtained from animals that are healthy or fit for consumption.

    An authorised veterinarian declares animals fit for human consumption if and when they pass an *ante mortem* and a *post mortem* inspection. These inspections will identify and therefore exclude from the human food chain, clinical BSE cases and any animals that show a behaviour or clinical sign that could be compatible with BSE. Clearly such inspections do not identify pre-clinical cases of BSE.

    In addition, the rapid BSE tests that have been recently developed provide an additional prospect for pre-clinical diagnosis **[EC, 1999; EC, 2002; EC, 2003].**

12. The **Specified Risk Materials and age of the animal [10 → 29]**

    12.1. Part II provides a chapter summarising the current knowledge on specified risk materials in cattle and in sheep.

    TSE infectivity is not distributed uniformly throughout the body and at all ages, but varies significantly according to the tissue, the species, the genotype (in sheep) and the age of the animal. The exclusion from consumption or recycling of tissues that pose a risk of containing BSE infectivity (the so-called "specified risk materials") will, therefore, significantly reduce or even exclude any human exposure risk. However, large differences exist between bovines and ovines:

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4 However, the evolution of BSE in Europe and especially the decline of the BSE epidemic in the UK, which represents more than 97 % of all BSE cases so far world-wide, does not support such routes or mechanisms, at least not at significant levels.

12.2. For ***cattle*** the infectivity distribution is mainly confined to a limited number of tissues and is predominantly in the central nervous system. In adult cattle at the end of the incubation period of BSE, approx. 95% of the total infectivity is present in central nervous system (brain, spinal cord, eye) and a limited number of other tissues (dorsal root and trigeminal nerve ganglia and ileum). The BSE infective load in infected animals early in the incubation period is much lower than in animals in later stages of incubation. In the earlier part of the incubation period low levels of infectivity are present in the intestine (ileum) and tonsil. By the clinical phase of disease infectivity is readily detectable in the central nervous system and dorsal root ganglia. The SSC considers, as a reasonable worst-case assumption, that infectivity may become detectable in CNS tissues as from half of the incubation period. Taking into account that in the UK, out of a total of approximately 180,000 BSE cases[5], only 0.17% were 35 months old or younger, 0.05% were 30 months old or younger and 0.006% were 24 months old or younger and that the corresponding data for BSE in other countries are similar, it can be accepted that the CNS-tissues of bovines younger than 12 months do not pose a risk.

12.3. For ***TSE-susceptible sheep*** (scrapie or experimental BSE), tissue infectivity distribution is much more widespread. As a result, sheep tissues that would pose a potential risk should BSE be present in sheep, cannot be listed by simple extrapolation from what is known about BSE infectivity distribution in cattle. **[11, 12, 30]**

Available findings indicate that, for TSE-infected sheep, infection may be widespread in the lymphoreticular system from a few months after exposure and detectable from two months of age in Peyer's patches and mesenteric lymph nodes. This being the case, there is currently no basis on which to recommend an age cut-off for the presence of BSE-infectivity in small ruminant tissues. In practice, for older sheep in an advanced stage of incubation, the larger fraction of the total infectivity would not only be

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5 Representing more than 97 % of all BSE cases recorded so far

present in what are currently listed as the "sheep specified risk materials"[6], but also in other tissues, particularly intestines and lymph nodes, and also enteric nervous system and associated autonomic nerves and blood[7]. In younger infected animals, not yet showing clinical signs, the non-CNS tissues would probably contain most of the infectivity and should also be considered as possible specified risk materials.

Note: In April 2002 the EU has launched a large campaign of rapid TSE testing of small ruminants (see Part II). It involves several hundred thousands of animals per year. Plans exist to submit a significant fraction of the tested animals to an additional PrP genotyping examination. It is expected that, once a statistical significant number of results of both surveys are available, it will also be possible to modulate the recommendations with regard to infectivity distribution in sheep tissues as a function of genotype and age.

13. **Appropriate processing of raw material into derived products [44 → 75]**

13.1. **Part II.C** provides summaries of current knowledge on the TSE infectivity clearance levels resulting from various types of **production processes.**

13.2. One might argue that no additional sourcing requirements are needed to produce a safe product from tissues or organs of potentially BSE-infected animals if a high starting infectivity titer in TSE infectivity clearance experiments[8] shows high levels of clearance (e.g. gelatine). The argument that this requirement is not strictly necessary is that the processing conditions are so harsh that no infectious TSE agents could survive. Moreover, the molecules that result from these processes (e.g. amino acids, alcohol's, …) are entirely different substances as compared to the raw material they were

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6 The skull including brain and eyes, the tonsils, the spinal cord of ovine and caprine animals aged over 12 months or which have a permanent incisor erupted through the gum; The spleen of ovine and caprine animals of all ages. (See also **Part II**)

7 See also the SSC Opinion of 12-13 September on The implications of the recent papers on transmission of BSE by blood transfusion in sheep (Houston *et al*, 2000; Hunter *et al*, 2002)

8 Simulating a worst case scenario of inclusion of brain and spinal cord in the raw material

derived from and could therefore be considered equally safe as their equivalents derived from plants or inorganic materials. For a number of substances (e.g. certain hydrolysed substances, bovine charcoal, …) no systematic sourcing from healthy animals would then be required, provided the production process (and if appropriate: the filtering) are adequate. The SSC, however, considers that careful sourcing of the raw materials, where needed in combination with appropriate processing, remains a key-factor for producing safe products from tissues and organs both of potentially BSE-infected animals. Within the current content of TSE knowledge, the approach to consider safe sourcing as less essential if a process has shown under experimental conditions that a product does not contain infectivity at detectable levels, is not scientifically acceptable since no experiments so far can positively prove the total absence of infectivity. Moreover, there is no evidence that experimental spiking of tissues with high BSE titers results in similar conditions as material from naturally infected animals or fallen stock.

Note: All experiments measuring destruction or removal of infectivity are constrained by the starting titre of the material to be treated and the sensitivity of detection of the assay to be used and the validation of the scale down. It must be assumed, in the absence of other evidence, that infectivity at levels below the limits of detection is present even if it cannot be detected. Inactivation experiments measure "clearance", the difference between input and output titres (assumed to be the limit of detection if no infectivity is detected). It is more effective to demonstrate a high clearance than to demonstrate that no infectivity has been detected but with a lower clearance because the input titre is lower or the sensitivity of the assay is poorer. It follows that in most cases it is not justifiable to conclude, that if no residual infectivity was found at detectable levels, a given production process results in total TSE clearance.

The alternative approach, to consider safe sourcing as less essential if a process has been shown under experimental conditions to produce a product that does not contain infectivity at detectable levels implies an extrapolation to the whole consumer community of experimental results on a comparatively small numbers of test animals.

Safe sourcing is still expected to be, apart from a few possible exceptions, the initial step in ensuring a safe product. The exceptions are when the source material itself does not pose a risk (e.g. pure fat from meat-grade materials fit for human consumption) or when the process results in break-down molecules with a molecular weight that is sufficiently low to exclude any risk (e.g. certain tallow-derivatives).

14. **Intended end-use of and exposure to a product** (human, animal, technical[9], etc.).

14.1. The intended end-use of a product will determine the modalities of use/application. Whether a product is used as food/feed, a cosmetic product or a medicinal product or a medical device, will determine the route and the length of possible exposure that can be oral, intravenous, topical, and/or inhalatory and also whether or not there may be a repeated exposure. In the absence of quantitative data on minimal infectious doses and species barrier, the SSC throughout all its opinions on product safety, has always opted for "reasonable" worst case scenarios implying that a product should be as safe as possible and did not allow for modulation of risk assessments according to the route of exposure or intended end-use, except for certain exclusive technical uses for which possible human and animal exposure to any residual BSE-infectivity was deemed to be insignificant.

14.2. Product safety, also for topical applications, should be guaranteed by appropriate geographical and tissue sourcing and by appropriate processing (including purification or filtration). The basis of all SSC opinions on product safety is that the combination of these conditions will result in a product that can be safely used, even for prolonged periods. Available data indicate that the combination of the following actions will minimise the residual risk deriving from the BSE agent.

– Safe geographical sourcing of animals (i.e. Exclusion of BSE risk countries, where no appropriate risk control measures have been adopted on time. By excluding such countries from sourcing, the risk deriving

9 Within the context of SSC opinions, "technical uses" explicitly exclude cosmetic or pharmaceutical applications, also if it concerns the use of a ruminant-derived product as a a material for the production of cosmetics or pharmaceuticals.

from clinically observed BSE cases and from non-detected sub-clinical cases likely present, is avoided.

- Safe sourcing of animals, implying the use of healthy animals or animals fit for human consumption and the exclusion of the "so called" risk animals, fallen stock, emergency, slaughter etc. As a result, the number of sub-clinically affected animals being utilised will be significantly reduced.

- Safe sourcing of tissues (exclusion of specified risk materials). The cattle specified risk materials (i.e. brain, spinal cord, etc.) represent more than 95% of the total detectable infectious load of an adult animal with clinical signs. In tissues such as skin, pure fat and bones no infectivity has been detected so far.

- Appropriate processing, including the avoidance of cross-contamination with infectious tissue material, cleaning, filtration and physical treatment will further reduce the risk of any residual undetected BSE-infectivity persisting. In Part II a summary of the experimentally observed infectivity clearance levels of a number of standard processes is provided.

14.3. **Part II** provides a summary overview of how the above scientific information has been translated into BSE safety criteria for a number of ruminant-derived products sourced from countries or regions where the presence of one or more cattle clinically or pre-clinically being infected with the BSE agent is highly unlikely (GBR I).

IV. FURTHER TSE RESEARCH RECOMMENDED IN SSC OPINIONS AND REPORTS OF THE TSE/BSE *AD HOC* GROUP

Research into the transmissible spongiform encephalopathies (TSE) or prion diseases has progressed rapidly in recent years providing much new information but several of the most critical elements of our knowledge of these diseases remain enigmatic. Many SSC Opinions and Reports have addressed practical issues and research recommendations arising from them have suggested further work in applied research areas such as diagnosis, decontamination, exposure risk etc. It needs to be emphasised however that successful applied research is dependent on an understanding of the basic mechanisms of the TSE, many of which are still unknown.

1. Fundamental research

Fundamental research on the molecular nature of the infectious agent, the physical substrate of agent "strain", pathogenetic mechanisms of infection, how the agent is amplified, spread of infection and initiation of pathology, the genes involved in susceptibility to TSE (infection and/or disease) and the molecular nature of the species barrier and the relationship between this and agent strain, are all prerequisite to the improvement of approaches for applied studies, including the development of more sensitive diagnostic procedures, and, eventually, therapies.

2. Broad areas of research recommended in SSC opinions and reports

The list hereafter summarises the main areas in which needs for additional research have been identified in SSC opinions:

Epidemiology and surveillance

- Investigation of the incidence of TSEs on a geographical basis, including investigation of the possible presence of BSE in sheep and the possible prevalence of TSEs in other species (e.g. deer, fish, pigs, ...); also the origin of BSE cases in animals born after reinforced feedbans and standards of case ascertainment, both for human and animal TSEs.

Pathogenesis, infectivity

– Further knowledge is required on several aspects of pathogenesis, including: characterisation of dose/response relationships, infectivity distribution in relation to incubation period in tissues following oral exposure, cumulative exposure effects and clearance phenomena, intrinsic age-susceptibility and carrier status.

Diagnosis

– Development and validation of more sensitive post mortem and in vitro diagnostic methods, including techniques for differentiating TSEs (e.g. scrapie versus BSE).

Therapy (treatment and prophylaxis)

– Inhibition of the conversion of PrP^{C} tot PrP^{Sc}; prevention of neuro-invasion.

Environment

– The evaluation of residual risk from burial, burning and incineration (e.g. via ashes) of infected animals and materials;

– Persistence of the infectious agent in the environment as a transmission factor.

Other areas

– Risk assessment techniques

– Further investigation of stunning and slaughter methods to avoid embolism.

– Evaluation of the impact of physical treatments to inactivate infectivity (e.g. "133°C/20'/3bar") on the nutritional value and quality of bloodmeal for animal nutrition.

– Quantitative assessment of the residual risk for humans and animals of ruminant-derived products.

– Speciation of materials in (by-)products for feed and food.

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PART II:

OVERVIEW OF SPECIFIC SUBJECTS REFLECTING SSC OPINIONS AND REPORTS

PART II A:

BSE IN FOOD ANIMAL SPECIES

THE ORIGIN AND TRANSMISSION OF BSE IN CATTLE

By R. Bradley

The origin of BSE and the infectious scrapie-like agent responsible for BSE is not known. The common source origin of the BSE epidemic in cattle via meat and bone meal contaminated with a TSE agent is widely accepted, but the events which preceded this to explain the ultimate origin of such an agent remain a subject of speculation.

The origin of BSE

The contribution on the epidemiology of BSE in bovines by J. W. Wilesmith elaborates in detail on the hypothesis that the unique combination of demography (the large sheep population compared to the cattle population and therefore the relatively large amount of sheep waste generated for rendering), the fact that scrapie is endemic in the UK sheep population and the conditions of rendering (changes in the process) provides a plausible explanation as to why BSE was initiated on such a scale in the UK and not elsewhere. This hypothesis also clearly implicates the sheep scrapie agent as the origin of the BSE agent.

Other hypotheses have been suggested including an origin of a TSE agent from several mammalian species other than sheep. For example, from cattle, implying a previously undetected form of BSE in this species. Theoretically, this could occur as a rare sporadic form of BSE akin to sporadic CJD of humans, or as a spontaneous case resulting from the change of normal bovine PrP into an infectious form. Other mammalian species, including possibly, captive exotic or wild animals whose carcasses were rendered into MBM have also been proposed as the potential origin of the BSE agent. There are insufficient data to either substantiate or to completely reject any of these hypotheses. Furthermore, all of these theoretical sources would indicate a point source of infection, whereas, when first recognised, the BSE epidemic was already presenting as an extended common source epidemic. An extended common source epidemic occurs more or less concurrently in multiple, widely dispersed different geographical locations inferring that at each location the same, or similar, exposure to the same infection occurred at approximately the same time. The hypothesis of an extended common source epidemic is consistent with the observations that BSE appeared in most parts of Great Britain within a

short space of time (i.e. shorter than the mean incubation period of BSE) with the epidemiological findings with respect to regional differences.

A point source epidemic is, on the other hand, one originating from a singleton event, or focus, with subsequent spread from that focus. The discrimination between a point source and common source is not easy because a point source epidemic, after spread, may take on the characteristics of a common source epidemic. For the origin of BSE, a point source epidemic is thus feasible, but it would imply that in the intervening years (say 10-15 years or 2-3 mean incubation periods) between initial exposure and the first detected cases, veterinarians did not recognise the novel disease occurrence. However, whether the nature of the epidemic was an extended common source from its origin or, it started as a point source followed by repeated recycling, to become indistinguishable from a common source event, before being recognised, cannot now be established.

Alternative hypotheses on the origin of BSE and its exclusively TSE agent causation have been documented. Some are not supported by scientific scrutiny and can be rejected (*e.g.* the autoimmune hypothesis, the bacterial (*Spiroplasma* sp.) hypothesis, the single stranded DNA hypothesis or an origin from *Coenurus cerebralis*). Some other hypotheses implicate a toxic (co-)factor (*e.g.* fat-associated chemical toxins in tallow or organo-phosphorous compounds) or a deficiency such as an inadequate exposure to natural prostaglandins and have also been regarded implausible. Once the nature of TSE agents is defined and accepted it may be important that certain potential aetiological factors in the original causation of BSE be reinvestigated.

BSE transmission

There is very clear and strong support from epidemiological studies, rendering studies and the effect of feed bans in all countries with BSE, for the hypothesis of infected mammalian protein in the form of MBM being the major vehicle for BSE transmission in cattle. It can enter the feed deliberately or, accidentally by cross-contamination. Experimental proof of this is however lacking since, given the low incidence of the disease, an experiment in cattle to formally test this hypothesis using compound feed containing BSE-infected MBM (as prepared commercially at the time of natural exposure), would require group sizes of the order of several thousand animals.

The actual occurrence of cross-contamination of ruminant diets with infected mammalian protein (especially MBM) is considered to be part of the feed route. Cross-contamination

can occur readily during feed preparation in feed mills, during transportation, or on farm, unless stringent measures are taken to avoid it.

The incorporation of infected ruminant- or mammalian-derived materials in feed other than MBM is another possibility of transmission. Such materials might have been gelatine, fat or blood (or protein products derived from them) in which the starting materials were contaminated. Effectively enforced SRM bans and alternative, improved and authorised ruminant stunning and processing methods (including for rendered products, fat and for gelatine manufacture) should now eliminate such causes.

Maternal transmission is theoretically a possible route of disease spread since it would appear to occur in natural scrapie in sheep. There is some statistical support for the possibility of some form of maternal transmission of BSE in cattle, but there is no evidence so far that this so called 'maternal transmission' occurs in the absence of a feed borne source and no plausible mechanism for the so-called maternal transmission has been identified. Nevertheless, maternal transmission cannot completely be excluded yet as an occasional, or rare, cause of BSE, but the incidence is so low that it cannot sustain an epidemic alone.

Any other cause than from feed or maternal transmission becomes a potential 'Third Way'. Possible genuine 'Third Ways' are discussed in the SSC opinion of 30 January 2001 **[2]** on hypotheses on the origin and transmission of BSE. But, if they exist, they are unlikely to contribute significantly to the BSE epidemic. Such causes may historically have been concealed by the overwhelming majority of feed induced cases but theoretically could be exposed as contributors once infected feed is completely eliminated.

Relevant SSC Opinions (see annex II): **1, 2, 6, 7, 8**

EPIDEMIOLOGY OF BSE IN BOVINES

By J. W. Wilesmith

BSE was first identified as a novel disease in Great Britain in November 1986 as a result of the routine animal disease surveillance activities. Affected animals were brought to the attention of veterinary surgeons, who in turn sought expert diagnostic help, as a result of herd owners discussing among themselves the unusual clinical signs, especially behavioural changes and the occurrence of multiple cases, over a few months, in one large dairy herd.

Epidemiological studies indicated that the disease occurred predominantly in dairy herds and only in adult animals. The geographical distribution of the disease was remarkable in two respects. The disease occurred simultaneously throughout Great Britain, including the Channel Islands. However, the incidence was significantly greater in the south of England. These studies were naturally also concerned with investigating the cause of the disease and whether it was truly a new disease in addition to gaining a full insight of the descriptive epidemiology and the clinical signs. The results did not provide any evidence that BSE was simply a genetic disease; neither was it shown that BSE was linked to an unrecognised toxicity, agrochemicals or therapeutic products. With respect to the possible role of a scrapie-like agent, direct contact with sheep scrapie could not account for the occurrence, nor could contamination of vaccines or biological products such as hormone preparations.

The only common factor was the feeding of commercial feedstuffs containing meat and bone meal (MBM), incorporated as a protein source, and tallow, as an energy source. Both were products of rendering animal carcass waste predominantly from slaughterhouses.

Tallow was not considered to be the vehicle of a scrapie-like agent because the geographical variation in incidence was not consistent with its distribution and use. MBM on the other hand was distributed and incorporated into animal rations within a relatively small distance of production. The epidemiological studies had indicated that BSE was a new disease with the first cases occurring in early 1985 and that effective exposure of the cattle population commenced in 1981/82. This did not coincide with the start of the use of MBM in cattle rations. It had been incorporated as a protein source for

several decades. Attention was therefore directed at the rendering industry and the processes that had been used to produce MBM. There was no evidence that a proportion, at least, of rendering plants had changed the species composition of the slaughterhouse waste such that there was a change in the concentration of sheep tissues. There had however been two changes in the processes used to render animal tissues. One was a change from batch rendering to continuous rendering. This change occurred in an attempt to reduce energy inputs. The other change was the cessation, except in Scotland, of the use of hydrocarbon solvents to maximise the extraction of tallow. There were a number of reasons for this change. The price difference between tallow and MBM (the two products of rendering) reduced during the late 1970s such that the world price of tallow reduced. It was therefore not profitable to maximise the extraction of tallow. In addition, the energy content of animal feedstuffs was increasing and this could be simply included by using MBM with a greater tallow concentration. There were also energy cost implications in using solvent extraction and health and safety issues.

Both changes could have favoured the survival of scrapie-like agents, but the abandonment of solvent extraction was probably most significant. It involved the application of additional heat, notable in the form of steam which is more effective than dry heat used in the rest of the rendering process. This heat was applied to an almost defatted material and the solvents themselves are likely to have had some effect on such agents. Evidence in support of this hypothesis was provided by the geographical distribution of BSE in Great Britain where the incidence was much lower in the north of England and Scotland. The continued use of hydrocarbon solvent extraction, in Scotland, was consistent with this distribution. In addition, the geographical distribution of MBM which was not transported long distances and was produced by the reprocessing of greaves and therefore subjected to double heat treatment, was found to be inversely related to the BSE incidence.

The emergence of BSE in Great Britain, and an absence in other countries in the late 1980s, is consistent with the disease having its origins in sheep scrapie. This is because of several interacting factors. Firstly the ratio of sheep to cattle (in favour of sheep) in Great Britain which was greater than in any other European country at least; secondly the fact that scrapie is endemic in the GB sheep population and probably is present at a greater prevalence than elsewhere (see however also the section with Epidemiological data) and lastly, conditions of rendering that favoured the survival of the scrapie-like agents. The origin of BSE has been widely discussed in the scientific community and it is

unlikely that it will be definitively identified. However, the sheep scrapie origin remains as the one hypothesis which explains the observed epidemiology of BSE.

The MBM hypothesis has been substantiated by a case-control study of calf feeding practices in BSE affected and BSE unaffected herds. Also, and significantly, the initial ban on feeding ruminant derived protein (RDP) to ruminants in July 1988 resulted in a decline in the incidence of BSE from 1993, that is after the average incubation period. This statutory ban was not, however, completely effective and cases of BSE in animals born after July 1988 occurred. These are referred to as born after the ban (BAB) cases. These stimulated additional epidemiological studies because of the concerns of other means of transmission. One study revealed that at the peak of feedborne exposure in 1988, offspring of clinically affected cows had an enhanced risk of developing clinical BSE themselves. As the epidemic has progressed, and there has been no evidence of true maternal transmission, it is likely that this enhanced risk for offspring is due to some as yet unidentified genetic component. However, this risk could not explain the occurrence of the BAB cases and no other means of transmission were identified. The BAB cases exhibited a different geographical distribution compared to the cases in earlier born animals. This was such that the incidence increased in the east of England, where the pig and poultry populations are concentrated. Specific analyses indicated that the incidence of BAB cases were associated with the ratio of cattle to pigs. The feeding of MBM to pigs and poultry was still allowed at this time. The specified bovine offal (SBO) ban introduced in September 1990 was intended to remove high-risk tissues from the feed chain. However, it eventually became apparent that there was incomplete compliance with this statutory ban. Therefore MBM produced from high-risk tissues was present in feedmills, the majority of which produce feedstuffs for all farm livestock species. Investigations of feedmills indicated that there was considerable opportunity for cross-contamination of ingredients especially at points of entry to storage areas. It was also a common practice to divert pig rations that did not meet commercial standards into cattle feedstuffs. Testing of feed ingredients for species specific proteins confirmed that cross contamination was occurring. There was no evidence of the deliberate and illegal inclusion of RDP in ruminant feedstuffs, and the exposure of the BAB cases was therefore due to cross contamination in the production of commercial rations and to a lesser extent cattle receiving feedstuffs intended for other species.

The incidence of BAB cases in GB continued to decline in successive birth cohorts, but represented a significant number of cases in the epidemic. The occurrence of vCJD in 1996 and the association with BSE, together with a realisation of the imperfections in the

ban on feeding MBM resulted in a total ban on the use of mammalian derived protein in the feedstuffs for any farm livestock species. This came into effect on 1 August 1996 in the UK (re-inforced MBM ban). In addition, since April 1996 all cattle over thirty months (OTM) were excluded from the human food chain and slaughtered in designated slaughterhouses. The high risk tissues, designated as specified risk material (SRM) were removed separately and rendered, as were the carcasses. The resulting greaves have been stored in secure containment whilst awaiting incineration. The auditing of the processes involved in the OTM scheme have not indicated any potential leakage of material into the food and feed chains.

The report of a case of BSE in an animal born after 31 July 1996 in Great Britain on 1 June 2001 was therefore a significant occurrence. Initial epidemiological analyses of the first 30 such cases born after the reinforced ban (BARB) indicated that they represent a third epidemiologically distinct series of cases that have occurred during the course of the epidemic in GB. The first series comprised those cases born before the initial feed ban in July 1988 whose incidence was greatest in southern England. The second series were the BAB cases which resulted in a marked increase in the incidence in the eastern part of England. The geographical distribution of BARB cases is consistent with the major risk factor being simply the number of cattle herds per county. This is suggestive of a random risk, consistent with a low risk of exposure, given the apparent incidence in these later born cohorts.

Thus there is no evidence of exposure to environmental contamination or maternal transmission. A feedborne source seems to be the most likely reason for exposure of these animals but the origin of such a source has as yet not been established.

By the beginning of 2003, cases of BSE in indigenous cattle had been detected in 21 countries worldwide. Infection was confirmed in ten of these following the EU-sponsored validation study of post mortem rapid screening tests developed by 1999, and their use from late 2001 in active surveillance. This involved testing animals at routine slaughter, fallen stock and casualty slaughtered animals, or one or more of these categories of cattle. There is little documented evidence as to how infection was introduced into each country, but it is widely accepted that importation of live cattle and/or MBM have been the principal factors. The epidemic of BSE indicates that amplification has occurred in a number of countries and stresses the fact that it was highly unlikely that any rendering system is capable of inactivating the BSE agent sufficient to preclude effective exposure of cattle.

The relatively high proportion of countries in which BSE was first detected by active surveillance rather than the detection of clinical cases is notable. This is, however, not unexpected given the difficulties of clinical diagnosis. BSE manifests itself clinically rather vaguely in its early stages, often with only behavioural changes, it occurs in relatively mature cows for which slaughter is likely to be the most economic course of action and occurs at a very low within herd incidence, with just singleton cases being common in countries with a low incidence. Screening fallen stock with the currently available rapid tests is clearly an effective means of detecting infection, where such animals can be made available for examination. However, the current EU BSE surveillance requirements should allow more to be learnt about appropriate surveillance strategies in countries with different abilities and budgets for testing the various potential target populations. BSE is likely to be detected in additional countries, but it seems unlikely that any country will experience an epidemic of the magnitude experienced in the UK.

Tabulations of the number of BSE in cattle, by country and year of reporting together with explanatory notes are annexed in the next section. Also tabulated are the cumulative numbers of cattle tested within the EU in 2002 for BSE, and the cumulative numbers of sheep and goats subjected to TSE testing in the same period.

Relevant SSC opinions (see annex II): 2, 67, 90, 91, 106, 108, 109, 112, 114.

STATISTICS ON THE INCIDENCE OF BSE IN BOVINES AND TSE IN SHEEP AND GOATS

PROVIDED BY THE EUROPEAN COMMISSION

Preamble by C. Ducrot

<u>Preamble:</u> Elements be taken into account in interpreting the attached statistics

There are major differences between countries in the implementation of the different aspects of the surveillance of BSE and other TSEs, which are important to take into account to avoid misinterpretation of the data.

Dates and targeted population

Rapid tests did not start at the same time in the different countries. For example Switzerland started a test program targeted at risk cattle in 1999, and France in 2000. Most EU countries started test programs at the abattoir in January 2001, and on cattle at risk in July 2001. It is hence obvious that the number of BSE cases detected with the rapid tests per country per year differs depending on the beginning of the test programs.

Sampling procedure

Sampling of cattle populations at the abattoir and as fallen stock for application of rapid diagnostic tests is subject to some variation among different EU-countries, so the number of BSE cases detected with the rapid tests per country per year cannot be compared only on the basis of their proportion to the overall cattle population.

Age limit and cohorts

The age limit to test animals at the abattoir differs between countries. The EU regulation sets this limit at 30 months old but some countries such as Germany or France have a lower age limit (24 months old). For this reason, the ratio of positive to tested animals at the abattoir cannot be compared directly between countries.

Survival curve of cattle

Finally, interpreting correctly the statistics between countries would require taking into account the age of slaughter of cattle. If animals are sent to the abattoir on average at a younger age, the probability that they reached the end of the incubation period (if they are infected) at the time of slaughter (so that they test positive) is lower. Precise comparative data between countries is lacking on this point.

Number of cases of BSE in cattle

Country	< 1988	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	Total
United Kingdom	442	2473	7166	14294	25202	37056	34829	24290	14475	8090	4335	3197	2281	1428	1194	1124	**181876**
Deutschland	0	0	0	0	0	1[(a)]	0	3[(a)]	0	0	2[(a)]	0	0	7	125	106	**244**
Österreich	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**1**
Belgique/België	0	0	0	0	0	0	0	0	0	0	1	6	3	9	46	38	**103**
Danmark	0	0	0	0	0	1[(a)]	0	0	0	0	0	0	0	1	6	3	**11**
España	0	0	0	0	0	0	0	0	0	0	0	0	0	2	82	134	**218**
Suomi/Finland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**1**
France	0	0	0	0	5	0	1	4	3	12	6	18	31[(b)]	162	277	240	**759**
Ellas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**1**
Ireland	0	0	15[(b)]	14[(b)]	17[(b)]	18[(b)]	16	19[(b)]	16[(b)]	74	80	83	95	149	246	333	**1175**
Italia	0	0	0	0	0	0	0	2[(a)]	0	0	0	0	0	0	50	36	**88**
Luxembourg	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	**2**
Nederland	0	0	0	0	0	0	0	0	0	0	2	2	2	2	20	24	**52**
Portugal	0	0	0	1[(a)]	1[(a)]	1[(a)]	3[(a)]	12	15	31	30	127	159	150[(b)]	113	86	**729**
Total min UK	**0**	**0**	**15**	**15**	**23**	**21**	**20**	**40**	**34**	**117**	**122**	**236**	**290**	**482**	**968**	**1001**	**3384**
Total EU/UE	**442**	**2473**	**7181**	**14309**	**25225**	**37077**	**34849**	**24330**	**14509**	**8207**	**4457**	**3433**	**2571**	**1910**	**2162**	**2125**	**185260**

Sources: < 1997: OIE

1997,... Systematic notification of animal diseases by MS, completed by monthly reports of the UK and Portugal, and since 2001, of the other MS; websites of the competent authorities of MS and the IOE.

(a) Imported cases

(b) Imported cases: Ireland: 5 in 1989, 1 in 1990, 2 in 1991 and 1992, 1 in 1994 and 1995
France: 1 in 1999 - Portugal : 1 in 2000 and 2002

Number of cases of BSE in cattle

Country	< 1988	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002[(b)]	Total
Isle of Man	0	6	6	22	67	109	111	55	33	11	9	5	3	0	0	0	**437**
Jersey	0	1	4	8	15	23	35	22	10	12	5	8	6	0	0	0	**149**
Guernsey	4	34	52	83	75	92	115	69	44	36	44	25	11	13	2	0	**699**
Japan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	**5**
Liechtenstein	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	**2**
Poland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	**4**
Slovakia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	6	**11**
Slovenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	**3**
Switzerland	0	0	0	2	8	15	29	64	68	45	38	14	50	33	42	24	**432**
Israel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	**1**
Czech Republic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	**4**
Others[(a)]	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	**4**
Total	**4**	**41**	**65**	**115**	**165**	**239**	**291**	**210**	**155**	**104**	**96**	**54**	**70**	**46**	**55**	**41**	**1751**
World	**446**	**2514**	**7246**	**14424**	**25390**	**37316**	**35140**	**24540**	**14664**	**8311**	**4553**	**3487**	**2641**	**1956**	**2217**	**2166**	**187011**

Sources: OIE

(a) Imported cases registered in 1989 (Falkland Islands :1; Oman:2) and in 1993 (Canada :1)
(b) Last report on cases in 2002: Japan (23/8), Poland (31/10), Slovakia (2/9), Slovenia (12/7), Israel (4/6)
Isle of Man, Jersey and Guernsey (provisional data at 31/5), Switzerland (3/1/ 2003)
Czech Republic: 1/10.

BSE Testing in Cattle

Cumul January-December 2002

	Adult cattle[1] (in million)	BSE Suspect Animals[2] Positive		Risk Animals[3]			Healthy Animals[4]			BSE Eradication[5]	
		Nr	Positive	Nr	Positive	Ratio[7]	Nr	Positive	Ratio[7]	Nr	Positive
Belgique/België	1.5	279	5	37,929	16	4.2	408,934	17	0.42	3,277	0
Danmark	0.9	37	0	35,995	2	0.6	231,597	1	0.04	2,643	0
Deutschland	6.3	241	11	257,940	50	1.9	2,758,351	42	0.15	2,629	3
Ellas	0.3	0	0	2,256	0	0.0	21,456	0	0.00	22	0
España	3.4	63	17	86,380	75	8.7	452,733	36	0.80	5,473	6
France	11.2	207	41	271,727	124	4.6	2,896,182	74	0.26	15,881	1
Ireland	3.6	511	108	78,372	187	23.9	610,002	34	0.56	18,659	4
Italia	3.4	104	0	101,910	15	1.5	621,005	21	0.34	3,909	0
Luxembourg	0.1	14	0	1,941	1	5.2	16,443	0	0.00	0	0
Nederland	1.7	39	1	64,321	13	2.0	491,069	10	0.20	3,000	0
Österreich	1.0	2	0	13,564	0	0.0	215,075	0	0.00	0	0
Portugal	0.8	150	23	14,190	24	16.9	66,721	38	5.70	1,163	1
Suomi-Finland	0.4	6	0	22,333	0	0.0	114,669	0	0.00	0	0
Sverige	0.7	33	0	25,426	0	0.0	12,073	0	0.00	0	0
United Kingdom[6]	5.0	872	467	221,089	635	28.7.1	171,585	14	0.82	945	0
Total	**40.4**	**2,558**	**673**	**1,235,340**	**1,142**	**9.2**	**9,093,284**	**287**	**0.32**	**57,601**	**15**

[1] Source: Eurostat

[2] Animals reported as BSE clinical suspects

[3] Dead-on-farm animals, emergency slaughtered animals, animals sent for normal slaughter but found sick at ante mortem inspection

[4] Healthy animals subject to normal slaughter

[5] Birth and rearing cohorts, feed cohorts, offspring of BSE cases, animals from herds with BSE

[6] GB & Northern Ireland

[7] Positives per 10,000 tested animals

TSE Testing in Goats

Cumul January-December 2002

	Adult goats[1] (in million)	TSE Suspect Animals[4]		Risk Animals[2]			Healthy Animals[3]			TSE Eradication		Total	
		Nr	Positive	Nr	Positive	Ratio[6]	Nr	Positive	Ratio[6]	Nr	Positive	Nr	Positive
Belgique/België	0.02	1	0	86	0	0.0	64	0	0.0	0	0	151	0
Danmark		4	0	95	0	0.0	51	0	0.0	0	0	150	0
Deutschland		31	0	1,119	0	0.0	506	0	0.0	0	0	1,656	0
Ellas	3.59	8	4	273	0	0.0	9,037	5	5.5	0	0	9,318	9
España	2.22	7	0	901	2	22.2	4,389	0	0.0	0	0	5,375	2
France	1.04	0	0	12,371	13	10.5	14,657	2	1.4	1,342	3	28,370	18
Ireland		0	0	1	0	0.0	0	0		0	0	1	0
Italia	1.15	3	0	469	0	0.0	2,787	3	10.8	20	3	3,279	6
Luxembourg		0	0	0	0		0	0		0	0	0	0
Nederland		0	0	932	0	0.0	3,120	0	0.0	0	0	4,052	0
Österreich	0.04	0	0	451	0	0.0	127	0	0.0	0	0	578	0
Portugal	0.42	0	0	372	0	0.0	188	0	0.0	0	0	560	0
Suomi-Finland	0.01	0	0	47	0	0.0	58	1	172.4	140	3	245	4
Sverige		2	0	41	0	0.0	33	0	0.0	0	0	76	0
United Kingdom[5]	0.04	0	0	6	0	0.0	9	0	0.0	0	0	15	0
Total	**8.53**	**56**	**4**	**17,164**	**15**	**8.9**	**35,026**	**11**	**3.2**	**1,580**	**9**	**53,826**	**39**

[1] Source: Eurostat December 2001

[2] > 99% on farm deads, some emergency slaughtered animals and some with clinical signs ad ante-mortem

[3] Healthy animals subject to normal slaughter

[4] Animals reported as TSE clinical suspect

[5] GB & Northern Ireland

[6] Positives per 10,000 tested animals

TSE Testing in Sheep

Cumul January-December 2002

	Adult sheep[1] (in million)	TSE Suspect Animals[4]		Risk Animals[2]			Healthy Animals[3]			TSE Eradication		Total	
		Nr	Positive	Nr	Positive	Ratio[6]	Nr	Positive	Ratio[6]	Nr	Positive	Nr	Positive
Belgique/België	0.11	9	2	737	2	27.1	2,131	1	4.7	428	20	3,305	25
Danmark	0.09	6	0	396	0	0.0	563	0	0.0	0	0	965	0
Deutschland	1.57	1,676	4	18,845	6	3.2	12,718	5	3.9	1,498	1	34,737	16
Ellas	7.55	88	33	439	8	182.2	22,915	46	20.1	0	0	23,442	87
España	17.67	79	8	10,905	4	3.7	31,484	8	2.5	2,270	19	44,738	39
France	7.13	142	124	17,607	121	68.7	33,829	32	9.5	12,688	166	64,266	443
Ireland	3.89	122	47	5,222	33	63.2	54,813	13	2.4	21,884	237	82,041	330
Italia	8.22	29	17	2,687	25	93.0	19,867	26	13.1	918	20	23,501	88
Luxembourg	0.01	0	0	79	0	0.0	214	0	0.0	0	0	293	0
Nederland	0.93	0	0	3,864	11	28.5	19,642	29	14.8	0	0	23,506	40
Österreich	0.21	49	0	2,232	0	0.0	2,017	0	0.0	0	0	4,298	0
Portugal	2.35	0	0	7,443	0	0.0	1,290	0	0.0	0	0	8,733	0
Suomi-Finland	0.05	0	0	348	0	0.0	2,053	0	0.0	16	0	2,417	0
Sverige	0.21	13	0	984	0	0.0	3,992	0	0.0	0	0	4,989	0
United Kingdom[5]	16.08	536	421	1,348	6	44.5	31,145	33	10.6	0	0	33,039	461
Total	**66.07**	**2,749**	**656**	**73,147**	**217**	**29.7**	**238,673**	**193**	**8.1**	**39,702**	**463**	**354,271**	**1,529**

[1] Source: Eurostat December 2001

[2] > 99% on farm deads, some emergency slaughtered animals and some with clinical signs ad ante-mortem

[3] Healthy animals subject to normal slaughter

[4] Animals reported as TSE clinical suspect

[5] GB & Northern Ireland

[6] Positives per 10,000 tested animals

PATHOGENESIS, TISSUE INFECTIVITY DISTRIBUTION AND SPECIFIED RISK MATERIALS

By G.A.H. Wells and H.A. Kretzschmar

The rationale for the control of BSE to protect human and animal health was necessarily based on knowledge of the pathogenesis of natural and experimental scrapie. Recent studies of natural and experimental scrapie, confirming earlier findings, suggest that, after oral exposure and LRS replication, there is a routing of the agent via peripheral nerves (autonomic) to the central nervous system (CNS) where the disease becomes manifest. Close similarities between scrapie and BSE in respect to the distribution and titres of infectivity in tissues was not borne out by the initial limited mouse bioassays of tissue in naturally occurring cases of BSE, where infectivity was detected only in the CNS. An experimental study of the pathogenesis of BSE found that infectivity in non-neural tissues was confined to the distal ileum (6-18 months and 36-40 months post-exposure) and sternal bone marrow (only at 38 months post-exposure). The infectivity in ileum may reasonably be ascribed to the presence of the BSE agent in Peyer's patches; these patches were later confirmed to be the location of accumulations of PrP^{sc} in the ileal tissue in the experimentally affected cattle. The underestimation of the infectivity titre of BSE tissue when titrated across a species barrier in mice, as determined experimentally, is a factor of 500 fold. This relative degree of insensitivity of the mouse bioassay can partially explain the absence of widespread LRS infectivity in BSE. While inoculation of cattle (i.e. within species assay) with tissues from the pathogenesis study has confirmed infectivity in certain tissues which were found to be positive by the mouse bioassay and has shown traces of infectivity in palatine tonsil of cattle killed 10 months after experimental oral exposure, negative results have been obtained with pooled lymph nodes or pooled spleens from clinical cases of BSE.

Evidence from these studies suggest that involvement of the LRS in BSE is relatively restricted as compared to that in natural scrapie. This apparently restricted distribution of the agent in tissues of BSE affected cattle does not seem to be an exclusive property of the BSE agent since evidence from the experimental transmission of BSE to sheep indicates that, after parenteral inoculation or oral exposure, the pattern of tissue distribution of infectivity in genetically susceptible sheep resembles that of scrapie.

Age-cut-off limits below which no tissue from bovine, ovine and caprine animals is considered a risk need to take into account the criteria of animal species, infectivity in relation to incubation period, factors associated with slaughter protocols and geographical risk level of the source country.

The earliest onset of clinical signs in the study of BSE pathogenesis in cattle was 35 months after oral dosing and there was a close temporal association between the detection of infection of PrP^{sc} and of pathological changes in the CNS, all first apparent only at a late stage (about 90 per cent) of the incubation period. This observation must be interpreted with caution since the sequential sacrifice design of this study did not permit detection of incubation period for all but a very few animals and therefore cannot provide any information upon the relationship between the earliest detectable infectivity in CNS (or any other tissue) and the incubation period. It is not possible to predict when a case of BSE in the field will first show infectivity in the CNS. From dose response data of cattle infected orally with a dose of BSE infectivity, closely similar to that administered to induce disease in the Pathogenesis Study, a mean incubation of almost 45 months (range 33-55 months) has been shown. In some experimental models and from naturally occurring sheep scrapie, CNS infectivity can first be detected about halfway through the incubation period, but it is not known whether this is also applicable to BSE. However, the implication of these data is that infectivity may be *detectable* in the CNS in natural BSE well in advance of clinical onset. This time interval might be as short as 3 months before clinical signs, but at least theoretically, it could be 30 months in an animal with an average estimated field incubation of 60 months.

Unlike the situation in experimentally-infected cattle, the distribution of infectivity in experimentally infected sheep tissues, at different time intervals from exposure, by the oral route, to a large dose of the BSE agent, indicate a widespread involvement of lymphoid tissues early in the incubation period. In fact, after only one month from exposure to the BSE agent, susceptible sheep show an estimated significant load of BSE infectivity, in the intestine, lymph nodes, tonsils, stomach and spleen. After 36 months from exposure the estimated total BSE infectivity load in the animal body is much higher and the distribution of infectivity very different. As compared to the central nervous system tissues, the PrP^{Sc} load in the intestine of BSE-infected small ruminants is relatively higher at the beginning of the incubation period and of the same order of magnitude toward the end of the incubation.

Age-thresholds for the removal of SRM are therefore only possibly appropriate in small ruminants of semi-resistant or resistant PrP genotypes and will need to be revised in the light of more information on genotype in relation to susceptibility to

BSE infection. Should the presence of BSE in small ruminants become probable, safety of sourcing of small ruminants materials could be improved by combining different approaches including removal of tissues known to pose a risk of infectivity as from a given age, testing for BSE, genotyping and breeding for BSE-resistance, flock certification and individual animal and flock tracing.

The Table (Overview of current knowledge with regard to possible TSE infectivity[†] in ruminant materials) provides a list according to cattle or small ruminant of those tissues in which the occurrence of infectivity or disease specific PrP has been recorded at **any time** in the coarse of the disease (i.e. throughout the incubation or clinical periods).

The list is based exclusively upon observations of naturally occurring disease, and, in cattle and sheep, only in relation to BSE, primary experimental infection by the oral route. It does not include data on models using strains of TSE that have been adapted to experimental animals, because passaged strain phenotypes can differ significantly and unpredictably from those in naturally occurring disease. The single exception is blood that has been shown to be infectious, in experimental BSE in genotypically susceptible sheep and in sheep with naturally occurring scrapie, after transfusion of large blood volumes.

Some entries rely on the results of single or a small number of tissue examinations but have are been included for completeness.

Overview of current knowledge with regard to possible TSE infectivity in ruminant materials.

The **Table** below is compiled from the SSC opinions on infectivity in tissues and some specific opinions on intestine, fats, etc. Recently available published and unpublished findings have also been added. It is necessarily a simplification of available data on BSE or scrapie infectivity detected in tissues as it provides no indication of the sensitivity of the assay used and where results between studies differ, only positive results are given.

<u>Table:</u> Overview of current knowledge with regard to possible TSE infectivity[†] in ruminant materials.

Symbols used:

NOS: Not otherwise specified; No entry indicates no data available/not tested

Yes/No: Presence or absence of detectable infectivity.

MATERIAL:	Cattle	Small ruminants
NERVOUS TISSUES		
Brain	**YES**	**YES**
Pituitary	**NO**	**YES**
Dura Mater		
Spinal cord	**YES**	**YES**
Eye/Retina	**YES**	**YES**
Optic Nerve	**NO**	
Nodose ganglia	**NO**	**YES**
Dorsal root ganglia	**YES**	**YES**
Stellate ganglia	**NO**	
Trigeminal ganglia	**YES**	**YES**
Cerebrospinal fluid	**NO**	**YES**
Ceoliaco-mesent. Ganglion		**YES**
Cauda equina	**NO**	
Sciatic nerve	**NO**	**YES**
Tibial nerve	**NO**	
Splanchnic nerve	**NO**	
Facial nerve	**NO**	
Phrenic nerve	**NO**	
Radial nerve	**NO**	
Vagus nerve		**YES**
LYMPHO-RETICULAR[10]		

[10] LN = Lymph node MP = Mesenteric/portal; PF = Prefemoral; PS = Prescapular; RP = Retropharyngeal; BM = Bronchomediastinal

MATERIAL:	Cattle	Small ruminants
Spleen	**NO**	**YES**
Tonsil	**YES**	**YES**
LN : Prefemoral	**NO**	
LN : Mesenteric	**NO**	**YES**
LN : Retropharyngeal	**NO**	**YES**
LN: Submandibular	**NO**	**YES**
Lymph node (RP/MP)		**YES**
LN: Mediastinal		**YES**
LN: Broncho-mediastinal	**NO**	**YES**
LN: hepatic	**NO**	
LN: prescapular	**NO**	**YES**
LN: popliteal	**NO**	
LN: (PS/PF)		**YES**
LN: supra-mammary		**YES**
LN: ileo-caecal		**YES**
Peyer's patch	**YES**[11]	**YES**
Thymus	**NO**	**YES**
ALIMENTARY TRACT		
Oesophagus	**NO**	**YES**
Reticulum	**NO**	**YES**
Rumen (pillar)	**NO**	
Rumen		**YES**
Rumen (oesophag. Groove)	**NO**	
Forestomaches		**YES**
Omasum	**NO**	**YES**

[11] Research results in print.

MATERIAL:	Cattle	Small ruminants
Abomasum	**NO**	**YES**
Duodenum	**NO**	**YES**
Proximal small intestine	**NO**	
Ileum		**YES**
Proximal colon	**NO**	**YES**
Distal colon	**NO**	**YES**
Distal ileum	**YES**	**YES**
Ileum-proximal		**YES**
Caecum		**YES**
Spiral colon	**NO**	**YES**
Rectum-distal		**YES**
Rectum	**NO**	
Intestine (NOS)		**YES**
REPRODUCTIVE TISSUES		
Testis	**NO**	**NO**
Prostate	**NO**	
Epididymis	**NO**	
Seminal vesicle	**NO**	**NO**
Semen	**NO**	
Ovary	**NO**	**NO**
Milk	**NO**	**NO**
Colostrum		**NO**
Uterine caruncle	**NO**	
Uterus		**NO**
Placental cotyledon	**NO**	
Placental fluids : amniotic	**NO**	
Placental fluids : allantoic	**NO**	

MATERIAL:	Cattle	Small ruminants
Placenta		**YES**
Udder	**NO**	
Mammary gland		**NO**
Foetus		**NO**
Embryos	**NO**	
BONES		
Femur (diaphysis)	**NO**	
MUSCLE TISSUES		
Muscle: semintendinosus	**NO**	
Muscle: diaphragm	**NO**	
Muscle: longissimus dorsi	**NO**	
Muscle : sternocephalicus	**NO**	
Muscle: triceps	**NO**	
Muscle: masseter	**NO**	
Muscle : skeletal		**NO**
Tongue	**NO**	
Heart	**NO**	**NO**
BLOOD		
Blood: buffy coat	**NO**	**YES**
Blood: clotted	**NO**	**NO**
Blood: foetal calf	**NO**	
Blood: serum	**NO**	**NO**
Whole blood		**YES**
OTHER TISSUES		
Lung	**NO**	**NO**
Bone marrow	**NO**	**YES**
Bone marrow (sternum)	**YES**	

MATERIAL:	Cattle	Small ruminants
Fat (midrum / perirenal)	**NO**	
Fats (NOS)	**NO**	**NO**
Pericardium	**NO**	
Mitral valve	**NO**	
Aorta	**NO**	
Kidney	**NO**	**NO**
Liver	**NO**	**YES**
Pancreas	**NO**	**YES**
Thyroid		**NO**
Adrenal		**YES**
Nasal mucosa	**NO**	**YES**
Salivary glands	**NO**	**NO**
Saliva		**NO**
Nictitating membrane	**NO**	YES
Skin		**NO**
Trachea	**NO**	
Collagen (Achilles tendon)	**NO**	
Urine	**NO**	
Faeces	**NO**	**NO**

† Where results of studies of tissues using PrP^{Sc} detection as a surrogate marker for infectivity have indicated a positive tissue, data has been included.

BSE infectivity distribution in bovine tissues

MAFF/VLA have carried out experimental oral challenge studies in cattle to determine the attack rate and incubation period for a range of doses of BSE infected cattle brain. In the first of two experiments, groups of 10 calves were dosed orally with 3X100g (100g on 3 successive days), 100g, 10g or 1g of brain tissue (titre of inoculum: $10^{3.5}$ mouse i.c/i.p ID_{50}/g) from clinically sick animals. All animals in the

two higher dose groups, 7 out of 9 in the 10 g and 7 out of 10 in the 1g trial groups developed BSE. A second experiment is extending these findings with lower doses (1g-1mg), but the final outcome of the study will not be available for at least 5 years. Interim results at approximately 5 years post exposure, with 2 out of 15 animals in the 0.1g group and 1 out of 15 in the 0.01g having been confirmed positive for BSE (G. A. H. Wells & S. A. C. Hawkins, unpublished data) give an estimated oral ID_{50} of clinically affected BSE brain (Titre, as above) for cattle of 0.67g with a confidence interval of 0.24g to 1.83g. This estimate assumes that a 1mg dose would represent the null effect dose level, a factor that is not yet known. However, on the basis of these data, the range (confidence interval) of cattle oral ID_{50}'s in 1g could be approximately 0.55 to 4.2, although with higher titres of BSE affected brain (e.g 10^5 mouse i.c./i.p. ID_{50}/g, as have been recorded) the range could extend to 200.

Note: the issue of carrier states remains a key uncertainty with regard to TSEs in animals. The theoretical possibility remains that so called "resistant" animals act as sub-clinical carriers of TSE infection, capable of maintaining and transmitting infection.

It is known that infectivity builds up in an infected animal over time, so that the infective load in any particular animal will depend on the length of time since that animal was infected with BSE, and what proportion of the incubation period that represents. However, little is known about the dynamics of this. Also, there is no way of knowing when any particular animal would have been infected and age is therefore only an approximation, assuming as a conservative assumption that the animal was infected in calfhood. The initial dose consumed and the route of transmission will also influence the infective load.

In addition to the total infective load, the distribution of the BSE-infectivity in the animal's body also changes over time. The MAFF pathogenesis experiment has shown that at early stages of the incubation, the intestines are infective while at later stages of the incubation, the CNS carries significantly higher infective loads. Little is known about the way by which the infectivity moves through the body. No infectivity was found in the other tissues that were tested; i.e. if present the level of infectivity was below that detectable by the mouse bioassay.

The infectious load of the cattle by-products varies thus with the type of tissue, the titre of infectivity, its weight and with the age of the animal, relative to incubation period. The majority of the infectivity (about 95%) in cattle with clinical disease is in the brain, the spinal cord, and the trigeminal and dorsal root ganglia (TRG & DRG).

The distal ileum also carries a measurable infectivity and for spleen[12] and eyes a low level of infectivity is to be assumed based on scrapie experiments. Together these tissues carry about 99% of the infectivity in a clinical BSE case.

Specified risk materials

What precedes forms the basis for the definition of the so called "Specified Risk Materials" which are listed in the Section on *Preventing recycling of infectivity* by Bert Urlings (See **Part II.B**)

Relevant SSC opinions (see annex II): ,**10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29**

[12] It should be noted that all infectivity transmission experiments so far with bovine spleen have given negative results.

DIAGNOSTIC TSE TESTS AND PROSPECTS FOR THE FUTURE

By T. Baron

The very unusual properties of the infectious agents causing the transmissible spongiform encephalopathies (TSE) have a number of consequences for the diagnosis of such diseases. While there still remains many uncertainties regarding the precise nature of these infectious agents, their clear association with a conformationally modified form of a normal host protein, the prion protein (PrP), now offers this as the main analyte for the diagnosis of TSEs in both animals and humans. These neurodegenerative diseases are characterised by the absence of an inflammatory process or overt immune responses. A major consequence is that the diagnosis cannot be obtained from evidence of immune responses as in conventional infectious diseases, by the detection of antibodies in the blood of infected animals or humans.

Prior to the discovery of the prion protein, diagnosis of these diseases relied upon the post mortem finding of specific neurodegenerative brain lesions. These brain lesions typically involve spongiform changes, visualised as vacuolation of neurones and of the neuropil, and neuronal degeneration and loss, but also activation and proliferation of glial cells. Generally, the diagnosis can only be established post mortem; biopsy of brain is only considered in human patients and then, only rarely, and always at an advanced stage of the clinical disease. It should be emphasised that the distribution of the brain lesions depends on the infectious agent and on the host of the disease. In some forms of these diseases, as in BSE of cattle, the distribution is very uniform in the host species and therefore the post mortem diagnosis can be established by the specific examination of well defined neuro-anatomical regions, e.g. the medulla oblongata at the level of the obex. In a number of other situations, as in scrapie in sheep and goats, the distribution of brain lesions can be quite variable between different individuals presenting difficulty in the histological diagnosis and sometimes requiring examination of several areas of brain. Furthermore, in all cases of these diseases, the intensity of brain lesions may vary from one brain region to another, so that the absence of lesions in a small sample of the brain, as in the particular case of a brain biopsy, does not provide evidence of the absence of disease. Another major feature and drawback associated with histological diagnoses is that the lesions occur late during the course of the disease and after very long incubation periods. During much of the incubation period, measured in years in some animals species and in decades in humans, infection is not manifest in the form of any pathology but may provide a source of transmission of the disease.

The demonstration that an abnormal form of a normal host protein accumulates in the brain of infected animals or human has opened new avenues for the diagnosis of these diseases. While the normal cellular host protein (PrP^{C}) is soluble and can be fully degraded by proteolytic enzymes, the abnormal form of the protein (often referred to as PrP^{Sc} (for scrapie), PrP^{BSE}, PrP^{CJD} according to the disease and species in which it occurs, or PrP^{d} for disease) is insoluble in the presence of certain detergents and is partially resistant to the degradation by proteolytic enzymes (hence also referred as PrP^{res} when the identification of this protein has involved a preliminary treatment demonstrating its protease resistance). This PrP^{d} protein can be identified in the brain of clinically affected animals or humans, by methods which involve treatments allowing its distinction from the normal host proteins and particularly from the normal prion protein. The identification of "scrapie-associated fibrils (SAF)" comprised essentially of accumulated PrP^{d} using electron microscopy following detergent extraction from brain homogenates and ultracentrifugation has been used for the diagnosis of natural diseases.

Biochemical methods now allow the direct identification of PrP^{d}. These methods can be performed in freshly sampled or in previously frozen brain tissues. The tissue is first homogenised into a buffer, and treated with the proteolytic enzyme proteinase K to digest other proteins including PrP^{c}. Some methods allow steps for the concentration of the protein based on its abnormal solubility. Following this extraction procedure, PrP^{d} can be identified using different formats of tests. These can be Western blot methods that first involve a separation of proteins according to molecular mass in a polyacrylamide gel, then, following a transfer of the proteins from the gel to a nitrocellulose membrane, the identification of PrP^{d} using antibodies against the prion protein. Western blot methods thus allow identification of the specific size of the previously proteinase K digested prion protein, enabling specific recognition of the disease associated protein. PrP^{d} can also been identified by Elisa methods in microtiter plates, which are easier to handle and more rapid than Western blot methods. These Elisa methods do not allow the recognition of the specific molecular mass of the disease associated protein and confirmatory steps using Western blot or immunohistochemistry allowing the recognition of specific features associated with PrP^{d} are required, when the results of Elisa tests do not give a clear negative result. Some methods have been described recently that could allow the highly sensitive detection of PrP^{d} in fluids, such as cerebrospinal fluid, using fluorescence spectroscopy.

Immunohistochemistry can also be used to identify PrP^{d} in brain sections from fixed tissues, as used for the identification of specific brain lesions. These methods again involve pretreatments of tissue sections (autoclaving, proteinase K treatment etc) that

are necessary for the identification of PrP^d using antibodies recognising the prion protein. These pretreatments increase the specific antibody labelling of PrP^d and some remove the labelling of the normal prion protein. A drawback of these methods is that the pretreatments are not necessarily easy to standardise between different laboratories. Furthermore, a significant experience of the reader is required to be able to recognise the disease specific morphological features of PrP^d deposits. Also, although these methods can be automated to some degree, they are not suitable for screening a large series of samples. However, the immunohistochemical methods certainly allow the identification of small deposits of PrP^d that would probably not be detected by some of the biochemical methods, and enable precise identification of the specific brain regions in which PrP^d accumulates. A similar method (pet-blot) has been developed and involves the use of tissue sections, prepared from paraffin-embedded fixed tissues collected onto nitrocellulose membranes before proteinase K treatment. Such a method allows both a precise localisation of PrP^d in the brain and the sensitive and specific detection of protease resistant PrP.

It should be strongly emphasised that, for all these methods, unfortunately, no antibody is yet available that can distinguish, without preliminary treatment, the disease associated protein from its normal counterpart.

The ability of different diagnostic methods to identify the disease at a preclinical stage, earlier during the incubation period, will be highly dependent on the pathogenesis of the disease. For instance in experimentally infected cattle, PrP^d could be detected 32 months following challenge using Elisa methods or immunohistochemistry, while the onset of clinical signs were first recorded 35 months following challenge in this experiment. However, major advances in the detection of small quantities of PrP^d, suggested from recent observations that amplification of misfolded PrP^d could be obtained *in vitro* following conversion of PrP, using cycles of sonication, promises marked improvements in sensitivity.

In some circumstances, the identification of PrP^d can be achieved outside the central nervous system and in tissues that may be sampled from the living animal or human. These tissues are essentially lymphoid organs or structures containing lymphoid tissues, some of which, like tonsils, and nictitating membrane (third eyelid) are accessible for biopsy. This can however only be considered in some forms of these diseases in which detectable levels of PrP^d accumulate in the lymphoid tissues, as in scrapie in sheep and goats or in Chronic Wasting Disease in cervids. At least so far, such an approach does not offer any possibility for the diagnosis of BSE in cattle, since no accumulation of PrP^d has ever been detected in peripheral lymphoid tissues, apart from the intestine of animals experimentally infected with high doses of the

agent. Also, even within species, variable results can be observed according to the infecting strain of TSE agent. In human TSEs for example, PrP^d is detectable in peripheral lymphoid tissues in variant Creutzfeldt-Jakob disease attributed to infection with the BSE agent, but not in other forms of the CJD, including those also associated with infection by peripheral routes such as in iatrogenic cases of the disease. Importantly, when PrP^d accumulates in peripheral lymphoid tissues, it is possible to identify the accumulation earlier than the appearance of clinical signs of the disease and sometimes considerably earlier. For instance in a study of Romanov sheep flock naturally infected by scrapie in which the most susceptible sheep (VRQ/VRQ sheep) showed clinical signs at the age of 18 months, PrP^d could be detected by immunohistochemistry in lymph nodes and spleen at the age of 3 months and in the third eyelid at the age of 5 months. Some situations have however been described in sheep in which no PrP^d accumulation could be detected in the lymphoid tissues, despite the occurrence of clinical signs of scrapie and the presence of detectable accumulation of PrP^d into the brain. These last observations involved sheep showing an allele of the prion gene associated with resistance to the development of scrapie (ARR allele). The possibilities for diagnostic approaches are thus highly dependent on the pathogenesis of the disease, which is influenced by both host factors, especially genotypes of the prion gene, and infectious agent strains. To what extent each of the available diagnostic methods will allow an early diagnosis of the infection is variable. Furthermore, it cannot be excluded that these factors may also have an impact on the validity of each of the different available diagnostic procedures, particularly in sheep and goats characterised by complex genetic features and variable infectious agents. While these approaches have already allowed preclinical identification of infected animals in the field, the precise evaluation of these methods remains incomplete.

While some recent results have emphasised the presence of infectious agents in the blood in sheep experimentally infected with scrapie or BSE, an approach has also been described that would allow identification of protease resistant PrP in the blood of sheep with scrapie using capillary electrophoresis immunoassay. The presence of an unusual form of PrP^d has also been reported in urine, not only in experimental hamster models, but also in human TSE and in cattle with BSE.

Alternative methods for the diagnosis of spongiform encephalopathies have been reported that are not based on the identification of PrP^d; they rely on differences in certain markers between clinically affected animals (following experimental infection) and normal animals. Some of these are linked to markers associated with neurodegenerative processes, e.g. 14.3.3 protein in the cerebrospinal fluid. This last method has mainly been validated for the diagnosis of classical forms of Creutzfeldt-Jakob disease in human.

Differences have also been found between experimentally infected and normal hamsters following the study of blood using infrared microspectroscopy on cryosections of brain tissues or in serum, even at an early stage during the incubation period. Another study has reported the decrease of an erythrocyte differentiation transcription factor in blood cells in experimentally infected mice and in sheep with scrapie. It should be emphasised that such approaches remain so far at a very preliminary stage, and have not been fully validated for the diagnosis of natural diseases. However, it should not be excluded that an approach based on the finding of a marker different from the prion protein could offer new avenues in the challenging question of the early diagnosis of spongiform encephalopathies from tissues that can be easily sampled in the living animal or human.

Relevant SSC opinions (see annex II): **31, 34, 85, 86, 87, 88.**

EVALUATION OF RAPID POST MORTEM TSE TESTS

By H.Schimmel and W.Philipp[13]

One of the important measures taken for monitoring the prevalence of BSE was the implementation of compulsory testing of cattle for BSE. The currently applied procedures include

i) that all cattle older than 30 months which enter the food chain must be tested for BSE in all EU member states

and

ii) the testing of fallen stock and sick/emergency slaughtered cattle older than 24 months. This led to more than 10 million rapid post-mortem BSE tests being carried out in 2002. However, the performance and reliability of diagnostic tests had to be evaluated before their introduction into the market.

In 1998, the European Commission (EC) designated the Institute for Reference Materials and Measurements (IRMM) of the Directorate General Joint Research Centre to organise and evaluate rapid tests for the post-mortem diagnosis of BSE. Following a world-wide call for the expression of interest, four tests were selected for evaluation, three of which (BioRad Platelia, Prionics Check Western, Enfer Test) performed sufficiently well to be approved for official use in the EU; and are still the only EU-approved assays.

In 2000, the European Commission organised a second open call to identify additional assays with a strong capacity for the rapid diagnosis of BSE. Five tests were selected to undergo a two-phase evaluation, which was again organised and technically-assisted by IRMM. The laboratory evaluation phase, which used a reduced number of test samples compared to 1999, was followed by an additional phase to test performance under field conditions. Two tests had completed their field trial by early 2003 for likely approval in spring 2003.

Transmissible spongiform encephalopathies affect not only cattle but also small ruminants such as sheep and goats. Consequently, the EC has adopted a monitoring

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13 European Commission, Directorate General Joint Research Centre, Institute for Reference Materials and Measurements, B-2440 Geel

scheme for the presence of TSEs (i.e. scrapie or other TSEs) in small ruminants as from 1 April 2002 on. All EU-approved tests for BSE screening of cattle received provisional approval for the screening of TSEs in small ruminants. IRMM has, however, set up an evaluation scheme for rapid post-mortem tests for the detection of the TSEs in small ruminants which is scheduled for early summer 2003. The overall scheme will basically follow the OIE (Office International des Epizooties, Paris, France) recommendations for the evaluation of qualitative diagnostic tests; and will be applied to central nervous and lympho-reticular tissues (1).

Evaluations of rapid TSE tests is complicated by problems such as sample specification, sample acquisition, sample storage and stability, heterogeneous distribution of prions, matrix effects and test performance, impacts on the homogenisation of tissues etc. Nevertheless, IRMM in co-operation with scientists and test producers has, over the last years, acquired extensive knowledge of the critical parameters to be respected in the design of a scientifically sound evaluation of rapid *post mortem* TSE tests.

Evaluation of BSE rapid tests in 1999

The first evaluation of rapid tests for the diagnosis of BSE in cattle was carried out in 1998 by the European Commission (2). It presented a major challenge conceptually and scientifically for all parties - the European Commission itself, the test developers and scientific experts - because rapid and scrupulous evaluation was primordial which necessitated the collection and processing of large numbers of specimen in a short period. Two of the EC-approved assays, the Enfer and the CEA test (commercialised by BioRad as Platelia) are quantitative assays, whereas the Prionics Check Western blot is purely qualitative.

The evaluation exercise comprised the analysis of 1300 tissue samples from brainstem, though 1000 negatives and 300 specimen from confirmed positive cases. These brainstems of BSE affected animals were collected and provided by the Veterinary Laboratory Agency in Weybridge, UK, and all negative specimens derived from New Zealand, internationally considered to be BSE free. These brainstems were then further processed into more than 13000 test samples at IRMM. A rigorous sampling scheme guaranteed full traceability of each single test sample.

Sensitivity (proportion of true positives which are test positive), specificity (proportion of true negatives which are test-negative) and a relative detection limit were assessed in the evaluation, see **Table 1**. Three tests correctly differentiated both all 1000 negative and all 300 positive samples and so recorded values of 100% for

sensitivity and specificity. The fourth test did not approach this level and was excluded from the approval process.

Table 1: A summary of the results obtained by four tests in the 1999 evaluation.

	Enfer	CEA	Prionics	Wallac
Specificity	100%	100%	100%	89.8%
Sensitivity	100%	100%	100%	69.8%
Dilution	1:30	1:300	1:10	-

Evaluation of BSE rapid tests in 2001

A second round of evaluations was organised to identify tests with a high sensitivity and specificity. Tests submitted by five organisations were selected for the evaluation following an open call for the expression of interest and underwent a laboratory evaluation in 2001 (3). The results are summarised in **Table 2**.

At the laboratory phase, all test developers analysed 48 brainstem tissue slices from confirmed BSE affected cattle to reliably determine the sensitivity, and 152 brainstem tissue slices from healthy cattle to determine the specificity of their tests.

The detection limits were analysed with serial dilutions of titrated nervous tissue (titre of $10^{3.1}$ mouse i.c./i.p. LD50/g tissue in RIII mice). In addition all test developers were offered the opportunity to prepare their own dilution series on site starting from the titrated positive tissue, to dilute it into a fresh pool of negative tissue homogenate and to analyse the serial dilutions directly. In general, this led to the detection of three times higher dilutions. In addition to a relative detection limit we gained further information on the behaviour of tests in heterogeneous samples, on storage effects and homogenisation effects.

Table 2: Results obtained with five tests and results for two already approved tests* obtained on the dilution series

	ID-Lelystad	PerkinElmer	Prionics		UCSF	Imperial College	BioRad Platelia*
			LIA	WB*			
Sensitivity (%)	97.9	100	97.9	n/t	100	100	n/t
Specificity (%)	100	99.3	100	n/t	100	100	n/t
IRMM homogenates	10	1	-	10	30	100	300
Fresh homogenates	91	9	243	81	-	270	243

The numbers in the rows with homogenates indicate the dilution at which a test still detects a large majority of test samples as positive. WB = Western Blot. N/t = not tested, these assays were evaluated in 1999.

Field trial

Based on a satisfactory outcome of the laboratory evaluation, all five tests could proceed to demonstrate their performance under field conditions and their non-inferiority compared to already approved tests (4). The developers of the Prionics ELISA format (LIA) and the aCDI test of the University of California San Francisco / InPro Biotechnology, Inc., completed the field trial and IRMM is currently analysing the data for concise scientific reporting to an expert group of the Scientific Steering Committee. The SSC will then recommend to approve or to decline these tests for official monitoring in the EU.

Lessons learned

1. Distribution of PrP^{Sc} : Analysis of the distribution of the quantitative signals identified gradients of PrP^{Sc} in the brainstem along the neural axis from the obex rostrally and caudally. This analysis underlined the importance of permuted randomisation for the provision of sets containing balanced numbers of sub-samples from different positions in the brainstems to minimise discriminatory effects. Heterogeneous distribution of PrP^{Sc} according to axial location may yield apparent 'false positive' or 'false negative' results, which do not automatically reflect on the capacity of an assay.

2. Homogenisation: Homogenisation is probably the most sensitive step in all current tests for rapid BSE diagnosis. It has a strong influence on the evaluation design

and on analysis of results obtained with homogenates as the assays have a different degree of susceptibility to homogenates which are not produced according to the test procedure. One test reacted with a strong decrease in signal using homogenates; another produced a high proportion of 'false positive' results on homogenates that had dried at the surface. It is important to note that these freeze drying effects presumably can lead to insufficient digestion during proteolytic treatment of homogenates. As a consequence signals increase considerably and lead to false positives.

One solution to the influence of homogenisation on the test performance is the use of tissue slices from one side of the brainstem and homogenates derived from tissues of the opposite location on the same brainstem. Here, most tests showed no significant difference in the test signals. Only one of the tests showed a drop in the signal of up to a factor of 40 with the pre-homogenised samples.

These test-specific homogenates are stable enough (more than 1 year at -70^0 C) to serve also as test material for other regulatory applications like batch controls.

3. Analytic sensitivity: IRMM has launched a research project on the use of brains of BSE infected transgenic mice by which we expect to identify a reference material that allows a direct comparison of tests, but it will need further efforts to come to a final conclusion on the feasibility of such material.

Other considerations

The two evaluation exercises made available reliable test materials for proficiency testing or ring trials. Ongoing research at IRMM is focused on the characterisation of surrogate materials such as brains of transgenic mice expressing bovine PrP, which should lead to an improved assessment of test sensitivity and might allow to compare different tests. Obviously, a need to continue the selection and evaluation of rapid tests with new assets exists, even if the further duration and volume of TSE testing in Europe cannot be predicted.

The new call for the expression of interest to participate in the evaluation of *post mortem* and live animal tests for ruminants will provide not only more sensitive tests, but also tests that could screen for a probable presence of BSE in sheep.

References

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(2) Schimmel, H., G.N. Kramer, J. Moynagh. 1999. The evaluation of tests for the diagnosis of transmissible spongiform encephalopathy in bovines. EUR report, in press.

(3) Schimmel, H., P. Catalani, L. LeGuern, J. Prokisch, W. Philipp, S. Trapmann, R. Zeleny, J. Moynagh. 2002. The evaluation of five rapid tests for the diagnosis of transmissible spongiform encephalopathy in bovines (2nd study). EUR report, in press.

(4) Design of a field trial for the evaluation of new rapid BSE *post mortem* test. Opinion of the Scientific Steering Committee, adopted on 22 February 2002.(5) Call for the expression of an interest to participate in a programme for the evaluation of tests for the diagnosis of transmissible spongiform encephalopathies (TSE) in ruminants. (2003/C 15/10) Official Journal 22.1.2003.

STATISTICALLY SOUND BSE/TSE SURVEYS

By S.Bird and C. Ducrot

The SSC addressed the following aspects related to organising and carrying out statistically sound BSE/TSE surveys:

1. Requirements for a statistically sound BSE survey to be used in assessing a country's BSE status;

2. Measures to be taken to ensure validity of the data;

3. Statistically valid design and sample size for TSE survey in small ruminants.

Valid interpretation of data from any TSE surveillance programme depends on the sampling being effectively random from the target population. Because TSEs have long incubation periods (mean of 5 years for BSE in cattle), the impact of a risk management measure will not be immediately apparent from TSE surveillance. This needs to be reflected in survey design and interpretation.

Statistically justified sample sizes per identified target population

From a statistical point of view, sample size calculations depend on the purpose of sampling, as follows:

Disease detection: Under reasonable assumptions, the sample size required to detect – with probability of at least $(1-\underline{\alpha})$ - least one positive animal if the true prevalence is p_0 or higher can be calculated as

$$n \geq \frac{\log \alpha}{\log (1 - p_0)} \qquad [I]$$

for example: $n \geq \frac{\log 0.05}{\log (1 - p_0)}$ for 95% probability.

This calculation yields the sample sizes *n* listed in the **Table 1** hereafter.

Table: **Sample size, n, for TSE detection according to likely prevalence p_0 & probability level**

Prevalence p_0	Required n so that, if likely prevalence is at least p_0, then probability of finding at least 1 TSE test positive is		
	90%*	**95%***	**99%***
1/1,000,000	2,300,000	3,000,000	4,600,000
1/100,000	230,000	300,000	460,000
1/50,000	115,000	150,000	230,000
1/10,000	23,000	30,000	46,000
1/5,000	11,500	15,000	23,000
1/2,000	4,600	6,000	9,200
1/1,000	2,300	3,000	4,600

* at most a 10%, 5% or 1% chance that nil/n positives would be observed if true prevalence $p>p_0$

The above formula [I] can be inverted so that if a Member State has observed 0 TSE positives out of n sampled animals [that is: 0/n tested BSE positive] then the Member State can report that if BSE prevalence were higher than :

$$p_0 = 1 - \alpha^{\frac{1}{n}}$$

the chance of observing 0/n TSE positives would have been α% or less.

Confidence interval estimation: Since surveillance has shown BSE prevalence in apparently healthy adult cattle to range from 10 to 100 per million adult bovines in most Member States, it is more appropriate to compute a 95% confidence interval for BSE prevalence in testees as approximately:

$$[(B-2\sqrt{B})/\text{number tested}] \text{ to } [(B+2\sqrt{B})/\text{number tested}]. \qquad [2]$$

based on B = number out of n sampled bovines which were BSE positive. For a 99% confidence interval, replace 2 by 2.58. When B is under 10, more exact methods are

needed. **Table 2** provides the required upper 95% and 99% confidence limits when B = 0, 1, . . . 9. For example, if nil / n tested bovines have been found BSE test positive, upper 95% confidence limit for BSE positivity should be taken as 3.7/n.

Table 2: 95% and 99% confidence limits for test positives when B = 0, 1, . . . 9.

B (Observed)	95% confidence limits		99% confidence limits
	Lower	Upper	Upper
0	0	3.7	5.3
1	0	5.6	7.4
2	0.2	7.2	9.3
3	0.6	8.8	11.0
4	1.1	10.2	12.6
5	1.6	11.7	14.2
6	2.2	13.1	15.7
7	2.8	14.4	17.1
8	3.5	15.8	18.6
9	4.1	17.1	20.0

Important other considerations

A. Target populations in cattle

The modal age at which clinical BSE is detected in cattle is 4 - 6 years. In the UK, 0.006 % of 177,500 BSE cases are detected at an age of 24 months or less and 0.17 % with onset at age 36 months or less. On this basis, BSE testing could be limited to bovines aged 30+ months. However, it has not [yet] been [fully] verified that:

1. the age distribution of BSE cases outside the UK is similar to the UK;

2. the age distribution of BSE in the sub-populations of risk animals follows the same pattern as in bovines offered for routine slaughter.

BSE prevalence in risk stock is roughly 10 to 15 times higher than in healthy adult bovines offered for normal slaughter. This BSE prevalence ratio for risk versus healthy stock may vary between countries according to: age limit for testing, BSE eradication schemes in place, and reliability of identifying/sampling risk stock.

Because prevalence of BSE in risk stock is substantially higher than in apparently healthy animals offered for normal slaughter, a statistically sound sampling scheme applied to risk bovines is a "worst case" indicator for the prevalence of BSE in less vulnerable sub-populations. Age threshold was set conservatively at 24 months for risk stock, to be revised as necessary.

For cattle, the minimal - and at least in theory sufficient - requirement is the establishment of a statistically sound surveillance programme for BSE in fallen cattle, sick slaughter and emergency slaughter animals (so-called risk stock) over the minimal age from which BSE, if it is incubating, has a reasonable chance to be detected.

B. Target populations in small ruminants

For small ruminants, the practicalities of TSE rapid test surveillance are different. Unless fallen sheep can reliably be traced and sampled, adult sheep need to be sampled from those sent for slaughter, which implies much higher sample sizes for disease detection or interval estimation than if a risk population is sampled. In theory, there is no age cut-off[14], but prudently initial surveillance targeted the age-group in which TSE test positivity was most likely, namely adults (above 12 months)[15].

Active TSE surveillance provides a prevalence rate among tested animals. For small ruminants, however, the unit of real interest for analysing TSE prevalence is the

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[14] See the SSC Preliminary opinion of 6-7 September 2001 on Stunning methods.

[15] The selected ages of the animals to be sampled may depend upon which tissue is being tested: if validated test are available that routinely can be applied to tissues such as tonsils, spleen or lymph nodes, animals below 12 months could be tested.

flock or farm. The set-up of a TSE surveillance programme should be such that TSE positive results can be linked to the farm or flock of origin. If test positive animals are found, and depending on the prevalence rate observed, a complementary surveillance design could be targeted at farms in order to estimate the percentage of affected ruminants per affected farm.

C. Sample size, taking into account possible temporal and geographical variation in challenge.

If information is needed about TSE prevalence in different subgroups of the target population (sub-grouping by age or region, for instance) then separate sampling schemes need to be set up specifying, for each subgroup, the prevalence to be detected and necessary precision. For example, cattle born before versus after the full implementation of a feed-ban constitute two separate, important sub-populations to be considered separately for surveillance purposes.

D. Level of possible risk, if any, to consumers resulting from BSE in small ruminants.

With the currently available rapid tests (January 2003), BSE surveillance of adult ruminants has to proceed in two stages: rapid TSE testing to identify TSE positives, and a second form of testing [to be determined, but preferably shorter duration than transmission studies in mice[16]] used to discover if any TSE positives were in fact BSE positive. From the above table it can be derived that, to exclude a BSE prevalence in TSE rapid test positive adult sheep of 1 in 200 TSE test positives, a Member State would need to apply second-stage BSE testing to between 600 (95%) and 920 (99%) TSE rapid test positives without finding any TSE positive small ruminant which is BSE positive. To exclude BSE prevalence in TSE rapid test positive adult sheep of 1 in 2000 TSE test positives, a country would need to apply second-stage BSE testing to between 6000 (95%) and 9200 (99%) TSE rapid test positives.

16 The SSC is currently preparing a specific opinion on this subject.

E. Genotyping of small ruminants

To enhance knowledge about susceptible and resistant genotypes per country and gradually to quantify the relation between genotype and TSE susceptibility in Europe, the SSC recommended that:

1. a random sub-sample of 500 from the first 100.000 routinely slaughtered native adult sheep which are subject to rapid TSE testing per country is genotyped.

2. every rapid TSE test positive adult animal is genotyped together with two set of three suitably sampled controls per TSE positive case

Countries which have not excluded that their TSE prevalence is 50 or more per 1 million adult sheep should continue rapid TSE surveillance until they have genotyped at least 100 TSE test positive adult sheep together with their associated controls.

F. Measures against diversion.

Even if the major target population consists of risk animals, the testing of healthy stock in parallel is recommended for at least the first year of active TSE surveillance for quality assurance in implementing the surveillance programme. Thereafter, active surveillance at slaughterhouses only needs to be sufficient to guard against diversion.

If the target population consists primarily of animals sent for routine slaughter (as may be the case for small ruminants) then escape routes, such as channelling of suspect animals for unmonitored disposal, should be controlled.

G. Quality assurance and reporting standards

1. **Practically oriented protocols** for random sampling from the target population should be properly documented and preferably peer-reviewed.

2. **Born After Real Ban (BARB)-controls study. A**ny BSE positive, whether a clinical case or surveillance-detected, born after the start date of a Member State's total feed ban should be followed up; together with suitable controls.

3. **Reporting format** should differentiate:

   - clinical TSE cases from TSE test positive surveillance-detected animals

- imported from native animals

and should include: month and year of birth, cause of death , month and year of death, age at death, region of slaughter or death; TSE rapid test result and type of test used; and, for small ruminants, flock, farm and genotype.

4. **The whole survey system** should be subject to regular and formal quality assurance.

5. **Number of BSE cases adjusted for surveillance-coverage**. Comparisons between Member States, or between reporting years per Member State, should be based on the Member State's surveillance-adjusted BSE cases.

H. Surveillance in small ruminants

TSE surveillance

Scrapie in sheep is under-reported. When clinical scrapie is followed up by veterinary surveillance of the host flock or post-mortem testing, additional clinical or sub-clinical cases have been discovered in sheep with non-resistant genotypes.

If a) correction is made for under-reporting and b) it is assumed (conservatively) that there is at least one additional rapid TSE test positive adult sheep per scrapie case, then TSE prevalence in adult sheep could range from 20 to 500 TSE positives per 1 million adult sheep according to Member State. In practice, TSE surveillance in healthy adult sheep has revealed these prior estimates to have been indeed under-estimates.

By analogy with cattle, TSE prevalence may be substantially higher in fallen sheep than in similarly-aged sheep which are being slaughtered for human consumption. Because of their lower value, sheep are seldom sent for emergency slaughter. They may be killed on farm, or die where they roam, or be sent directly to a rendering plant or disposal site. Thus surveillance of risk sheep, is unlikely to be comprehensive. The target group of risk animals in small ruminants is therefore not comparable to the corresponding target group in cattle.

TSE surveillance in sheep and goats should with the currently available tests target the age-group in which TSE test positivity is most likely, probably adults.

Active rapid TSE test surveillance of native adult sheep at slaughterhouses is therefore proposed as the first step in improving scrapie surveillance. Escape routes should be controlled. Additional surveillance schemes for imported sheep may need to be considered.

Later stages of active TSE surveillance may be envisaged, as follows:

– surveillance based on rapid TSE testing in the spleen of sheep under 12 months which have been sent for slaughter, if suitable tests are available.

– surveillance based on flocks, because scrapie eradication policies are flock-based, and making use of genotyping and, potentially, tonsil-based TSE testing of live sheep to limit within-flock culling.

Table 1 provides the numbers of adult sheep brains for TSE detection according to likely prevalence & probability level for Member States whose national flock is under 1 million. Interval estimation of TSE prevalence rates with adequate precision, rather than scrapie detection, is likely to be the surveillance goal in most member states, however.

Relevant SSC opinion (see annex II): **88**

RISKS OF BSE IN PIGS

By G.A.H. Wells.

The recognition of bovine spongiform encephalopathy (BSE) in domestic cattle in the United Kingdom (UK) in 1986 inevitably led to concerns about the potential risk of similar diseases occurring in non-ruminant livestock or farmed food species. Research was quickly directed toward the investigation of the susceptibility of pigs to infection with the bovine agent. Investigations into processing and trading practices within the rendering and feedstuffs industries in the UK identified the fact that consumption of meat and bone meal must have led to significant exposure of pigs to the agent of BSE.

The ban on the use of ruminant protein in ruminant feed in the UK in July 1988 raised concern about inter-species recycling. Also in the UK, between 1990 and 1996, some feed companies stopped using animal proteins, other than fish meal and milk products, in feeds for pigs and poultry. Others continued to use these ingredients until the use of mammalian meat and bone meal in livestock feed was banned in 1996. Despite the 1996 ban in the UK, the feeding of mammalian meat and bone meal to pigs and poultry remained legal in other countries of the European Union (EU). Since January 2001 the use of all processed mammalian protein in feeds for farmed animals has been banned throughout the EU with periodic adjustments, but its use in pig and poultry feeds in other parts of the world continues.

Experimental studies of the transmissibility of BSE to pigs

Studies to test the transmissibility of the BSE agent to pigs began in the UK in 1989. Parenteral inoculation of the agent to 10 pigs, by three routes simultaneously, produced disease with an incubation period range of 69 -150 weeks. Pre-clinical spongiform encephalopathy was detected in two pigs killed 105-106 weeks post-inoculation (p.i.). Infectivity was detected by bioassay in inbred mice in the central nervous system (brain and spinal cord) of all pigs which developed spongiform encephalopathy. Infectivity was also found in the stomach, jejunum, distal ileum and pancreas but not in other tissues assayed (spleen, thymus, mesenteric lymph node, liver and kidney) of the terminally affected pigs. These findings show that pigs are susceptible to BSE and although infectivity was present in all the CNS tissues from exposed pigs that were tested, not all of the assay mice injected with brain from clinically-affected pigs developed the disease,

suggesting the existence of a species barrier to the transmission of BSE from pigs to mice which reduced the sensitivity of the bioassay. What was unexpected was the relatively few peripheral tissues in which any infectivity was detected. This finding again suggests that a large species barrier compromised the sensitivity of the bioassays.

In contrast to the transmission of BSE by parenteral inoculation, disease failed to occur in 10 pigs retained for seven years after exposure by feeding BSE affected brain on three separate days, at 1-2 week intervals. The amounts fed each day were equivalent to the maximum daily intake of meat and bone meal in rations for pigs aged eight weeks. No infectivity was found in tissues (brain, spinal cord, semitendinosus muscle, spleen, thymus, retropharyngeal, mesenteric and popliteal lymph nodes, stomach, distal ileum, pancreas, liver and kidney) assayed from the pigs exposed orally. It is suggested that these pigs did not become infected. That exposure of pigs to the BSE agent by feeding did not transmit the disease to pigs is in marked contrast to the now considerable body of evidence that BSE has transmitted, by natural or accidental means, via foodstuffs to several other animal species and to man and indeed has been transmitted by feeding BSE-affected brain tissue to several additional animal species.

Other studies make it likely that the effective exposure of pigs was further reduced by a species barrier to the oral transmission of BSE from cattle to pigs. The existence of such a barrier can be inferred from comparisons of the study findings with the results of an oral titration, in cattle, of a pool of 60 BSE-affected brain stems. All the calves exposed to the 100g dose of brain material developed clinical signs and histopathological lesions of BSE. The amount of the brain pool required to cause BSE in 50% of the exposed cattle is estimated to be less than 1g. The fact that none of the pigs appeared to become infected after being fed an average of 400 g of brain on each of three successive occasions (a total of 1,200 g) suggests the existence of a cattle-pig species barrier that reduced the effective oral exposure to BSE by as much as 100-fold, or even more.

The absence of a naturally occurring TSE cases in pigs

There have been no reports of a naturally occurring TSE in pigs in the United Kingdom even though in the period that cattle were being exposed to contaminated MBM, pigs were also being exposed. Moreover, the inclusion rates of MBM in commercial pig feeds were usually greater than in ruminant rations. It is difficult to estimate the degree of BSE contamination of MBM but approximations suggest that the experimental exposure to CNS tissue by feeding was 50,000 times more than the calculated exposure in the field.

The experimental exposure of pigs on just one of the three occasions was probably well in excess of the average life-time global exposure of pigs in the field to BSE.

If pigs were as susceptible to BSE by the dietary route as are cattle, with a similar median incubation and assuming that highest level of prevalence of infection was 1 per cent, then over 1,000 cases of BSE in pigs should have occurred by 2002.

The possibility of subclinical infection of pigs

It is possible that in the experimental exposure of pigs by feeding infection occurred but did not produce clinical or pathological evidence of disease and the mouse bioassay, across the pig-mouse species barrier, was too insensitive to detect infectivity in any of the tissues. But had primary infection of pigs from cattle with BSE occurred, there would have been the potential for recycling, as occurred in cattle, and, hence, amplification of a porcine-adapted BSE agent because of the inclusion in pig rations of MBM of porcine origin. Also, pig material contributed in greater proportion to MBM and therefore, any infection in pigs would have been transmitted to pigs with no species barrier effect and, had disease resulted, it might have been expected to occur with shorter incubation periods than primary foodborne transmission to pigs. The failure of recycling and amplification to produce clinical disease in pigs both before and, currently, six years after the end of such exposure, tends to negate the hypothesis of inapparent BSE infection in pigs. Experimental investigation of possible subclinical infection in pigs would require sub-passage of selected tissues, notably those of the alimentary tract, from the orally exposed pigs, employing the same species, or possibly transgenic mice expressing porcine PrP.

It can be concluded from the studies of the transmissibility of BSE to pigs that although pigs are susceptible to BSE when injected by combined parenteral routes, there is no evidence of transmission after exposure by feeding three doses of BSE-infected brain in amounts equivalent to the maximum daily intake of MBM formerly used in commercial pig rations. The simplest explanation of this finding is that the effective exposure of pigs by the oral route was insufficient to establish infection. These observations are in contrast to the susceptibility of cattle to oral infection with gram quantities of BSE-affected brain and to the major feedborne epidemic in the UK.

Present knowledge therefore does not provide scientific justification to include certain tissue of pigs in an SRM-ban.

Relevant SSC opinions (see annex II): **42**

RISKS OF BSE IN FISH.

By E.Vanopdenbosch

1. Introduction

Mammalian MBM and other mammalian products have historically been fed to farmed fish. Furthermore, intra-species and intra-order recycling via feed is common practice in fish farming. It was therefore important to address the question whether the latter practice could enable mammalian TSE agents to establish themselves in fish and for species adaptation of such agents to occur. This could lead to the development of a TSE in fish that might lead to a TSE epidemic in fish and/or create a health risk for the consumer. An assessment was made to advise whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSEs and, if appropriate, to suggest examples of conditions under which intra-species or intra-order recycling of fish could be allowed.

2. Relevant data and risk assessment

Feeding of farmed fish

The feeding with fishmeal raises the question of intra-species or intra-order recycling of fish tissues. Generally, although recycled fish in the form of fishmeal is the principal ingredient of feed for farmed fish, available information indicates that recycled farmed fish tissues are normally not used as an ingredient of fishmeal produced for fish feeds.

Research on TSEs in fish

The limited transmission studies that are currently in progress, i.e. the EC FAIR CT97 3308 project: "Separation, identification and characterisation of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish" have so far not provided evidence of TSE disease or infectivity replication in fish. However the possibility cannot be ruled out totally as PrP immune reactivity with an antibody that detects several mammalian PrPs has been reported in salmon (Gibbs and Bolis,1997) and Suzuki *et al.* (2002) found a candidate PrP-like gene in pufferfish *(Fugu rubripes)*, based on partial nucleic acid sequence homology.

However, Joly *et al.*(2002) concluded from their studies of PrP primary sequence that the PrP from fish is different from that in mammals and would be unlikely to share the pathological properties of mammalian PrP^{sc}. Both from the literature and from limited observations on fish, there is no evidence that TSEs would naturally exist in fish but the possibility cannot be totally excluded.

The risk of recycling of fish with regard to TSEs

Intra-species recycling could be regarded as more dangerous than producing feed for phylogenetically less related species, because of possible species barrier effects. However, in the absence of any data on species barrier effect in fish, the potential importance of intra-species recycling versus intra-order recycling cannot be estimated at present and neither are indications available that recycling in fish can be considered in the same context as is done for the domestic animal situation. Nevertheless, as long as the TSE problem is not relevant for fish and meat and bone meal from other possibly TSE infected species is not used as feed in aquaculture, recycling would not create an increased risk in respect to TSE in fish. The assessment would have to be reviewed, in line with the general principles of intra-species or intra-order recycling, if evidence is found of replication of TSE agent in fish.

The safest way for treating organic wastes of animal origin is processing at 133 °C under 3 bar steam pressure for at least 20 min. If this causes technological problems which might be expected with fish material, other time/temperature relationships may be applied but they have to be validated.

Possibilities of TSEs being recycled in fish

a. Wild fish

Many species of wild fish are carnivorous. There are two main scenarios that may result in a build-up of TSEs in wild fish.

Firstly, it is possible to hypothesise that a spontaneous TSE could develop in wild fish and that wild sea or river fish would have the capacity to recycle a TSE. However, is likely that natural predation would offer limited scope for amplification of the agent and the “infectivity" could remain confined to a small number of the sea or freshwater fish or mammals.

The second scenario involves direct exposure to TSE infected mammalian carcasses or their parts. Such an exposure could, as with the case of a spontaneous development of a fish TSE, initiate a cycle which could be propagated to other pelagic, demersal, freshwater (coarse or game) fish or marine of freshwater mammals. However, as for spontaneous development and under natural predation conditions, it is unlikely that significant amplification would occur among wild fish.

Dumping fish waste/offal at sea or in fresh water is likely to increase any theoretical possibility of recycling a TSE among wild fish as all ages, and sizes of fish could consume the waste.

b. Farmed fish

Farmed fish in general, need a protein source in their feed that originates from fish and is generally provided by a diet based on fishmeal. For this reason the possibility of recycling a TSE in farmed fish would be greater than is the case for wild fish.

To date, there is no evidence of a TSE in wild fish and therefore, no obvious possibility of "infected" wild fish being caught and processed into fishmeal. Likewise, although scavengers such as crustaceans or even marine mammals could also be infected, such fish or animals generally have a limited contribution to fishmeal. However, even a low-grade infection in the source fish could initiate a cycle in farmed fish if entire, or parts of, "infected" farmed fish were recycled without measures being taken to inactivate TSEs.

It is possible that without treatment to inactivate infectious prions, fishmeal and fish oil could transmit "infectious" prions to farmed fish. Intra-species recycling, due to the absence of a species barrier could increase the risk that TSE cases occur or undetected pools of infectivity develop. However, although intra-species recycling could be regarded as more dangerous than producing feed for phylogenetically less related species, because of possible species barrier effects, in the absence of any data on species barrier effect in fish, the potential importance of intra-species recycling versus intra-order recycling cannot be estimated at present and neither are indications available that recycling in fish can be considered in the same context as is done for the domestic animal situation.

Farmed fish could likewise be directly exposed to a mammalian TSE by direct exposure to an infected dead animal or its parts. This is an unlikely, but possible scenario.

3. Conclusions

Very little is known about the possible occurrence of TSEs in fish, but the possibility cannot be totally excluded. On the other hand, intra-species and intra-order "recycling" of fish materials occurs naturally in most if not all fish environments. It is therefore likely that natural predation would curtail amplification of any naturally occurring fish TSE agent. This principle may, however, not apply if the TSE agent were external to the fish environment/ecosystem and it is therefore justified to avoid the introduction of such agents to the fish environment, as this could possibly result in fish presenting a risk to other animal or human health vis-à-vis TSEs. It is further appropriate to highlight a number of additional uncertainties, such as the unknowns regarding the structure of putative fish PrP's, the level of the barrier in respect to intra-order recycling versus intra-species recycling, assuming that this is determined in fish by the PrP gene sequence, and the possibility that TSEs, if naturally present, may not manifest themselves in the same way as the known TSEs of mammalian species.

From the limited available research results, scientific literature on TSEs in fish and routine examinations of fish brain in the course of fish disease diagnosis, it can be concluded that there is no evidence that a natural TSE exists in fish and that there are no indications of replication of scrapie or BSE agent in experimental transmission studies.

On the question whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSEs, it is therefore concluded that there is currently no evidence of any such risk existing although the data from the transmission experiments and from other sources are still very limited and incomplete.

Regarding the conditions under which intra-species or intra-order recycling of fish could be allowed, the following has to be considered:

– The risks caused by recycling in general, are addressed in the SSC opinion of 17 September 1999 on Intra-Species Recycling - the risk born by recycling animal

by-products as feed with regard to propagating TSE in non-ruminant farmed animals.

With regard to the specific TSE issue, some theoretical risks could exist, linked to feeding possibly TSE-contaminated feeds to animals currently believed to be not susceptible, including fish.

The possible TSE risks resulting from intra-species recycling of fish are therefore low if a number of conditions are complied with, as described in the SSC opinion of 22-23 July 1999 on Fallen stock, namely: safe sourcing [from an epidemiological point of view] with regard to the possible presence of TSE infectivity, of the material of origin: no fish should be recycled if it has been fed potentially contaminated mammalian MBM; appropriate treatment of the starting material

Relevant SSC opinions (see annex II): **41, 90, 91, 103, 104.**

BSE IN POULTRY (DOMESTIC FOWL OR CHICKENS)

BY: G. WELLS AND E. VANOPDENBOSCH

Concerns have occasionally been raised as to the theoretical risk that poultry could play a role in the exposure of, or the spread of TSEs, notably BSE, to humans by contracting the disease or by spreading the agent passively in excreted waste from consumed contaminated feed.

As far as these BSE risks in relation to birds are concerned, experimental data on the susceptibility of avian species are available only for the domestic fowl. They show that as yet there is no experimental evidence that BSE can be induced in this avian species by parenteral inoculations (which have included i/c injection), or oral challenge. Similarly, chickens inoculated i/v with the agent of transmissible mink encephalopathy (TME) did not develop disease but the agent could be recovered from their lymphoid tissues five months after they were inoculated. However, this persistence of agent could be explained as residual inoculum. The possibility of *active replication of agent* in birds is thus considered to be remote, if it occurs at all.

It has to be assumed that in the UK poultry were exposed (as were, for example, pigs) to high amounts of BSE-infectivity before their feeding with ruminant MBM was banned. Current disease monitoring systems are regarded to be unlikely to identify cases of TSEs in poultry, not least because of the short life-span of most commercially reared birds. However, higher incidence levels and shorter incubation periods, which could be anticipated with the occurrence of within-species re-cycling of agent, had poultry become infected, would probably not have gone unnoticed under all circumstances.

The possibility that poultry (as might be proposed for pigs or fish) to act, after oral challenge under field conditions, as healthy silent carriers in the spread of TSE-agents is still hypothetical and no results of experiments conducted as yet are available to support this hypothesis.

Birds may potentially ingest BSE infectious material[17] and *spread* ingested agent through the dissemination of faeces as it is unlikely that the pathological prion protein would be completely destroyed in the digestive tract. Moreover, plumage, claws and beak may also be contaminated with infectious material, which is then released into the environment.

If poultry are fed with animal-derived products that may contain BSE infectivity the following measures are considered to reduce such recycled infectivity:

– exposing the recycled animal material to a treatment by 133°/20'/3b or equivalent conditions,

– excluding those tissues known to carry the highest infectious load (ruminant Specified Risk Materials),

– excluding risk waste and fallen stock from the production of feed,

stop feeding poultry possibly contaminated feed for a sufficiently long period of time before slaughter in order to reduce the risk of recycling infectivity via the gut-content.

Relevant SSC opinions (see annex II): **43, 90, 91, 103, 104.**

[17] This may be via concentrate feed diets or, in the case of necrophagous and some omnivorous species of birds, through direct consumption of parts of infected bovine carcases or offals.

BSE IN SMALL RUMINANTS

by E Vanopdenbosch and G.A.H. Wells

Sheep and goats in many countries have probably been exposed to the BSE agent through MBM as a result of past feeding practices[18]. Because it has been experimentally demonstrated that BSE can be orally transmitted to certain genotypes of small ruminants, it should be assumed that BSE could have been introduced into the sheep and goat population. If the agent behaves like scrapie in these species it is possible that it has then been maintained, propagated and/or recycled by horizontal and vertical transmission[19]. Hence the risk could persist, even after effective implementation of the ruminant feed bans, which bans the feeding of ruminant meat-and-bone meal to small ruminants. At present however there is no evidence that BSE is present in small ruminants under field conditions and no indications pointing toward an increased likelihood of this being the case.

At present the Scientific Steering Committee (SSC) considers that the risk of BSE in small ruminant is "possible". Should the risk become "probable", current practices of safe sourcing of small ruminant materials by exclusion of certain Specified Risk Materials would no longer be adequate, but a more comprehensive approach for sourcing small ruminants materials would be needed. Such an approach should combine different strategies including removal of tissues known to pose a risk of infectivity from a given age, testing for BSE, genotyping and breeding for BSE-resistance, flock certification and individual animal and flock tracing.

To establish such a comprehensive approach, consideration would need to be given to the issues discussed below.

1. Distribution of infectivity in experimentally infected BSE-susceptible animals

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[18] The actual feeding practices of small ruminants, e.g., the age and extent of MBM feeding, are nonetheless different from cattle. They will also vary depending on whether the animals are to be used for meat, wool or dairy purposes.

The amounts, distribution and kinetics of accumulation of PrP^{Sc}, and by implication presumably BSE infectivity, differ in sheep experimentally infected with BSE by the oral route from those in cattle. Data indicate a widespread involvement of lymphoid tissues early in the incubation period. After one month from exposure to the BSE agent, susceptible sheep show an estimated significant load of BSE infectivity in the intestine, lymph nodes, tonsils, stomach and spleen. Data from experimental BSE in one sheep breed suggests that after 36 months of exposure the estimated total BSE infectivity load in the animal body is much higher and the distribution of infectivity very different. However other breeds may differ. When compared to the central nervous system tissues, the PrP^{Sc} load in the intestine of BSE-infected small ruminants is relatively higher at the beginning of the incubation period and of the same order of magnitude toward the end of the incubation.

The tissues/organs of BSE-infected susceptible small ruminants that, according to current knowledge, contain, or may contain BSE-infectivity are as follows: the head, the spinal cord and associated dorsal root ganglia, peripheral nervous tissues, the spleen, other lymphoid tissues (e.g. tonsils) and lymph nodes (e.g. prescapular lymph nodes and supra mammary lymph nodes), liver, pancreas, placenta, the alimentary tract from oesophagus to rectum**,** (i.e. not only the intestine but, the forestomachs and the abomasums and closely related lymph nodes, including the mesenteric lymph nodes and the mediastinal lymph nodes; also the innervation of the entire alimentary tract.

2. Scrapie and BSE- resistant and susceptible small ruminant genotypes

It has been demonstrated in experimental models of TSE diseases that the combination of the infecting strain of TSE agent and the genotype of the host PrP gene play a major role in determining relative incubation periods between model systems. Together these two factors also affect the targeting of infection to different organs and to different parts of the brain. The relative dose required to infect the host is also affected by these two factors.

The use of the words “susceptible” and “resistant” in what follows requires careful definition. They should be seen as relative terms in a continuum of susceptibility, not as absolute statements. By “more susceptible” it is implied that animals can be

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[19] Maternal transmission is unproven in goats.

infected by a relatively small amount of infectivity, even by a relatively inefficient route (e.g. the oral route). By contrast "more resistant" implies that a larger dose of infective material is required to infect the animal and possibly by an efficient route (e.g. the intracerebral route). Although it is often the case that more susceptible models have relatively short incubation periods, susceptibility and resistance should not be confused with length of incubation period, since in some cases highly susceptible animals can have long incubation periods.

As far as the *genetic susceptibility of* ***sheep*** *to BSE* is concerned, sheep PrP genotypes and their effect on incubation period and pathogenesis are very complex and poorly understood. The available knowledge is based on a few published experiments carried out on small numbers of animals involving only a very small proportion of sheep breeds. Further studies are in progress. The results obtained indicate variation such that it is difficult, at present, to draw specific conclusions, or to make generalisation on host susceptibility to BSE in sheep. What follows should therefore be interpreted in this context.

Results to date have been interpreted that, in general, the relationship between PrP genotype and susceptibility is similar for scrapie and BSE in some breeds (e.g. in Suffolks). Susceptibility to these two TSEs is linked to PrP genotype, with codons 136, 154 and 171 being of major importance. In some breeds (e.g. Suffolks) sheep which are homozygous for glutamine (Q) at codon 171 are more susceptible to scrapie than other genotypes and can also succumb to experimental BSE. In other breeds e.g. Cheviots there other genotypes (those with Valine at codon 136 are more susceptible to natural scrapie. Nevertheless Cheviots with Alanine (A) at 136 have shorter BSE incubation periods. Available findings indicate that, after an exposure to a high dose of BSE-infectivity, detectable infection may be widespread in the lympho-reticular system a few months after exposure. Furthermore, in natural scrapie of Romney sheep (to which pathogenetically experimental BSE in sheep bears a resemblance), PrP^{Sc} can be detected from two months of age in Peyer's patches and mesenteric lymph nodes in the VRQ/VRQ genotype.

Available evidence indicates that sheep that are homozygous for the arginine (R) allele at codon 171 are the most resistant to development of the disease upon challenge with BSE-infected material. Infection of this genotype has been shown to occur after intracerebral infection but the development of the disease in these sheep,

if it occurs at all, would probably be slow and not result in significant infectivity levels in young animals.

Sheep that are heterozygous with one arginine (R) at codon 171 show an intermediate degree of resistance to BSE-infection and a distinct pathogenesis, as indicated by a different pattern in levels and distribution of infectivity in tissues and a much longer incubation period compared to that of genotypes which have a shorter incubation period. In consequence, for any given level of exposure to the BSE agent, the probability of finding clinical BSE or infectivity in tissues is lower in these sheep than in susceptible animals. Moreover, during the pre-clinical phase, PrP^{Sc} has not been detected, so far, in the enteric (autonomomic) nervous system of heterozygotic ARR/ARQ or ARR/VRQ sheep.

Until demonstrated otherwise in several models of sheep TSEs it must therefore be assumed, as a reasonable worst case, that after infection, there may be a rapid rise in the amount of infectivity in lymphoid and other peripheral organs of both susceptible and semi-resistant sheep genotypes but that resistant sheep may harbour less infectivity early in the incubation period.

3. Rapid tests to identify BSE-affected small ruminants

The currently available rapid post-mortem tests for detection of bovine BSE would certainly be useful to identify affected small ruminants. However, they would not offer the same degree of consumer protection as for bovines, because of the pattern of pathogenesis in BSE-susceptible small ruminants which may result in the presence of infectivity in peripheral tissues very early in the incubation period.

Tests for use on tissues that show infectivity in the early stages of incubation such as the lymphoid tissues are still being developed and will probably not be available for routine applications in the immediate future. Such tests would only permit an early identification of the infected susceptible small ruminants that pose a BSE risk, if sensitive enough to detect low levels of BSE-infectivity. On the other hand, only tests applied to CNS at the end of the incubation period are likely to be useful to detect BSE-affected semi-resistant sheep because detectable infectivity may be absent in certain lymphoid tissues of these genotypes.

4. **Breeding for TSE resistance in small ruminants**.

Since available data indicate that the relationship between sheep genotype and susceptibility to a TSE is similar for scrapie and BSE, breeding for scrapie resistant sheep is also expected to result in BSE-resistant sheep.

Breeding for resistant PrP genotypes is now being carried out in a number of countries, including the UK, the Netherlands and France. Concerns have been expressed about the potential long-term effects of such nation-wide and generalised programmes. These include the possible emergence of a TSE strain to which ARR sheep are highly susceptible, the possible deleterious effect of R171 on normal PrP function, and possible co-selection for negative traits. It may be expected that breeding for ARR/ARR genotypes in some breeds of sheep would be a multi-step process involving (a) ram genotyping scheme to increase frequency of the ARR allele in healthy flocks, (b) monitoring for scrapie on farms taking part in the programme and (c) dealing with scrapie affected flocks. Such a programme should initially be targeted at risk population or risk areas and would require:

- The availability of an acceptable method of identifying individual sheep (for example, electronic chips or boluses);

- For each important breed, an approximate knowledge of the frequency of ARR/ARR sheep to give an estimate of how quickly the breed would be able to move towards use of ARR/ARR rams only.

- An agreed procedure on scrapie monitoring, taking into account that very young animals or animals of heterozygous genotype may not show easily identifiable PrP^{Sc} in peripheral tissues.

- A programme of genotype monitoring of scrapie cases as recommended in the SSC's opinion of 30 November 2001 on Requirements for statistically authoritative BSE/TSE surveys, in order to have warning of the potential emergence of new scrapie strains able to cause disease more easily in the heterozygous genotypes (at the moment judged to be of intermediate susceptibility). Also the monitoring of the PrP^{Sc} profile will be needed in conjunction with strain typing.

- With respect to the occurrence of possible adverse effects, an effective monitoring of breed characteristics in scrapie resistant genotypes to obtain

reliable information on any undesirable changes (e.g. in birth weight, growth rates, strength and resistance to particular other diseases).

– Careful monitoring for comprehensiveness of protection against infection within the flock.

– Embryo storage for important pedigree flocks should be considered to protect against loss of important genetic traits.

5. **Flock certification**

Animals from a certified "Scrapie/BSE-free" (or preferably: "Scrapie/BSE-negligible risk") flock would represent no risk. However, infectivity can be present for years in animals and flocks that were apparently TSE-free in terms of clinical manifestation before coming under observation. The implementation of a comprehensive programme leading to the possible certification of flocks would therefore, in most cases and for most countries require many years. An approach of less stringent "provisional certification", is a possible alternative in the short term if, where necessary, it is applied in combination with other criteria such as testing and genotyping. The Section on TSE certification of bovine herds and small ruminant flocks (Part II.B) by E.Vanopdenbosch provides details on possible approaches to flock certification, which apply to both sheep and goats.

6. **Culling strategies**

Because of the transmissibility of the infection within a flock and between flocks by direct or indirect contacts, the elimination of the index case only will not eliminate the enhanced risk in a flock (of sheep and/or goats) where a clinical or sub-clinical TSE case has been confirmed. Therefore, a culling strategy could be considered which covers the entire flock where the index case was found and, in the case BSE was confirmed, the flocks that were in contact with the original flock via other small ruminants[20] or via grazing areas. Such culling would, however, have little or very

[20] Including via the offspring of the case

little risk reducing effect for sheep of the ARR/ARR genotype[21] or if the risk of transmission to other flocks was negligible.

The assessment whether the risk for transmission to other flocks was negligible would require that the animals introduced into a flock are identifiable and their history traceable and that they are genotyped. The risk would, for example, be negligible in the case of contacts with or imports from flocks certified to represent a negligible risk, if the contact only concerned the use of breeding rams (as compared to pregnant ewes) or if the imported animals tested negative with a validated in vivo test (once available).

Much of the above described approach will have to depend on the availability of detailed records and identification, and it may be impossible to trace sheep that have moved out of a flock or cohort historically, or indeed, to identify of cohort and offspring. If no tracing of animals exported from a BSE infected flock is possible other approaches (e.g. ad hoc epidemiological investigations) could be helpful to identify the potentially exposed flocks.

Whole flock slaughter might also turn out to be counter-productive for various scientific, economic and social reasons (e.g. it encourages even more under-reporting)**.** A more effective policy is to identify infected animals as soon as possible and therefore remove them from the flock combined with a programme of genetic selection for resistant PrP genotypes. The development of an antemortem diagnostic test would facilitate this policy[22].

7. Geographical sourcing of small ruminant materials

The possible risk of materials sourced from small ruminants potentially being infected with BSE is likely to change with the geographical origin of the animals, depending on factors such as possible local unsafe feeding practices, possible episodic imports of BSE-affected animals and differences in the reliability of the

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21 Rapid TSE testing at slaughter of the spleen or brain of ARR/ARR animals above the age of 18 months from flocks with TSE would gradually provide conclusive evidence / reduce to negligible the risk that this genotype is a carrier of detectable infectivity levels.

22 Woolhouse, M. E., Stringer, S. M., Matthews, L., Hunter, N. & Anderson, R. M. (1998). Epidemiology and control of scrapie within a sheep flock. *Proc R Soc Lond B Biol Sci* **265**, 1205-10.

existing surveillance system. The **section on the Geographical BSE risk in Part II.B** elaborates further on this aspect.

Note on TSEs in goats.

Much less is known on TSEs is goats than on TSEs in sheep. In terms of risk management, the approach taken is to consider the conclusions for sheep as applicable to goats as well, at least until sufficient evidence will have become available to underbuild a possible goat-specific approach. The following evidence may nevertheless be mentioned:

- Scrapie occurs less frequently in goats;
- Maternal transmission has not been confirmed as occurring in goats ;
- Perhaps all goats may be susceptible to BSE or scrapie by the oral route under certain conditions. It is noted that the dimorphism in codon 142 of the caprine PrP gene appears to be associated with different incubation periods in goats experimentally infected with BSE or scrapie. Recent research has shown that goats have similarly complex PrP genetics as sheep. However, the relationships between breed, PrP polymorphisms and susceptibility to scrapie are not yet as well understood as in sheep and therefore a breeding programme towards TSE resistance in goats is not feasible on the basis of the present knowledge.

Relevant SSC opinions (see annex II): **30, 31, 32, 33, 34, 35, 36, 37, 38.**

PART II B

BSE RISK REDUCTION STRATEGIES

OVERVIEW OF THE APPROACH FOR THE GEOGRAPHICAL BSE RISK ASSESSMENT OF BSE IN BOVINES AND IN SHEEP

By V. Silano

The geographical BSE risk of BSE in cattle

1. Definitions

The Geographical BSE-Risk in cattle (GBR-C) is a qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE, pre-clinically as well as clinically, at a given point in time, in a country. Where presence of disease is confirmed, the GBR-C gives an indication of the level of infection as specified in the table below.

Table 1: Definition of geographical BSE risk (GBR-C) and its levels

GBR level	Presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a region or country
I	Highly unlikely
II	Unlikely but not excluded
III	Likely but note confirmed or confirmed, at a lower level
IV	Confirmed, at a higher level

2. Underlying hypothesis

The SSC-methodology for the assessment of the GBR-C is based on the assumption that BSE arose in the United Kingdom (UK) and was propagated through the recycling of bovine tissues into animal feed. Later the export of infected animals and infected feed provided the means for the spread of the BSE-agent to other countries where it was again recycled and propagated via the feed chain.

For all countries other than the UK, import of contaminated feed or infected animals is the only possible initial source of BSE that is taken into account. Potential sources such as a spontaneous occurrence of BSE at very low frequency or the transformation into BSE of other (animal) TSEs (scrapie, CWD, TME, FSE) arising in a country are not considered, as they are entirely hypothetical events. Blood, semen and embryos are not seen to be effective transmission vectors[23]. Accordingly, blood-meal is not taken into account, neither.

Cross-contamination of feed can be a way of propagating the disease. However, the influence of cross-contamination on the GBR-C has to be considered in the light of the risk that the animal protein under consideration could carry BSE-infectivity.

The possible impact of maternal transmission on the GBR-C has not been taken into account in this methodology because its occurrence is unconfirmed and its potential minor role in comparison to feed, also the qualitative nature of the GBR exercise. Similarly no "third route of transmission" was taken into account.

3. Information factors and model of the BSE cattle system

The methodology is based on 8 factors that were originally identified by the SSC in January 1998 as the most relevant information for carrying out the assessment (see **Table 1 and Figure 1**).

In order to clarify the often-protracted interaction between these factors, the SSC has adopted a simplified qualitative model of the cattle/BSE system (**Figure 1**) which focuses on a feed-back loop that is required to be activated to initiate a BSE-epidemic. This feed-back loop consists essentially of the processing via rendering of (parts of) cattle that carry the BSE-agent into feed and then the feeding of this contaminated product to cattle which then become infected and amplify the BSE-agent.

This feed-back loop is influenced by a number of factors that, on the one hand, may activate the loop and, on the other hand, might prevent this activation or slow down or reverse the build-up of BSE-infectivity within the system.

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[23] See SSC-opinion on vertical transmission, 18-19 March 1999 and on the safety of ruminant blood (13/14 April 2000)

Table 2: Information factors for assessing the GBR-C

Structure and dynamics of the bovine population – Number and age distribution of beef and dairy cattle, both alive and slaughtered – Husbandry systems, proportional to the total cattle population.
Surveillance of BSE Measures in place to ensure detection of BSE-cases: – Identification system and its tracing capacity – Date since when BSE is compulsory notifiable and criteria for a BSE-suspect – Awareness training (when, how, who was trained) – Compensation (since when, how much in relation to market value, payment conditions) – Other measures taken to ensure notification of BSE suspects – Specific BSE-surveillance programs and actions – Methods and procedures (sampling and laboratory procedures) used for the confirmation of BSE-cases Results of BSE-surveillance: – Number of cattle, by origin (domestic/imported), type (beef/dairy), age, method used to confirm the diagnosis and reason why the animal was examined (CNS, BSE-suspect, BSE-related culling, other) – Incidence of reported BSE-cases by year, by birth cohort, and – if possible – type of cattle
BSE related culling – Culling schemes, date of introduction & criteria used to identify animals that are to be culled – Information on animals already culled in the context of BSE

Import of Cattle and meat-and-bone meal (MBM)

- Imports of live cattle and/or MBM from UK and other BSE-affected countries
- Information that could influence the risk of imports to carry the BSE agent (BSE-status of the herds of origin of imported cattle, precise definition of the imported animal protein, etc.)
- Main imports of live cattle and/or MBM from other countries
- Use made of the imported cattle or MBM

Feeding

- Domestic production of MBM and use of MBM (domestic and imported)
- Domestic production of composite animal feed and its use
- Potential for cross-contamination of feed; measures to reduce and control it, results of the controls

MBM-bans

- Dates of introduction and scope (type of animal protein banned for the use in feed in different species, exceptions, etc.)
- Measures taken to ensure and to control compliance
- Methods and results of compliance control

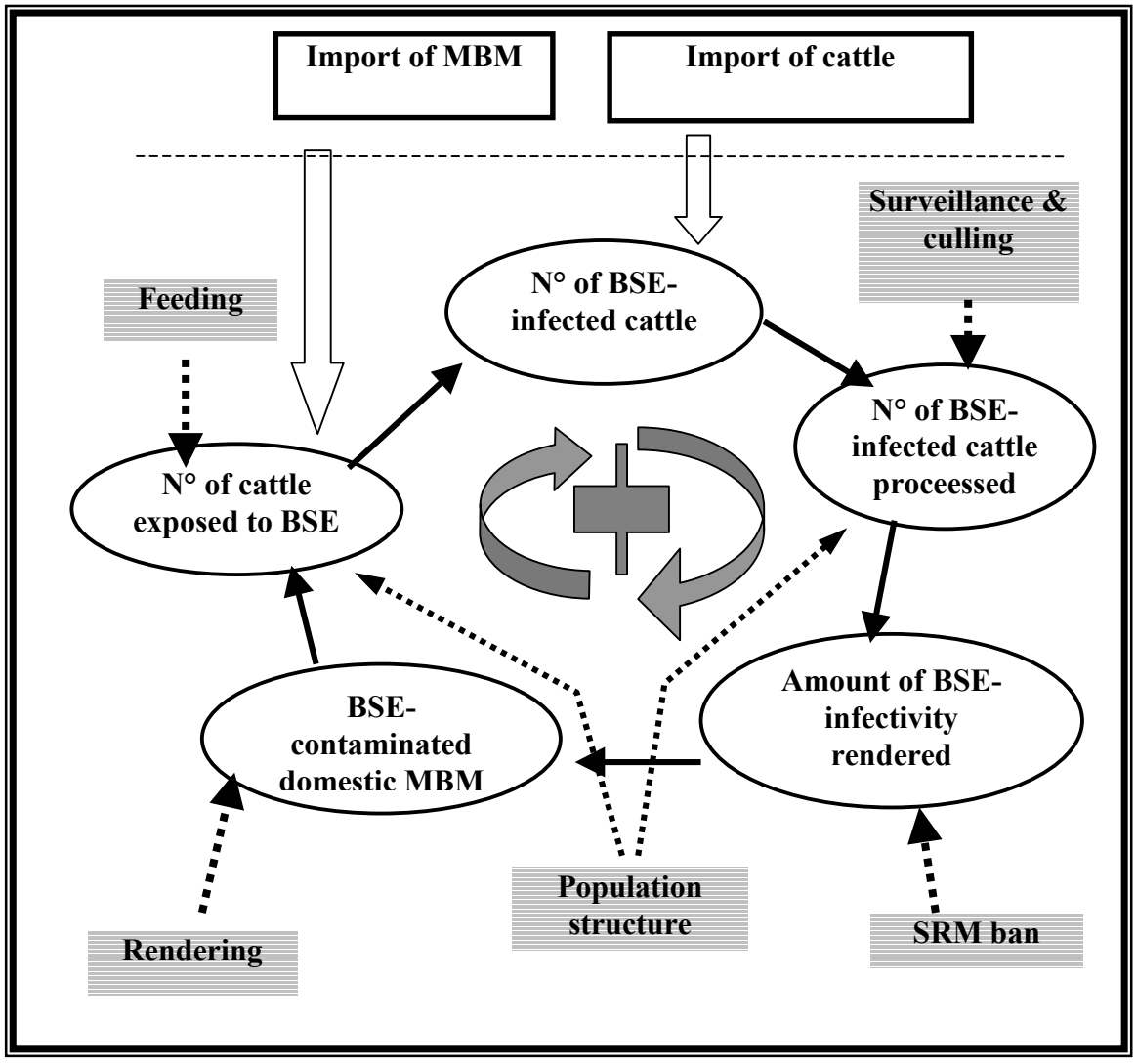
SRM-bans (SRM: Specified Risk Material)

- Dates of introduction and scope;
- Measures taken to ensure and to control compliance
- Methods and results of compliance control

Rendering

- Raw material used (type; annual amounts by type)
- Process conditions applied and their share of the annual total domestic production.

Figure 1: The model of the BSE/cattle system used by the SSC



In the model used by the SSC the initial introduction of the BSE-agent has to come from outside the country under assessment – it is therefore called an external challenge of the system. For the UK it is assumed that the initial introduction of the agent happened before the period taken into account in this model. Two possible routes of introduction are considered: import of infected cattle or import of contaminated MBM. The factors assumed to be able to prevent the building-up of BSE-infectivity in the system are the following:

- Surveillance and culling;
- SRM-removal; exclusion of fallen stock;
- Appropriate rendering and processing methods;
- Appropriate feed bans.

In summary, the model can be basically broken down into two parts relating to challenge and stability, and the model assumes a mechanism for their interaction. "External challenge" refers to both the likelihood and the amount of the BSE agent entering into a defined geographical area in a given time period through infected cattle or MBM. "Stability" is defined as the ability of a BSE/cattle system to prevent the introduction and to reduce the spread of the BSE agent within its borders. Stability relies on the avoidance of processing via rendering of infected cattle and the avoidance of recycling of the BSE agent via the feed chain. A "stable" system would eliminate BSE over time; an "unstable" system would amplify it.

4. **Main results obtained**

The usefulness of the methodology is multiple. Firstly, it allows prediction of presence of BSE in the bovine population long before BSE-infected animals are discovered through passive *ad hoc* surveillance programmes; so far this has proved the case for several countries (i.e. Czech Republic, Germany, Italy, Slovakia, Slovenia, Poland and Israel). Secondly, it allows the understanding of the weaknesses of any specific country's system with respect to BSE and therefore is an effective guidance for the identification of the additional control measures needed to prevent BSE from entering the country and being amplified. Thirdly, the work carried out so far has produced the most powerful world-wide data basis on ruminant husbandry and feeding and on animal waste recycling and disposal. If properly exploited, this database is likely to prove to be very helpful for the control of other ruminant diseases and of other animal health problems. However, the most relevant implication in public health terms of the GBR methodology is the translation of this scientific evaluation into BSE safety criteria for a number of ruminant-derived products in different countries (See **Part II.C** on Safety of Products).

The GBR level of the countries that have been assessed so far by the Scientific Steering Committee is as shown in **Table 3**[24]:

Table 3: GBR status of 60 countries. The GBR methodology has also provided for each country an evaluation of the expected development (trend) of the GBR with time.

N°	Country name	Current GBR	Remarks
1.	Andorra	III	
2.	Albania	III	Re-assessment ongoing
3.	Argentina	I	
4.	Australia	I	Re-assessment ongoing
5.	Austria	III	
6.	Bellarus	III	
7.	Belgium	III	
8.	Botswana	I	Re-assessment ongoing
9.	Brazil	I	
10.	Bulgaria	III	
11.	Canada	II	Re-assessment ongoing; own risk assessment provided
12.	Chile	I	

24 These assessments can be found at: http://europa.eu.int/comm/food/fs/sc/ssc/index_en.html.

N°	Country name	Current GBR	Remarks
13.	Colombia	II	Re-assessment ongoing
14.	Costa Rica	I	
15.	Croatia	III	
16.	Cyprus	III	
17.	Czech Republic	III	Re-assessment ongoing;
18.	Denmark	III	
19.	El Salvador	I	Re-assessment ongoing
20.	Estonia	III	
21.	Finland	III	
22.	Former Yugoslavian Republic of Macedonia	III	
23.	France	III	
24.	Germany	III	
25.	Greece	III	
26.	Hungary	III	Re-assessment ongoing
27.	Iceland	I	
28.	India	II	Re-assessment ongoing
29.	Ireland	III	

N°	Country name	Current GBR	Remarks
30.	Israel	III	
31.	Italy	III	
32.	Kenya	II	Re-assessment ongoing
33.	Latvia	III	
34.	Lithuania	III	
35.	Luxembourg	III	
36.	Malta	III	
37.	Mauritius	II	
38.	Namibia	I	Re-assessment ongoing
39.	Netherlands	III	
40.	New Caledonia	I	
41.	New Zealand	I	
42.	Nicaragua	I	Re-assessment ongoing
43.	Nigeria	II	
44.	Norway	I	Re-assessment ongoing
45.	Pakistan	II	Re-assessment ongoing
46.	Panama	I	Re-assessment ongoing

N°	Country name	Current GBR	Remarks
47.	Paraguay	I	
48.	Poland	III	Re-assessment ongoing;
49.	Portugal	IV	
50.	Romania	III	
51.	San Marino	III	
52.	Singapore	I	
53.	Slovak Republic	III	Re-assessment ongoing;
54.	Slovenia	III	
55.	Spain	III	
56.	Swaziland	I	Re-assessment ongoing
57.	Sweden	II	Re-assessment ongoing
58.	Switzerland	III	Re-assessment ongoing
59.	Turkey	III	
60.	United Kingdom	IV	
61.	Uruguay	I	
62.	USA	II	Re-assessment ongoing; own risk assessment provided.
63.	Vanuatu	I	

THE GEOGRAPHICAL BSE RISK OF BSE IN SMALL RUMINANTS

BSE in sheep has not been proven under field conditions, but information obtained so far can be used as a scientific plausible stepping-stone for a hypothetical model for the occurrence and spread of a BSE epidemic in small ruminants, if indeed it did occur. This model for hypothetical BSE in sheep, combined with the experiences from the assessment of the geographical BSE risk in cattle, has led to the framework for assessing the geographical BSE risk in sheep and goats (GBR-S) described in the report adopted on 8 November 2002.

1. Definitions and methodology

A stepwise approach was developed to assess the geographical BSE-risk for sheep and goats (GBR-S) based on the exploitation of the geographical BSE-risk for cattle (GBR-C) in order to make possible public health decisions while the very time consuming tests now being proposed for the discrimination of BSE from scrapie are carried out.

For sheep, the same classification of GBR already in use for cattle, i.e. Levels from I to IV with exactly the same definitions after substitution of the word "cattle" with the word "sheep" is used. It acknowledges the peculiarities of sheep, as compared to cattle, concerning:

(a) **routes of infection** (not only contaminated feed, but also direct and indirect contact);

(b) **prevalence of BSE in a sheep scrapie population** (upperbound BSE prevalence assumed to be 1%);

(c) **prevalence of scrapie in small ruminants** (as a reasonable worst case hypothesis it is assumed a prevalence of 0.5% of scrapie in small ruminants);

(d) **prevalence of BSE in small ruminants** (assumed to be 0.05% as a maximum).

(e) **information factors and model of the BSE sheep system** The methodology proposed to assess the GBR-S is a stepwise systematic process that follows steps:

Step one - Countries in GBR-C levels III and IV

Based on the above mentioned assumptions, it is concluded that countries with GBR-C levels III or IV should be classified, even in the absence of notified BSE or scrapie-cases among small ruminants, into GBR-S level III unless data can be provided showing that, since 1980, it was very unlikely or unlikely that significant levels of potentially-infected MBM reached small ruminants through the feed chain. The methodology for assessing the data provided by a country to show that small ruminants were not exposed to significant levels of potentially-infected MBM since 1980 would be the one already developed for cattle by making use of the available information highlighted in **Table 4**. It should be understood that it would be extremely unlikely that such data would be available for most countries and that, in practice, classification in the level III GBR-S would be the most common and logical consequence for all these countries.

Table 4: Information elements for assessing the GBR for sheep and goats(a)

Structure and dynamics of the ovine/caprine population

- Number and age distribution of sheep and goats, both alive and slaughtered
- Information on husbandry systems used for sheep and goats

- type of main product: wool/meat/milk,
- intensive/extensive,
- productivity of milk-sheep/goats,
- co-farming of pig/poultry/cattle with sheep/goats,
- geographical distribution of sheep/goats, cattle and pig/poultry populations,
- size distribution of sheep flocks and goat herds,
- Internal animal trade: (n° and age distribution of sheep/goats annually traded

between flocks/herds, and between different husbandry systems and/or between different regions of the country.

Surveillance of TSEs in small ruminants

Measures in place to ensure detection of TSE (scrapie)-cases:

- Identification system and its tracing capacity (for sheep and goats)
- Date since when TSEs are (scrapie is) compulsory notifiable and criteria for a TSE (scrapie)-suspect
- Awareness training with regard to TSEs (scrapie) in small ruminants (when, how, who was trained)
- Compensation for animals culled in the context of scrapie eradication (since when, how much in relation to market value, payment conditions)
- Other measures taken to ensure notification of scrapie suspects
- Specific TSE/scrapie-surveillance programs and actions (detailed description, plans)
- Methods and procedures (sampling and laboratory procedures) used for the confirmation of TSE-cases

Results of TSE/scrapie-surveillance:

- Number of examined sheep and goats, by origin (domestic/imported), type (wool/milk/meat), age, method used to confirm the diagnosis and reason why the animal was examined (CNS, TSE-suspect, TSE-related culling, other)
- Result of the surveillance efforts
- Incidence of reported TSE-cases/n° of newly infected flocks by year of confirmation, by birth cohort of the confirmed cases, and – if possible – type of use (wool/meat/milk).

TSE related culling

- Eradication measures, including culling schemes, date of introduction & criteria used to identify animals that are to be culled

- Information on animals already culled in the context of TSE

Import/export of live animals (bovine/ovine/caprine) and of MBM (<u>Note:</u> Blood, semen, embryos or ova not seen as an effective transmission route. MBM is used as proxy for mammalian protein (other than milk) as animal feed)

- Imports/export of live animals (cattle/sheep/goats and/or MBM from/to UK, from/to other BSE-affected countries[25] and from/to other "BSE-free" countries; provide annual data per partner-country)
- Information that could influence the risk of imported live animals or MBM to carry the BSE agent (BSE-status of the herds/flocks of origin of imported cattle/sheep/goats, precise definition of the imported animal protein, information on the process conditions and raw material used for imported MBM, etc.)
- Use made of the imported animals and of the imported MBM.

[25] BSE-affected countries are all countries with confirmed BSE-cases and all countries classified by the SSC as GBR III, even if they have not notified any cases.

Feeding and cross-contamination

- Composition of the feed for ruminants (for cattle/sheep/goats give the percentage of grass/pasture, roughage, industrial feeds, protein concentrates used in on-farm preparation of compound feed for ruminants, feed additives, … per species) and measures taken to control this composition
- Use of MBM (domestic and imported: for farmed animals (ruminant/non-ruminant), in pet food, fertilizer, or in other uses (please specify); information on how this use was controlled)
- Domestic production of composite animal feed and its use (type of feed mills (single line/multiple line plants, single/multiple species production), annual production of feed by target species and by feed mill, information on how the use of the produced feed was controlled).
- Potential for cross-contamination of feed for ruminants with MBM or blood during feed production, during transport and on-farm,

- measures taken to reduce it (labelling, awareness raising, technical installations);
- measures taken to control it (feed sampling (specify n° of samples taken from compound feed for ruminants per year and species, method of examination, place of sampling (feed mills, during transport, on-farm), other controls in feed mills, during transport or on-farm);
- results of the controls, handling of breaches.

Step one - Countries in GBR-C level I and II

To assess the GBR-S of countries with GBR-C levels I or II, it would be necessary to check that the challenge deriving from potentially-BSE-infected materials, already assessed for cattle as being negligible or very low, remains as such even after consideration of the additional challenge for the feed chain that might have occurred since 1980 through live sheep imported from BSE risk countries (this import, in fact, might have given origin to an internal production of potentially-infected MBM which could have reached both small ruminants and cattle). Should the challenge through the feed chain due to live small ruminants be found to be negligible throughout, the GBR-S classification would remain identical to the GBR-C classification? Otherwise the combined external challenge should be assessed and a

stability analysis conducted for the sheep feeding system since 1980, resulting most probably in a higher GBR-S level. The issue depends crucially on the stability of the system with the exclusion of any possibility that BSE infectivity can contaminate the feeding systems for small ruminants.

In order to apply to small ruminants the methodology already developed for cattle, one could use the same external challenge categories in use in the GBR-C, taking advantage of the available information on the imports of live small ruminants (this is potentially very important as the EUROSTAT data reveal very large number of animals being traded every year) from BSE-risk countries and on the reasonable worst case assumption for the prevalence of BSE in small ruminants. The data reported in **Table 5** should be fed as applicable to the GBR model and examined consequently.

Table 5. Level of external challenge resulting from import of live small ruminants from the UK or other BSE-risk countries.

Level of external challenge	Live small ruminants from the UK, 1988 – 1993	UK	Other countries
Extremely High	>10.000.000	**UK-imports before 88 and 94-97: *10; after 97: *100**	**Imports from other countries with a BSE risk: R1*1000, R2* 100**
Very High	1.000.000-<10.000.000		
High	100.000-<1.000.000		
Moderate	20.000-<100.000		
Low	10.000-<20.000		
Very low	5.000-<10.000		
Negligible	0-<5.000		

Step two

For countries that at the end of step one remain classified as GBRS level I or II, it would be necessary to estimate whether BSE might have entered the country through live small ruminants and transmitted through horizontal or vertical routes. To this end, use should be made of, the information when available on the numbers of imported live small ruminants from BSE-risk country and dates. The intended use of these animals is important because it is expected that a substantial proportion of these animals are scheduled for slaughter, but experience suggests that an appreciable proportion of the animals imported into one country may be rapidly

exported to another country. This will reduce the risk in the first country, but amplify the potential spread of BSE infectivity.

In order to develop different challenge levels for the horizontal transmission of BSE in small ruminants, it could be considered, as a starting point, that information derived from scrapie indicates that even a small number of infected sheep (according to a worst case hypothesis, even 1 animal can be at the origin of disease spread within a flock) is sufficient to generate and sustain an epidemic and that such a probability increases with the number of potentially–infected animals imported. This evaluation should be based on the same prevalence factor reported above. Therefore, significant probability of a of BSE epidemic in small ruminants would be associated for example with the import into a given flock of a few thousands breeding or milking sheep, whereas sheep imported for immediate slaughter would not be expected to make any major contribution to the risk.

The SSC stresses that this GBR-S model will need adjustments if or when new scientific data regarding probable/possible presence of BSE in small ruminants under field conditions become available, but supports the further development (and its application) of the present model if an acute situation concerning discovery of BSE in sheep under field conditions would occur.

Relevant SSC opinions (see annex II): **118 to 260**

PREVENTING RECYCLING OF INFECTIVITY: CULLING STRATEGIES

By D. Heim

Impact of culling

The probability that "at risk animals" epidemiologically linked to BSE-index cases are infected with BSE is somewhat higher than for the rest of the healthy cattle population. Culling therefore can avoid that some potentially infected animals enter the human food and animal feed chains and can therefore reduce the risk for humans and animals.

However, the impact of BSE-culling on the current pre-clinical BSE-incidence and the future clinical BSE-incidence is dependent on many factors and cannot easily be assessed.

Assumptions

Bovine Spongiform Encephalopathy (BSE) is not transmitted horizontally and the only significant transmission route is feed; it is hence not comparable to contagious diseases.

It is assumed that the majority of infections normally take place in the first months of life of calves.

As the incubation period of BSE is between 2 and 14 years (mean 60 months) with the vast majority of clinical cases being 4-6 years at clinical onset, the exposure event that lead to the development of a clinical case must have taken place 4-6 years previously for the majority of animals.

Current diagnostic tools do not allow the identification of animals in the early phases of the incubating period. The available methods (PrP^{Sc} detection) are able to detect a proportion of BSE a-symptomatic infected animals, not previously identified as suspects, i.e. a-symptomatic animals, but only then in the late stages of the incubation period.

BSE is a rare event. With the exception of the UK in the years of the height of the epidemic, the yearly incidence remained below 0,1 % (1.000/million) of the adult (>24 months) cattle population.

Factors that influence the efficiency of a culling policy.

The efficiency of any culling scheme is critically depending on the ratio of identified number of BSE-cases to the real number of cases. It seems logical that the willingness of farmers to notify a suspect case is influenced by the impact that this would have on his farm. Culling of a-symptomatic animals will make the impact more severe and less easily acceptable. A herd culling policy can be assumed to be a greater disincentive to notify a suspect than a birth-cohort culling.

On the other hand, culling only parts of the herd could be economically problematic for some farmers, e.g. If the industry denies taking milk or meat from herds where BSE has occurred.

Appropriate compensation schemes may buffer the impact of the culling scheme on the notification of BSE-suspects to some extent. The acceptance of any culling scheme depends on its assumed cost/benefit ratio. The cost depend mainly on the number of culled animals, the compensation paid, and the cost of collecting, culling, testing and disposing of these animals. The benefits of culling are determined by its "hit-rate": number of incubating animals per total number of culled animals, and hence the reduction of the current prevalence and of the future clinical cases saved per number of animals culled.

The public will not accept a culling scheme unless convincing and sound evaluation is provided of the efficiency of different culling schemes with regard to preventing a BSE-epidemic and reducing the risk for man.

The SSC has examined data from Switzerland, Ireland, Belgium, France and Portugal and theoretical back-calculations from the UK. The available data shows that the vast majority of the additional cases found in the population of cattle that were culled under the applied (herd-) culling-scheme, while not showing signs of BSE, fell indeed into the birth-cohorts as defined above. In the second SSC-opinion it was confirmed that data from France, Germany, Portugal, Spain and Ireland showed that all secondary cases found when testing animals culled under the herd culling strategy belonged to the birth cohort of the index cases.

Recommendations

Ideally, all cattle exposed to the same feed as the index case should be culled but this target population may be difficult to be identified.

The limited available information indicates that herd culling is already having some effect both in terms of eliminating otherwise not identified (pre-clinical) cases and in terms of preventing future cases.

However, the data also indicate that largely the same effect can be reached by birth cohort (see definition below) culling, i.e. only culling about 1/3 of the animals that are culled under a herd-culling scheme.

In view of the limited data available, the impact of the epidemiological situation in a country on the relative efficiency of practically possible culling schemes cannot be fully assessed. It is, however, likely that birth cohort culling is in most cases the more cost-efficient approach.

The SSC recommends the application of birth-cohort culling whenever a domestic index case appears, irrespective of the prevailing epidemiological situation and has stated that cohort culling is apparently as effective as herd culling. All animals from these cohorts should be traced, killed and destroyed, independent of their current localisation.

This position is based on the definition of a birth cohort including all animals born and/or raised in the same herd as the confirmed case within approximately 12 months before and after the date of birth of the index case.

The SSC further recommends that all members of these birth cohorts that are older than 24 months are systematically examined for the presence of PrP^{Sc} in their brain or spinal cord using a validated method.

Relevant SSC opinions (see annex II): **76, 77, 83.**

TSE CERTIFICATION OF BOVINE HERDS AND SMALL RUMINANT FLOCKS

By Emmanuel Vanopdenbosch

TSE certification of bovine herds

1. Terms of reference and scope

In the medical, pharmaceutical sector, one of the preconditions for putting animal derived products on the market is that they are derived from safe sources. Safe sources might be countries, accepted to be BSE-free. For countries, which may have or have had BSE at some point in time, the practical concept of "negligible BSE-risk" herds, sometimes called "closed herds" needed to be developed and the SSC addressed the following question:

"*Under what conditions could it be considered that the concept of 'Closed herds' (where there are controlled and documented conditions of breeding and slaughter), offers the same guarantees as the so called 'BSE-free regions'.*"

In this context, negligible BSE-risk implies that all the animals alive at the moment of certification have never been exposed to any source of infection and have no epidemiological link with TSE cases.

2. Critical factors in the establishment and maintenance of "Closed herds"

Feeding of Meat and Bone Meal (MBM)

It is generally accepted that BSE is mainly, if not entirely, initiated by exposure to contaminated feed where inappropriately prepared MBM is assumed to be the most probable source. MBM should not be fed as long as no guarantee can be provided that it is made solely of animals or materials that presents no risk and are processed appropriately without subsequent (cross-) contamination with TSE infectivity. It is therefore proposed that a negligible BSE-risk herd must be able to prove that no MBM has been fed to any cattle in that herd for at least 8 years. This period is chosen in order to provide a safety margin in comparison to the average incubation period of 5 years. On the level of an individual animal it has to be guaranteed that it never has been exposed to MBM.

Semen and embryos

The SSC considers that the risk of transmission of BSE via semen and embryos is unlikely As a precautionary measure, however, no embryos or semen originating from donor animals, which developed BSE, should have been used in the herd in the previous 8 years. If this happened, all progeny (first generation) should be eliminated.

Live animals

No animal should have been introduced in the preceding 8 years into the 'closed herd' unless sourced from a herd with an equivalent status or from a country or region classified as "negligible till zero BSE-risk".

Vaccines and veterinary medicaments

Vaccines produced in accordance with requirements of the CVMP, are regarded to be safe with respect to the risk of transferring BSE.

Other feed components

Although the risk is regarded to be low, tallow, gelatine, hydrolysed proteins and feed of unknown origin, such as waste food, should not be given.

3. **Information permitting to establish and maintain a "negligible BSE-risk herd"**

a. Disease history

In the previous 8 years no BSE case must have been diagnosed in the herd. Brains from all died or slaughtered bovines from the herd, at an age over 1 year, must be examined in an approved BSE-reference laboratory.

For newly established herds guarantees are needed that the herd is constituted only of animals from a country of negligible to zero BSE-risk or from herds of an equivalent status.

b. Records, surveillance and management

Complete records of births, deaths and all movements of the individual animals for the past eight years are needed.

Veterinary surveillance for recognition of neurological disorders has to be guaranteed.

The herd has to be separated from other domestic species, especially sheep and must have no contact with potentially infected materials.

TSE certification of small ruminant flocks

1. **Definition**

A certified TSE-negligible risk flock is a flock of small ruminants, which gives sufficient guarantees of absence of TSE in the flock after the date that the flock was closed. Guarantee is supported by documented total elimination of all TSE infected and possibly exposed animals and with documented proof of absence of those factors, which could introduce the TSE agent into the flock.

2. **Factors affecting the TSE status of a small ruminant flock**

No TSE may have been diagnosed in the herd since its establishment.

Sheep management and feeding of concentrates possibly containing MBM.

Main differences in small ruminant management and feeding practices are based on its purpose, i.e. meat, wool or dairy, e.g. sheep kept mainly for wool, are most often managed extensively on pasture and not fed concentrates. Hence the risk from feed is expected to be smaller for such sheep as compared to the more intensively managed meat or milk-producing breeds.

Goat management and possible feeding of concentrates containing MBM.

Under certain management regimes, goats are highly at risk if infected MBM is fed.

Feed components other than MBM

Although the risk is regarded to be low, tallow, gelatine, hydrolysed proteins and feed of unknown origin, such as waste food, should not be given.

Feeding and scrapie

It is theoretically possible but not proven that index cases of scrapie could arise from exposure to scrapie-infected MBM. If so, there would be no intra-species barrier for transmission of scrapie via MBM. Initial introduction of scrapie through infected MBM could lead to a smaller or larger epidemic, dependent on the prevailing genotypes of the actual sheep in the region.

Feeding and BSE in small ruminants, should it occur.

Present evidence suggests that index cases of BSE in sheep, if they occurred, would likely be due to exposure to BSE-infected MBM. Current risks would depend on the effective enforcement of MBM bans. Other factors would include cross contamination , bans of specified sheep risk materials (SRM) , rendering parameters, feed processing and scrapie related culling.

Horizontal transmission

Transmission of disease from one animal to another can occur by direct or indirect contact . Potential methods are *via* placenta (proven), milk, faeces or nasal discharges (all unproven). The risk for horizontal spread is the highest when sheep are kept together, for example at lambing time.

Vertical transmission

Infectivity was not found by bioassay of ovine semen from a ram with scrapie, in injected lambs. It remains unclear whether scrapie can be transmitted by embryos.

Environmental and other forms of transmission

Common grazing could constitutes a risk factor, especially around the lambing period but also permanently because of the persistence of infectivity via soil or vectors (hay mites, nematodes, etc). However, the evidence for transmission of natural scrapie from an infected environment is circumstantial.

The evidence for transmission of scrapie via vectors such as hay mites, fly larvae, protozoon parasites and nematodes, is limited. However, this form of transmission cannot be entirely ruled out.

Iatrogenic exposure of scrapie has probably occurred with a louping ill vaccine and a vaccine against *Mycoplasma agalactiae* both prepared from sheep tissues.

Genotype of the flock animals with regard to TSE susceptibility

A flock entirely composed of resistant or semi-resistant genotype(s) is much less likely to have an occurrence of a TSE. If present, infectivity levels in younger animals are likely to be lower as compared to animals of a susceptible genotype. However, according to current knowledge, genotypic resistance will not [yet] provide a 100% full proof of not being a potential carrier of infectivity.

Culling strategies applied to eradicate or control TSE in a flock

Ideally, all animals exposed to the same source of infection as the index case should be culled. Therefore, a culling strategy for small ruminants should cover whole flocks, i.e. where the index case was found and flocks that were in contact with that flock . An exemption could be made for animals of an ARR/ARR genotype.

3. **Information needed to establish and maintain status of a "Small ruminant flock certified as of TSE-negligible risk"**

a. Records

- showing that no clinical cases occurred in the flock.
- indicating that there was a negligible risk that TSE cases were present and that no infectivity was introduced during a given period.
- showing that each animal has been identified and monitored beyond doubt.
- guaranteeing, for newly established flocks, that the flock is constituted only from animals from a country of negligible to zero TSE-risk or from flocks of an equivalent status.

b. Surveillance data

Veterinary surveillance of the flock should be of such level that it is guaranteed that all cases of neurological disorders, for which TSE cannot be

excluded, are immediately recognised. Rapid TSE testing would significantly increase the trustworthiness of a certification..

c. Management

Contact with other flocks is strictly limited to (i) exchanges via artificial insemination, (ii) exchanges between certified flocks and (iii) introduction of ARR/ARR rams for breeding and reproduction.

4. **Elements of an accreditation scheme for maintaining a provisional certificate of representing a negligible TSE risk**

To maintain a certificate of "TSE negligible risk flock", the flock must be kept closed and a number of conditions must be fulfilled:

- marking of all animals
- availability of reliable records
- management practices showing that the risk of introduction of infectivity was/is reduced to a negligible level
- testing of brains from all that have died and from a statistically appropriate number of small ruminants (> 6 months) from the flock slaughtered.

Scenarios for certifications are described in the SSC opinion of 4-5 April 2002 on safe sourcing of small ruminant materials.

Relevant SSC opinions (see annex II): **32,36.**

PREVENTING RECYCLING OF INFECTIVITY: FEED-BANS AND REMOVAL OF RISK MATERIALS

By B. Urlings

The broader issue of recycling animal by-products as feed

The main part of animal by-products is by-products originating from healthy[26] slaughtered animals. An average of 30% of the slaughtered weight composes the volume of slaughter by-products not intended for human consumption. Large volumes of these by-products are processed into highly nutritious animal feed constituents. These processed feed constituents represent very often an ingredient for animal feed production. This can be used in feeding of several species of animals, including petfood and fur animal feed. Recycling of animal by-products as feed should thus be evaluated in a broader context:

- The experience of the emergence of BSE is a vivid illustration of the need to consider precautionary measures before one has absolute proof that a problem has occurred. The possibility of emerging of viruses and other biological agents with unusual characteristics would similarly needed to be evaluated.
- It is also recognised that TSEs occur in many species and experimental evidence that a particular species can develop infection whatever the route of administration (e.g. the intra-cerebral and intravenous routes), is cause for concern, because as yet we have so little information about the natural occurrence of TSEs in different species. Survival of animals in experimental inoculation studies, even for life time, does not provide proof of absolute resistance to infection. Nevertheless a very slow development of disease suggests that the multiplication of the agent is only limited and that the reproduction ratio (R_0) of the disease in a population could be very low, resulting in a fade out of the disease. However a long persistence of a pathogen in a population provides good

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26 Healthy animals are defined as animals which have undergone an ante mortem inspection by an official veterinarian where it was determined that the animals were not suffering from a disease which is communicable to man and animals and that they do not show symptoms or are in a general condition such as to indicate that such disease may occur and they show no symptoms of disease or of a disorder of their general conditions which is likely to make their meat unfit for human consumption. (Definition as given in Directive 64/433/EEC, laying down the rules for ante mortem inspection)

possibilities for the agent to adapt on its host and thus challenging the population again.

– It should also be noted that recycling is a means, whereby such unusual infectious agents can accumulate and/or be amplified in a susceptible species without necessarily presentation of disease.

– Recycling might also similarly lead to biomagnification of toxic substances.

– Many infections are totally or partly species-specific, but infectivity may in some cases require to adapt to new host species on passage, as in experimental models of TSEs. In this context the possible emergence and propagation, after several cycles of recycling, of micro-organisms that are resistant to the standard recycling/rendering processes could also be mentioned.

– The supplementary feeding of herbivorous animals with animal proteins derived from the same or from different species has presented new biological challenges to species that originally evolved to cope only with plant proteins.

With regard to TSE risks resulting from recycling animal by-products, the following elements can be summarised from the various SSC opinions which have addressed this issue:

A. So far there exists no scientific evidence of natural occurrence of TSE in farmed pigs, poultry and fish, which may create a basis for an intra-species progression of a TSE infection due to intra-species recycling.

B. Given the limitations of the surveillance in certain areas, and the length of the incubation time in relation to the normal (=economic or commercial) life span of the animals, it can not be excluded that cases occur and if so, an undetected pool of infectivity could be present.

C. It cannot be entirely excluded, on the basis of the available evidence, that TSEs are already present (albeit undetected) in non-ruminant farmed animals. This is in particular so if there is reason to assume that these species have been (and might still be) exposed to BSE-contaminated feed (produced from ruminants).

D. Recycling of animal material, in general, will increase the risk that cases occur or undetected infectivity pools develop, in particular if potentially BSE (TSE) contaminated material is recycled to ruminants or (possibly) susceptible non-ruminants.

E. Intra-species recycling will, due to the absence of a species barrier, increase the risk further.

F. If recycling, and in particular intra-species recycling, of animal material to farmed animals can not be avoided, all measures that reduce the recycled infectivity would reduce the risk.

G. Measures that reduce the recycled infectivity include[27]:

- exposing the recycled animal material to a treatment by 133°/20'/3b or equivalent conditions,
- excluding those tissues known to carry the highest infectious load (ruminant SRMs[28]),
- excluding[29] fallen stock from the production of feed,
- discontinue feeding pig, poultry or fish potentially contaminated feed a sufficiently long period of time before slaughter in order to reduce the risk of recycling infectivity via the gut-content.

H. It has to be understood that

- the possible measures would not be able to reach a zero risk should infectivity enter the recycling loop, and
- that due to the long incubation time of this type of disease a significant risk would have build up before an incidence becomes visible (as has been seen in the case of BSE in the UK). This proves again the necessity of an effective regional monitoring programme of animal diseases, in order to detect and combat as soon as possible new emerging diseases. Any delay in the control of new emerging, including unknown, diseases poses a risk to human and animal health.

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[27] See also the various opinions of the SSC on the safety of products.

[28] Disease and species dependent, at current only defined for BSE and cattle and cattle, sheep and goats.

[29] For detailed recommendation s see the "Fallen Stock" opinion of the SSC, July 1999.

Ruminant feed-bans and TSE risk reduction

A large number of experiments, abundantly reported in the scientific literature, has shown that cattle and sheep are susceptible to TSEs originating from their own species and that ruminants in general fed with infectious material originating from the same species can be infected with TSEs. Also, experimental evidence shows that BSE can be transmitted to sheep (and goats) via the oral route[30]. Appropriate measures with regard to the avoidance of the intra-species recycling of ruminant by-products will thus play a key role in the prevention of recycling and propagation of TSE infectivity.

If no feed potentially carrying the BSE-agent reaches bovines, the risk of new infections in the cattle population would be negligible. However traces of infectivity may result from cross-contamination of MBM-free cattle feed with MBM-containing pig or poultry feed, e.g. in feed mills that produce both types of feed in the same production lines. Apparently flushing batches that are often used as safeguard against such cross-contamination are not sufficient. Also recipients used for the transport of feed and feed ingredients (boats, containers and trucks) can pose a risk to the transmission of infectivity through cross-contamination. This conclusion from the practical experience is supported by the results of the feeding experiments in UK that have shown that already as little as 0.01 g of infected brain is enough to infect cattle orally.

Removal of ruminant specified risk materials from any feed chain

In BSE infected cattle that approaches the end of the incubation period about 99% of the infectivity is concentrated in the Specified Risk Materials (SRMs). The **Table** hereafter lists the Specified Risk Materials as they are currently defined listed according to GBR categories. Removing these from the feed or food cycle reduces the amount of infectivity by up to two logs. However, small breaches of such a removal mitigates this factor significantly.

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30 See also Section 2 Scope, on other ways of transmission.

Table: **Specified risk materials listed according to GBR categories.**

	Specified risk materials (for animals fit for human consumption)
GBR I	**Cattle:** none **Small ruminants:** none
GBR II and GBR III	Cattle: The skull, including the brain and eyes, tonsils The vertebral column excluding the vertebrae of the tail and the transverse processes of the lumbar and thoracic vertebrae and the wings of the sacrum, but including dorsal root ganglia, and spinal cord of animals above 12 months. Intestine from duodenum to rectum and the mesentery of animals of all ages. **Small ruminants:** the skull including brain and eyes, the tonsils, the spinal cord of ovine and caprine animals aged over 12 months or which have a permanent incisor erupted through the gum; The spleen of ovine and caprine animals of all ages.
GBR IV	**Cattle, in addition to the above:** the entire head excluding the tongue, including the brain, eyes, trigeminal ganglia and tonsils; the thymus, the spleen and the spinal cord of animals above 6 months **Small ruminants:** as above for GBR II and III

SRM are not only removed from slaughtered healthy cattle but also from fallen stock or cattle dead at arrival or condemned in ante mortem inspection. If BSE is present in a cattle population the prevalence of infected cattle approaching the end of the BSE incubation period is significantly higher in the sub-population of fallen stock and emergency slaughter than in normal slaughter. Hence excluding fallen stock from the feed chain is effectively reducing the risk of recycling the BSE agent. However, any occasional rendering of fallen stock could clearly pose a high risk.

Relevant SSC opinions (see annex II): **89, 90, 91.**

INACTIVATION BY PROCESSING AND SENSITIVITY OF EXPERIMENTAL ASSAYS

By R.A. Somerville

Despite the application of the principles of safe sourcing of raw materials for the production of animal or human derived products there remain actual or perceived risks of the presence of TSE infectivity. Further risk reduction is often possible through production processes. There are several areas to be considered when evaluating the degree of risk reduction obtained.

The problem

TSE infectivity is notoriously difficult to inactivate. Some TSE infectivity will survive standard autoclaving conditions. The BSE agent strain is particularly thermostable. Under dry heat conditions TSE infectivity shows even greater survival properties, e.g. some infectivity surviving temperatures of 200°C or more. Recent research has started to define these properties and to explore reasons for such high thermostability. It is becoming clear that partial inactivation in some cases with heat or high pH can stabilise residual infectivity. The infectious agent also shows great resistance to chemical inactivation. Although strong protein denaturants such as SDS or Guanidine chloride can be effective, high concentrations and/or long exposure times are required to achieve significant inactivation. Alternatives to inactivation include the use of separation technologies such filtration or phase partition technologies. These can be effective but are sometimes compromised by the heterogeneous properties of TSE infected material.

Methods of spiking

To test an inactivation procedure or a production process it is necessary to prime the input material with a high titre of TSE infectivity. The best source of high titre infected material is from infected brain, using rodent models which achieve and can be assayed to demonstrate high titres relatively quickly and cheaply compared to other possible models. The ideal is to present the infectivity at as high titre as possible but in a form that most likely mimics that of any endogenous infectivity that may enter the process under test. Thus the spike should be from a TSE model that is as similar as possible to the spiked sample with respect to TSE strain, tissue properties and species. However compromise is often required in order to produce an experiment which will produce usable data. Hence

the starting material for the production process (e.g. blood plasma) may bear little resemblance to brain homogenate so the addition of significant amount of brain tissue may compromise the subsequent procedures and the consequent effects on the purification process will have to be assessed. Some prior purification of infectivity may be of value but may also alter its fractionation properties.

Assays

Titration

Titration is the method of choice but is slow and expensive. To determine the amount of infectivity serial dilutions (usually 10 fold) are prepared and injected into groups of recipient animals. Mouse models are preferred. Titrations have to be monitored for prolonged periods to ensure that long incubation period cases are observed. Indeed in some cases infectivity in partially inactivated samples has only been detected after all the animals in control titration of the spike succumbed.

The advantage of full titrations is that they determine the minimum absolute number of infective units present in the sample, although without knowing the efficiency of infection it is not possible to determine the ratio of agent particles to number of infectious units. However it is known that in most intra-species systems the intracerebral route is the most efficient. Other routes of infection are less efficient as are systems in which a species barrier is crossed.

Incubation period assays

Since incubation period is usually inversely correlated to dose, it is possible to estimate the amount of infectivity from a calibrated dose response curve. However the dose response curve is sometimes altered, particularly by some partial inactivation procedures. Accordingly although using incubation period assays can be informative, in experiments where the effect of the test treatment on the dose response curve has not been determined, estimates of titre calculated from an incubation period assay may be compromised.

PrP^{Sc} assays

The protein PrP (sometimes called the prion protein) is found in an abnormal form, denoted PrP^{Sc}, in infected brain. PrP^{Sc} tends to co-purify with infectivity and it is thought that the protein may be a component of the infectious agent. However the relationship between PrP^{Sc} and infectivity is complex and poorly understood. Under some conditions a

substantial proportion of the PrP can be separated from infectivity. In addition some of the abnormal properties of PrP^{Sc} do not necessarily correlate with those of infectivity. Accordingly PrP^{Sc} assays should only be used with great caution as a marker of infectivity since the presence or absence of the protein may not necessarily correlate with infectivity. Moreover the sensitivity of PrP^{Sc} assays is several orders of magnitude less sensitive than infectivity bioassays.

Measuring residual TSE infectivity, and the interpretation of the information obtained

No experiment can demonstrate the absolute destruction or removal of TSE infectivity. What is feasible is the demonstration of a qualitative or quantitative reduction in the amount of infectivity. Such measurements depend on determination of the amount of infectivity used to spike the experiment and compare that with the amount detected after treatment. If no infectivity is detected then it must be assumed that the minimum detectable amount in the assay is the amount remaining. The difference between these two values gives a measure of the clearance factor. In some processes there may be sequential steps that could reduce the amount of infectivity. These steps can be assessed separately but it cannot be assumed that sequential reductions are additive and an overall measurement of the process should also be performed.

Methods of reducing infectivity

Two approaches, destruction or removal are possible. Destruction can include heat denaturation, chemical denaturation with strong detergents or chaotropes, or under more extreme conditions alkaline hydrolysis. Most methods are harsh and may well damage most biological products. Removal by differential centrifugation, precipitation or filtration is also possible. However the efficiency of these methods is often poor. Moreover accumulation of infectivity on filters may cause disposal problems. Infectivity may break through occasionally due to filter failure.

Conclusions

The demonstration of sufficient clearance of TSE infectivity from a product should be only part of risk reduction strategies used. Clearance measures should be based on an assessment of reasonable worst case situations, e.g. where the starting material accidentally came undetected from a highly infected animal. They should remove sufficient infectivity to reduce infectivity in such a reasonable worst case scenario to an

acceptable value. Assessment of the efficacy of the methods should include an assessment of the minimum desirable clearance, the theoretical maximum clearance that the experiment could demonstrate and actual clearance achieved.

Table: Summary overview of current knowledge with regard to TSE infectivity clearance by processing ruminant materials *

Production process	**Infectivity clearance factor**	**Ref: SSC opinion**
Gelatine, alkaline and acid procceses	At least $10^{4,5}$	**46**
Gelatine, heat pressure	At least $10^{6,5}$	
Final production steps of gelatine: filtration**, ion-exchange, rapid UHT sterilisation.	$10^{1.2} – 10^{2.2}$.	
Dicalcium phosphate	At least $10^{3,8}$	**45**
Tri-calcium phosphate	Approx. $10^{4,0}$ (estimate)	
Collagen from hides	No research available, but: hide & skin are not risk materials if contamination is avoided.	**53**
Saturated steam heat/pressure (133°C at 3 bars during 20 minutes) applied on mixture of tissues.	At least $10^{3,0}$	**68, 71**
Tallow, post-sterilisation	Not quantified: >1 and probably $\leq 10^3$.	**52**
Tallow, filtration 0,15% **	$10^{2.8}$ [see also full SSC opinion]	
Tallow, filtration 0,02% **	$10^{3.7}$ [see also full SSC opinion]	
Alkaline hydrolysis at high temperature (150°C) and high pressure.	$10^{3.5} – 10^{4.5}$.	**93**
Tallow derivatives	Total safety assumed under certain conditions.	**44**
Hydrolysates from hair and skin: 1M hydrochloric acid for an hour at temperatures of 65°C or higher leads to almost complete inactivation	Almost complete inactivation, but: hide & skin are not risk materials if contamination is avoided.	**57**

Production process	Infectivity clearance factor	Ref: SSC opinion
Hydrolysates from hair and skin: hydrolysis with 6M hydrochloric acid for six hours at a temperature of 100°C.	Almost complete inactivation, but: hide & skin are not risk materials if contamination is avoided.	**57**
Hydrolysation of proteins by heat/pressure/time conditions of ≥140°C/≥3.6bar/≥30minutes	At least 10^3	**58, 65**
Alkaline treatment of hydrolysed proteins at pH≥11, ≥3h at T≥80°C	"further reduces risk" (not quantified)	

* Maximum clearance factors are based on data for reductions achieved from high titre material. The efficiency of clearance is generally reduced when the clearance process is applied to low titre material.

** Inactivation of the agent is considered to be preferable to elimination.

Relevant SSC opinions (see annex II): **44 to 75,103,104.**

DISPOSAL OF RISK ANIMALS (FALLEN RUMINANT STOCK) AND RISK WASTE; A FRAME FOR RISK ASSESSMENTS OF WASTE DISPOSAL PROCEDURES

By J.W. Bridges

Risk animals (fallen ruminant stock) and risk waste and its disposal

At its meeting of 24-25 June 1999 the Scientific Steering Committee adopted a substantial Scientific Opinion on the risks to the public, to animals and to the environment from transmissible biological and chemical agents which may be present in fallen stock and dead animals, including farm animals, fur animals, wild, exotic and zoo animals, laboratory animals, cats and condemned materials as well as on recommendations on how such risks can be minimised. In the light of experience with BSE, this opinion includes consideration of unconventional and as yet unknown agents.

It is known that about fifty per cent of more than 1700 known microbial pathogens can be transmitted by animals to human beings (i.e. They are known to be zoonotic). Human beings may also be exposed to a variety of chemical agents present in food products of animal origin. In some instances biological and/or chemical contaminants have been shown to undergo modification between farm and plate with significant alterations of their risks to health.

Fallen stock dead mammals and non mammals and condemned materials may arise due to a variety of circumstances and can contain one or more of a very wide variety of chemical contaminants and / or biological agents.

Risk to man from dead animals and condemned materials depends on:

– The nature and level of the pathogenic or toxic agent(s) present in the dead animal / fallen stock, which in turn relies on accurate diagnosis and measurement;

– The prospect of intra and interspecies transmission;

– The actual processing / disposal method used;

– The prospects of human exposure as a consequence of the processing / disposal.

The use of Hazard Analysis and Critical Control Points (HACCP) will help to identify critical and other risk conditions. A case by case risk assessment should be conducted whenever possible.

Humans should not be exposed to hazardous agents *via* products recycled from fallen stock and condemned materials. If an animal died or was sacrificed because of a toxic chemical or of a pathogenic biological agent, the fallen stock or suspect condemned material should be disposed of in such a way that any processing into human or animal consumption products is avoided. As it is currently not practicable to expect a surveillance scheme to be applied under all circumstances to guarantee that only fallen stock and condemned material of proper quality are recycled in feed and in view of the potential for post slaughter infection or contamination of low risk material as a consequence of handling, transport and / or storage, the S.S.C. recommended that all material from dead animals where the causes cannot be specified should be considered as condemned.

Regarding the risks from TSEs and unconventional agents, according to current scientific knowledge, inter and intra-species transmission may occur across a range of animal species. The rendering standard of at least 133°C/20'/3 bars cannot, based on currently available data, be considered as totally effective in destroying TSE infectivity possibly present in animal species susceptible to TSE infectivity. Thus, additional protection measures are required to ensure absence of TSE infectivity.

Direct incineration of carcasses and incineration or burning under appropriate controlled conditions of rendered material are economically-feasible technologies for safely disposing of TSE risk materials. A further, but less well evaluated, potentially-suitable method is the treatment of rendered material with lime followed by encapsulation and disposal in a controlled landfill.

Less rigorous requirements, which may include recycling, may be acceptable for TSE-free condemned materials . However, this will depend on the nature and characteristics of the agent involved.

The SSC recognised that in emergency situations it may be necessary, as a short term measure, to seek alternative routes of disposal and it urged that any decision be based on a proper risk assessment to avoid unsafe practices. The competent authorities should carry out such assessments as part of their contingency planning work.

A frame for risk assessments of waste disposal procedures.

The SSC proposed a standard *framework for the assessment of the risk from different options for the safe disposal or use of animal waste which might be contaminated with microbiological agents including TSE.* This provides a structured approach to the assessment of the direct and indirect risks involved in the treatment of materials (potentially contaminated with TSEs or with other pathogens). The framework can be applied to identify suitable processes to be used in a routine situation or in an acute emergency and is intended to assist those preparing a dossier on the assessment of safety of specific processes and/or equipment relating to microbiological agents, including TSE. The proposed framework, however, only covers the assessment of risks directly resulting from the presence of microbiological agents (including TSEs). This framework does not directly address other risks possibly associated with the treatment of animal waste, which may result from chemicals (e.g. hyperchlorite) used in the treatment of the carcass or the material. Moreover, the framework does not address [31] toxic substances possibly present, neither the formation during the treatment of new toxic substances, which may pose a risk to human health and the environment as airborne emissions (for example, dioxins), as effluents or as residues in the treated material (for example, heavy metals).

Safe disposal alternatives to high temperature incineration, in addition to addressing methods for treatment of MBM, should also cover processes for volume reduction of carcasses. It is relevant, in considering safe disposal methods, to identify also the application of any process to pathogens other than prions.

Any decisions on the safety of a particular technology must be based on a sound scientific risk assessment. An essential requisite in utilising any risk assessment framework is to ensure that human health (both health of workers and the general public), animal health and the environment are properly protected. This assurance should be available prior to the widespread adoption of any process for dealing with animal carcasses and derived materials. Although it may be argued that in an emergency situation there is insufficient time for a risk assessment, this practice should be a normal part of contingency planning.

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31 It is understood that the assessment of such risks is covered by other frameworks or scientific opinions and/or by European and/or national legislation for the authorisation of waste treatment, recycling or disposal plants.

According to the legal requirement is defined in Article 4 of the Framework Directive on Waste (96/350/EC), processes and methods, which could harm the environment, should not be used

Typically, the risk assessment of any equipment/facility/ process has two stages:

– Identification of the *generic risk* (i.e. The one intrinsically associated with the specific equipment/ facility/process);

– Identification of *situation specific risks* which may include site sensitivity, effectiveness of the local management systems, etc.

The SSC framework addressed the generic risks only. For a framework to be employed for risk assessment purposes, it must identify each source of human, animal and environmental risk in the risk management chain (See **Figure**). The proposed process as a whole and each of its steps need to be described along with the key operating parameters. In addition, the availability of a flow diagram describing the process as a whole is viewed as most helpful.

The framework comprises the following six components.

1. **Identification of the risk category/categories**

   The categories should preferably be defined according to the 3 levels given in the Animal By-products Regulation (EC) 1774/2002 of the European Parliament and of the Council of 3 October 2002 lays down the health rules concerning animal by-products not intended for human Consumption. These levels are largely based on the aforementioned SSC opinion of 24-25 June 1999 and can be summarised as follows:

   (a) ***Category 1*** comprises of ABP regarded as ***high risk***. This includes amongst others any animals or parts thereof suspected of being infected by a TSE or killed in the context of TSE eradication measures, specified risk material or animals containing such material.

   (b) ***Category 2*** material consists of ABP posing a risk not quite as high as category 1 material but still a high risk. This group includes for example fallen stock and animals killed to eradicate an epizootic disease (other than those under category 1) and products of animal origin containing residues or drugs.

(c) ***Category 3*** material are ABP presenting ***a low risk***. In general, Category 3 ABP are derived from animals or products thereof considered as fit for human consumption but not intended for this use. This category would for example include by-products from the slaughtering process, former foodstuffs of animal origin, fresh fish by-products or catering waste.

2. **Identification and characterisation of risk materials**

Each significant risk material should be identified and an assessment made of the likelihood of human/environmental exposure of 'at risk' groups under:

– normal operating conditions

– emergency/abnormal operating conditions

If significant exposures are deemed possible, an assessment will be needed of the potential risks involved.

3. **Agent risk reduction**

An estimate is required of the degree of the risk reduction (in terms of human health, animal health and the environment) which can be achieved by the process.

This may be based on one or more of the following:

– Direct measurements (preferably, or otherwise:)

– Modelling

– Extrapolation from procedures which were previously proved to be effective in another context.

In each case the evidence to support the estimate must be cited. Where measurements have been made, information on the methodology used should be provided. This would include sensitivity and reliability of the methods used, the nature of samples which have been analysed and evidence that these samples are representative (relevant real samples and the number of tests performed). If surrogates for prion measurement are used, for example analysis of peptide levels, an explanation should be given of their relevance. In any case it is necessary to provide an evaluation of the validity with the uncertainties involved.

4. **Risk containment**

An analysis should be made of the likely effectiveness of the technical measures used to ensure that the risks are contained. It is also necessary to evaluate how these containment measures will operate in the event of the breakdown of the process. Monitoring and surveillance procedures to demonstrate containment need to be specified. If full containment is not achievable, an assessment is required of any potential risk.

5. **Identification of interdependent processes**

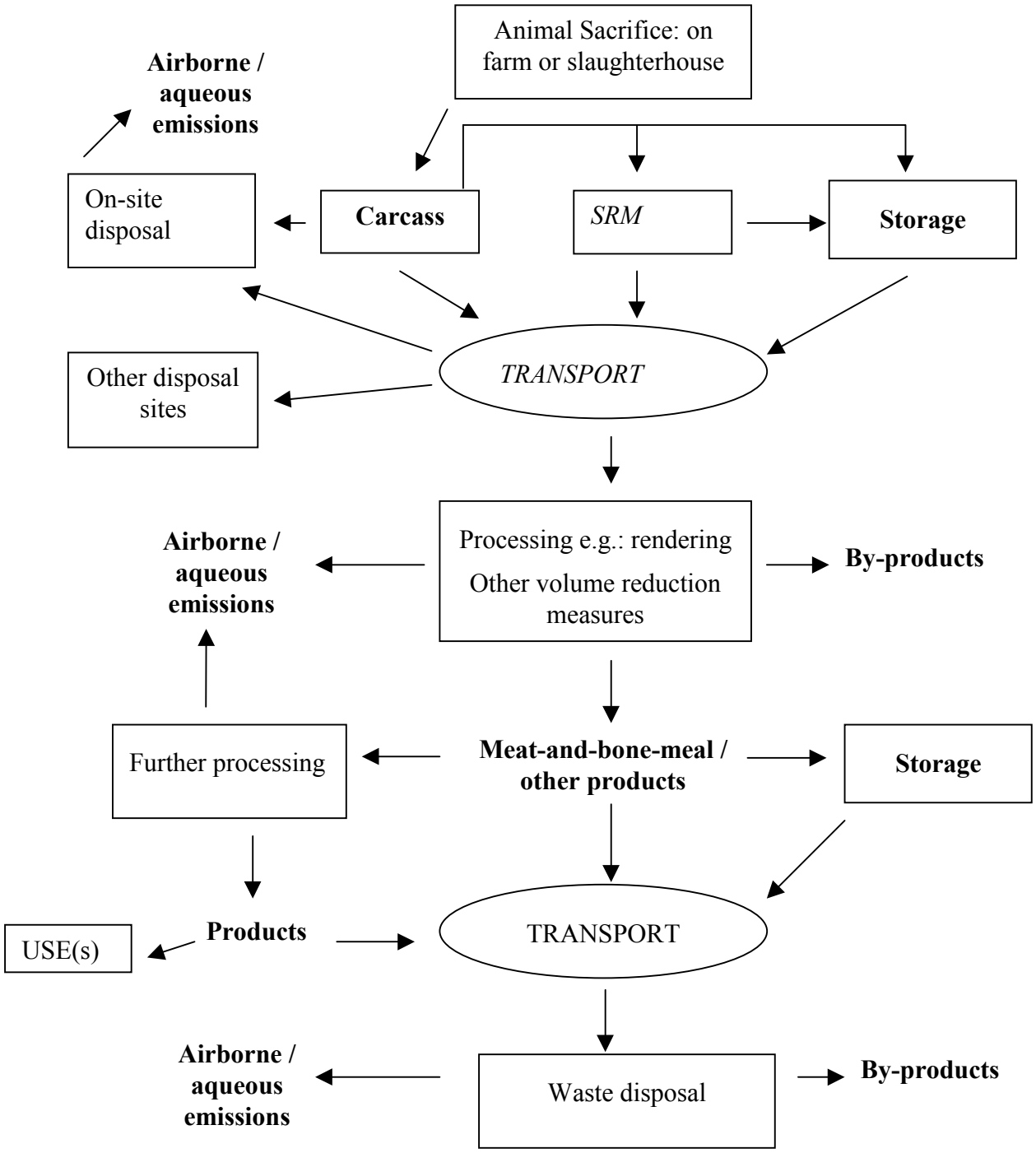
From a risk assessment viewpoint, any process identified to reduce the risk from the agent cannot be considered in isolation from indirect impacts, due to transport, storage and final disposal of the end –products and by-products. These particular aspects need to be evaluated to identify whether an increased indirect risk may occur. For example, risks arising from the increased demand for storage capacity. (See **Figure**)

6. **The intended use of the end-product(s)**

The anticipated uses (e.g. recycling or disposal) of the end-products need to be specified. From the estimated risk reduction (see 2 above), the potential exposure of workers or the public, animal health and/or the environment should be estimated if significant levels of exposure to the product(s) may arise.

Relevant SSC opinions (see annex II): **94, 100, 101, 103, 104.**

Figure: **Risk sources in relation to possible disposal routes for animal derived material, which might be contaminated with a microbiological agent.**



Note: The risk to workers in any of these processes and in handling materials must be assessed fully.

PART II C

SAFETY OF PRODUCTS

SUMMARY OVERVIEW OF SSC OPINIONS ON PRODUCT SAFETY

By M. Vanbelle

The basis of microbiological safety for human consumption of animal-derived products is that the *combination* of several risk reduction strategies will result in a safe end product. This basic principle serves also as the overarching guidance for assuring product safety with regard to the BSE risk. Assessing and reducing the risk of exposure to BSE in ruminant derived products can be divided into five parts:

I. Appropriate sourcing of the animals. [Could the geographical source of animals possibly indicate a BSE risk?]

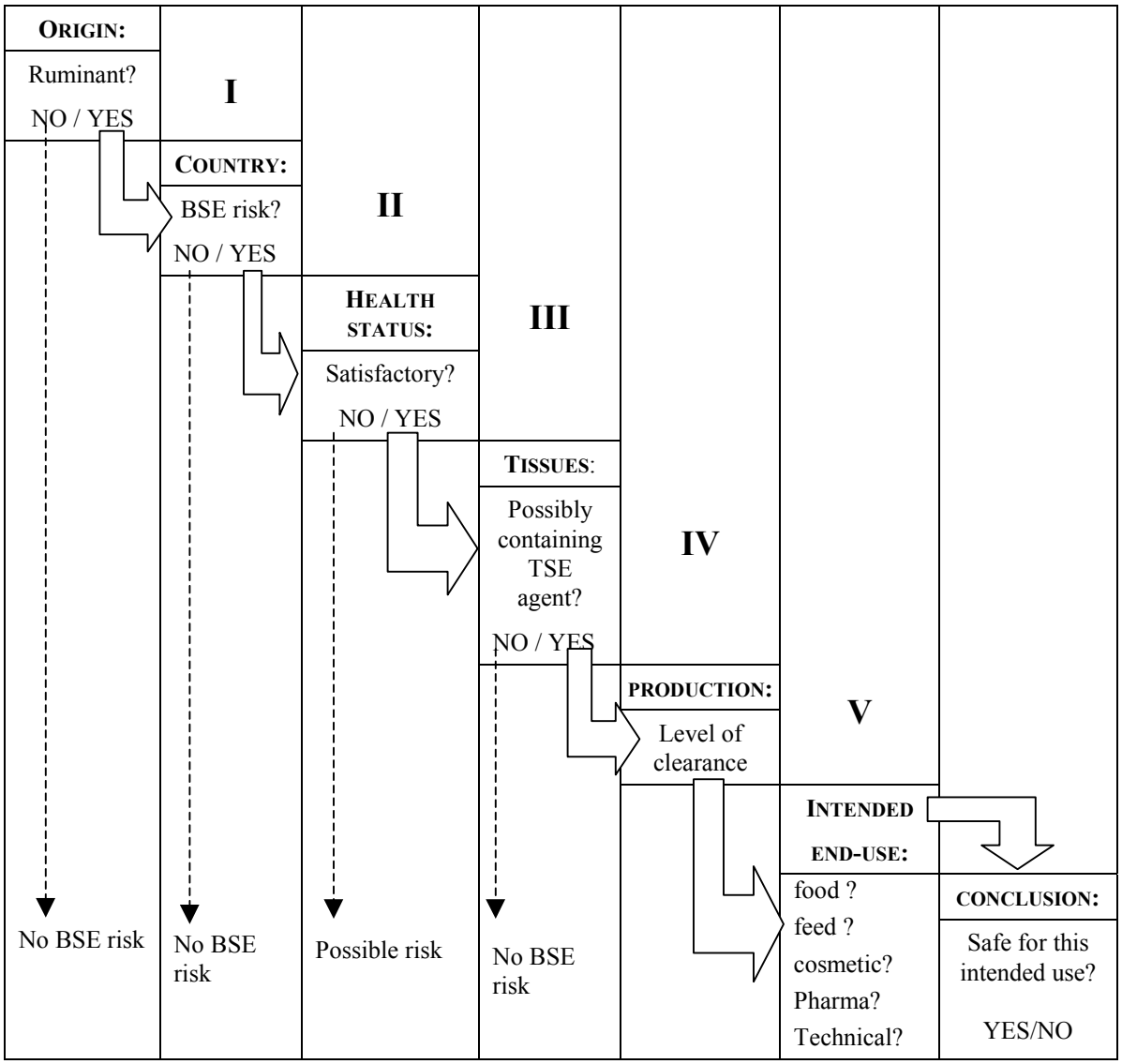
II. Veterinary inspection assuring that the animal is healthy or fit for human consumption. [Does the animal itself possibly poses a BSE risk?]

III. Appropriate sourcing, from a given animal, of the tissues. [Should certain tissues of the animal possibly be excluded for further use? [Are there tissues likely to be infected?]

IV. Appropriate processing of the raw material, resulting in elimination or reduction below significant levels of agents that may still be present after the above steps. [Will the production process remove or destroy TSE infectivity?]

V. Exclusion from certain (human, animal) uses of the product if a doubt remains about the safety of the end product (i.e. certain materials or products should be entirely disposed of or only find applications that exclude human or animal consumption such as certain technical uses.)

The risk of human exposure to BSE infectivity is therefore considered to be reduced to insignificant levels by the *combined* action on all parameters that have a possible impact on the level of BSE infectivity in a cattle-derived product (and in small ruminants products *in case* BSE is detected under natural conditions). This can schematically be presented as follows:



However, sometimes, the application of *all* the above steps is not always required. For example, animals sourced from a country that is proven to be exempt of a certain infectious agent would not need to undergo further risk reduction measures with regard to that agent. On the other hand, certain risk reduction measures may, on their own, result in a very large risk reduction and reduce or eliminate the need for additional measures. An example would be production processes that result in a substantial elimination of an agent.

In the following tables, a summary overview is presented of the safety aspects of a number of ruminant-derived products. In the tables, the following content is given to the notions “specified risk materials”.

	Specified risk materials (for animals fit for human consumption)
GBR I	**Cattle:** none **Small ruminants:** none
GBR II and **GBR III**	**Cattle:** The skull, including the brain and eyes, tonsils, the vertebral column excluding the vertebrae of the tail and the transverse processes of the lumbar and thoracic vertebrae and the wings of the sacrum, but including dorsal root ganglia, and spinal cord of animals above 12 months. Intestine from duodenum to rectum and the mesentery of animals of all ages. **Small ruminants:** the skull including brain and eyes, the tonsils, the spinal cord of ovine and caprine animals aged over 12 months or which have a permanent incisor erupted through the gum; The spleen of ovine and caprine animals of all ages.
GBR IV	**Cattle, in addition to the above:** the entire head excluding the tongue, including the brain, eyes, trigeminal ganglia and tonsils; the thymus, the spleen and the spinal cord of animals above 6 months **Small ruminants:** as above

GELATINE FROM BOVINE BONES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants: If: NO						No BSE risk
If: YES	If GBR I:					No BSE risk
	Otherwise	Fit for human consumption? If: NO				Possible risk
		If: YES	Tissues used: long bones Not used: Specified risk materials are removed, including vertebrae, spinal cord, skull, brain; 98% of marrow, lipids, etc. attached to bones are removed by the degreasing step	degreasing, alkaline and acid processes, final filtration and/or sterilisation: Total process clearance: at least $10^{4,5}$. Or: Or: Degreasing, heat/pressure process, final filtration and/or sterilisation: Total process clearance: at least $10^{6,5}$.	Food, feed, cosmetics, pharmaceutical, technical (photographic), etc.	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.
3) Gelatine from bovine bones only represents 23% of total gelatine production in Europe and from this 23%, 50% is used in the photochemical industry

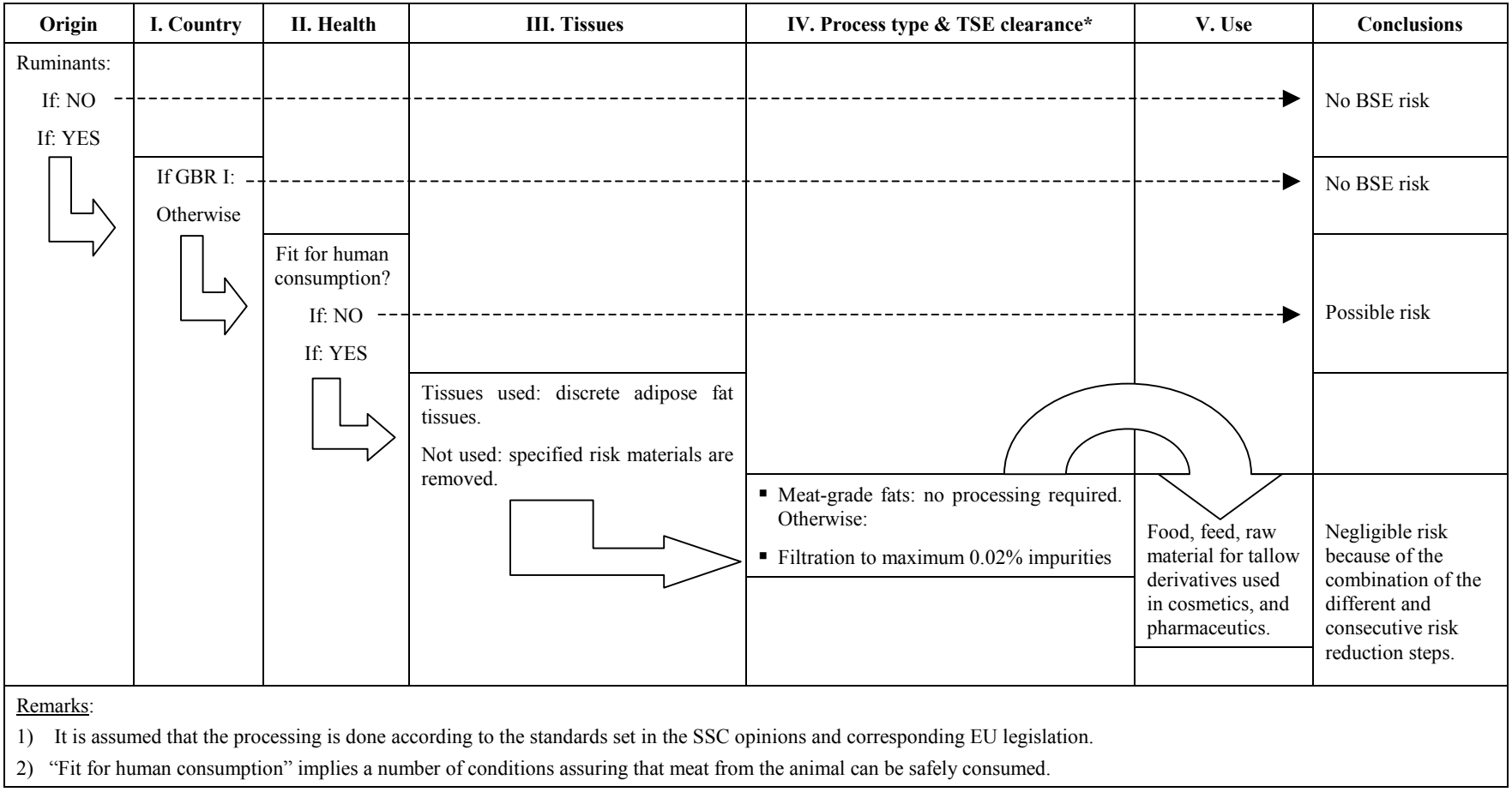
GELATINE FROM BOVINE HIDES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants: If: NO						No BSE risk
If: YES	If GBR I:					No BSE risk
	Otherwise	Fit for human consumption? If: NO				SSC recommends excluding fallen ruminant stock or risk animals from further processing
		If: YES	Raw material used: Bovine Hides (3) Slaughter methods should be such that the risk of contamination with SRM's or potential infected material is prevented. (4)	<u>Process types like as for bones:</u> • Degreasing, alkaline or acid processes; final filtration and/or sterilisation. (Total process clearance: at least $10^{4.5}$) or: • Degreasing, heat/pressure treatment, final filtration and/or sterilisation (Total process clearance at least $10^{6.5}$).	Food, feed, cosmetics, pharmaceutical, technical (photographic) etc	Negligible risk because of the combination of the different and consecutive risk reduction steps.

<u>Remarks</u>:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.
3) Should sheep hides be used and should BSE be found in sheep under natural conditions, an additional risk assessment would be needed for sheep hides with regard to peripheral nerves in subcutaneous layers of the hide.
4) Exclusion of animals that initially passed ante mortem but later tested positive with BSE will further reduce the risk.

TALLOW FROM DISCRETE ADIPOSE TISSUES: RISK ASSESSMENT



Remarks:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.

TALLOW FROM MIXTURES OF TISSUES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants:						
If: NO						No BSE risk
If: YES						
	If GBR I:					No BSE risk
	Otherwise					
		Fit for human consumption?				
		If: NO				Possible risk
		If: YES				
			Tissues used: mixture of tissues from which the fat is melted out.			
			Not used: specified risk materials are removed.	• Pre-sterilisation at 133°/20 minutes and at 3 bars and filtration to maximum 0.15% insoluble impurities	Feed; raw material for derivatives used in cosmetics, and pharmaceutics.	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.

TALLOW DERIVATIVES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants: If: NO						No BSE risk
If: YES	If GBR I:					No BSE risk
	Otherwise	Fit for human consumption? If: NO				SSC recommends excluding fallen ruminant stock or risk animals from further processing
		If: YES	Raw material used: food- or feed-grade tallow. Not used: specified risk materials are removed.			
				Hydrolysis at > 200°C for 2 hours and corresponding pressure, followed by either: ▪ <u>To obtain fatty acid esters</u>: Distillation > 200°C. The distilled fatty acids undergo esterification > 200°C with alcohols, followed by a purification to remove (insoluble) impurities; or: ▪ <u>To obtain glycerides</u>: distillation at 140°C. The distilled glycerine undergoes esterification > 200°C with organic acids, followed by a purification to remove (insoluble) impurities.	Food, feed, raw material for tallow derivatives used in cosmetics, and pharmaceutics.	Negligible risk because of the combination of the different and consecutive risk reduction steps, in addition to requirements imposed on tallow used as staring material.

<u>Remarks</u>:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.

2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.

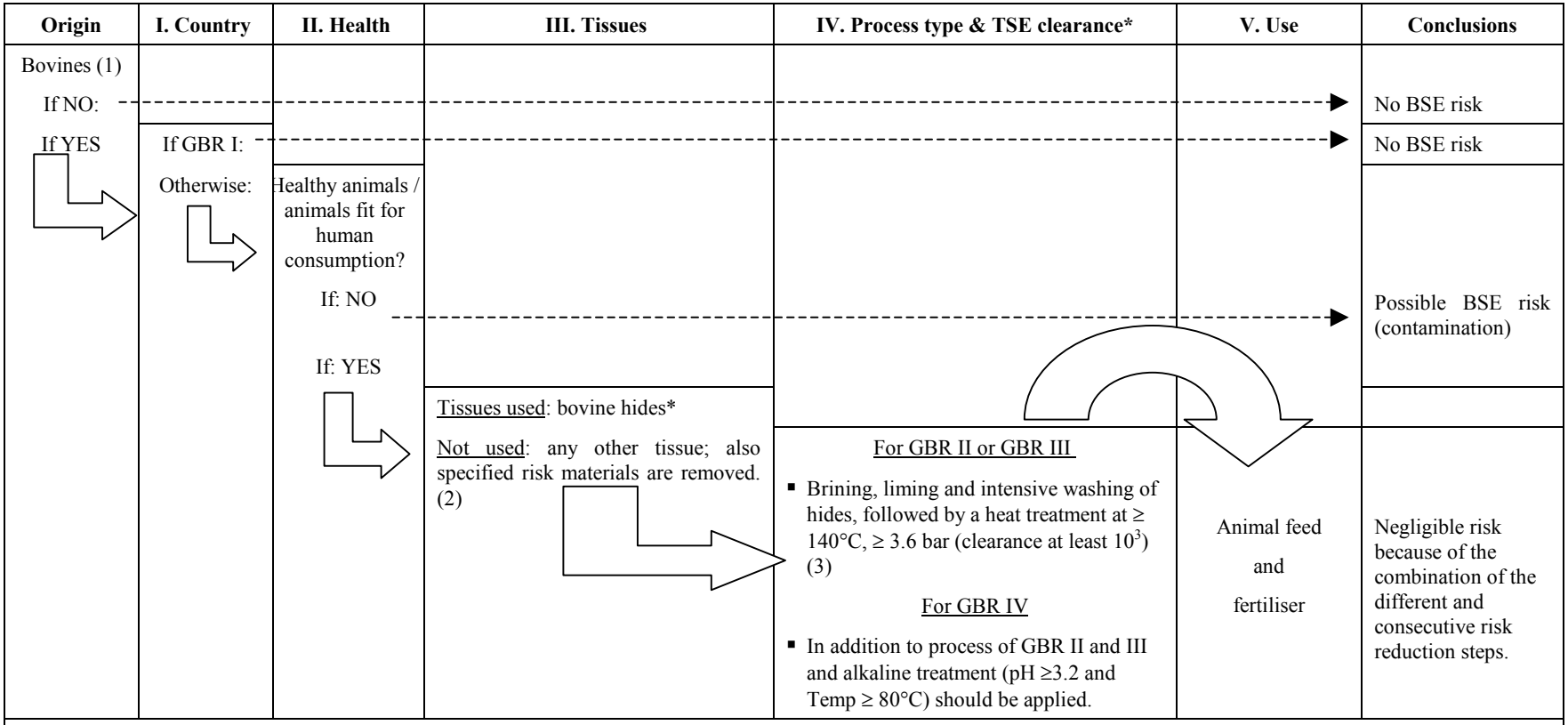
COLLAGEN FROM BOVINE HIDES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Bovines*: If: NO If: YES						No BSE risk
	If GBR I: Otherwise					No BSE risk
		Fit for human consumption? If: NO If: YES				Possible risk; SSC recommends excluding fallen ruminant stock or risk animals from further processing
			Raw material used: bovine hides* Slaughter methods should be such that contamination with SRM's or potential infected material is prevented. Exclusion of animals that initially passed ante mortem but later tested positive with BSE will further reduce the risk.	Collagen production generally involves an alkali step followed by an acid extraction step: as for gelatine, the production processes will reduce partially TSE infectivity. No TSE inactivation experiments have so far been carried out with ruminant hides.	▪ Food, feed, cosmetics, pharmaceutical, technical; ▪ Case by case risk assessment for: parental or ophthalmic administration; vaccines; topical application; implantable devices; special grade collagen.	Given the uncertainties/ unknown TSE inactivation capacity of the various collagen processes only an appropriate combination of safe sourcing and end-use will guarantee a reduction of the residual risk to nearly zero

Remark:

* Should sheep hides be used and should BSE be found in sheep under natural conditions, an additional risk assessment would be needed for sheep hides with regard to peripheral nerves in subcutaneous layers of the hide.

HYDROLYSED PROTEINS (PETIDES AND AMINO-ACIDS) FROM BOVINE HIDES: RISK ASSESSMENT



Remarks:

1) Should sheep hides be used and should BSE be found in sheep under natural conditions, an additional risk assessment would be needed for sheep hides with regard to peripheral nerves in subcutaneous layers of the hide.

2) Exclusion of hides from animals that successfully passed *ante mortem* inspection, but later tested positive with a *post mortem* test will further reduce risk.

3) A molecular weight of the end product below 10.000 Dalton may be used as an indicator of processing conditions but can not be seen as an absolute guarantee for safety.

AMINO ACIDS FROM HUMAN HAIR HYDROLYSATES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Human	GBR I, II, III or IV:	Healthy living individuals?				
		If: NO				TSE risk possible?
		If: YES	Tissues used: Human hair collected by hairdressers and in barbershops, eventually contaminated by skin tissues	▪ Example of a production Process: acid Hydrolysis with 20%, Hydrochloric acid at 100° C, 6 hours;* ▪ Product only containing free amino acids; ▪ Contamination with risk tissues is minimised or excluded; ▪ Crystallisation gives additional purification.	Incorporation into human hair- and skin care products for topical applications **.	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

* A molecular weight of the end product below 10.000 Dalton may be used as an indicator of processing conditions but can not be seen as an absolute guarantee for safety.

** No opinion available for other applications / uses.

DICALCIUM PHOSPHATE FROM BOVINE BONES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Bovines?						
NO						No BSE risk
YES	If GBR I:					No BSE risk
	Otherwise	Healthy animals / animals fit for human consumption?				
		If: NO				Possible BSE risk
		If: YES				
			Tissues used: Bovine bones, excluding skull and vertebrae Not used: any other tissue; also specified risk materials are removed and cross-contamination is prevented.	▪ Processing starting with degreasing, followed by acid treatment, liming at pH 4 to 7, purification and drying; ▪ Production process as a whole will reduce the infectivity up to $10^{3.8}$. ▪ Residual proteinaceous fraction not exceeding 0.6 % with 98% having a molecular weight below 10.000 Dalton.	Animal feed; fertiliser	Negligible risk because of the combination of the different and consecutive risk reduction steps.

THE SAFETY OF BOVINE MEAT

By D. Dormont and G. Wells

In the current stage of knowledge, no infectivity has ever been identified in skeletal muscles of naturally TSE-affected animals. Skeletal muscle homogenates from cattle naturally infected with BSE have been assayed in mice. Potential infectivity of skeletal muscles has also been evaluated through the pathogenesis experiment: no infectivity was recorded in several muscles by mouse bioassay, or, to date (Jan. 03), by assay in cattle (intracerebral inoculation), but the latter studies are incomplete (pooled skeletal muscles assays from cattle at different time points in the pathogenesis study are currently 48-76 months post inoculation).

One publication reported the presence of PrP^{Sc} and infectivity in the hindlimb of rodents inoculated with rodent-adapted scrapie strains. Another recent report describes widespread PrP^{Sc} in muscles of hamsters orally infected with a hamster-adapted scrapie strain. PrP^{Sc} was also detected in the tongue of hamsters inoculated by the intracerebral route, with several TSE strains. However, a pilot study was conducted by AFSSA in France, and no PrP^{Sc} was evidenced in either BSE infected mice or BSE-affected cattle.

From these studies it can be hypothesised that very low levels of infectivity may be detectable in skeletal muscles in some experimental models of TSE (rodents). This does not preclude the possibility of the presence of infectivity in skeletal muscle in natural diseases, but meat continues to be considered as not infectious per se.

Relevant SSC opinions (see annex II): **11, 19.**

BSE-RELATED SAFETY ASSESSMENT OF COSMETIC PRODUCTS

By I.R. White and F.H. Kemper

Cosmetic products are normally applied topically (although others may be used on the lips and for oral hygiene purposes). From a human risk exposure point of view, cosmetics are expected to pose less concern than for food or pharmaceutical products in view of the permeability characteristics of human skin including to high molecular weight proteins.

1. Definition

For the purposes of this report, cosmetics are defined as in the Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products:

"A 'cosmetic product' means any substance or preparation intended for placing in contact with the various external parts of the human body (epidermis, hair systems, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or principally to cleaning them or protecting them in order to keep them in good condition, change their appearance, perfume them or correct body odours."

Additionally,

A natural 'ingredient' is understood to mean a substance, a complex of substances or preparations of natural origin, which is used in a cosmetic formulation. (...)"

The above definition also corresponds with the definition used in Council of Europe (2002).

2. The safety assessment of cosmetic products

In general, the assessment of safety-in-use of cosmetic products containing natural ingredients requires integration of two types of data, i.e. those related to toxicity of individual ingredients, and those related to the extent and route(s) of exposure. "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" were adopted on 24 October 2000 by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SC-CNFP).

For the assessment of possible residual BSE risks, additional principles and criteria need to be considered. The scientific principles and criteria used since 1997 by the SSC as the bases for its BSE risk assessments are summarised in Part I of this Overview.

A critical factor in the assessment of safety-in-use of a cosmetic ingredient is the extent to which a consumer is likely to be exposed. Exposure to a specific ingredient can be estimated on the basis of:

- *types* of cosmetic containing it;
- *quantity* of the ingredient present in each product;
- *quantity* of each product used by the consumer in each application;
- *duration* and *frequency* of applications of the different products containing the ingredient;
- total *area* of the body exposed to the product in each application, and
- foreseeable misuses which may increase exposure.

Once the exposure has been estimated, the amount likely to enter the body can be estimated on the basis of bio-availability studies.

Other important considerations concerning exposure include the characteristics (e.g. age, atopic status) of the exposed population and other sources of potential exposure to the same ingredient (e.g. by professional/occupational exposure).

3. For assessing the possible residual BSE risk in cosmetic raw materials and ingredients of animal origin, sourced from countries where the BSE risk is *not highly unlikely*, a risk assessment along the scheme presented in the *Summary overview of SSC opinions on Product Safety* by M. Vanbelle (**Part II.C**) is required. If, following this assessment, it appears that a *non-negligible* residual BSE risk may (still) be present in the end product, further evidence supporting the safety of the product and/or a risk assessment is required. Additional key-elements of such evidence may (depending upon the ingredient) include:

   - Whether or not the raw material was sourced from animals fit for human consumption or healthy animals. F*or example:* sourcing of wool for lanolin

from healthy live sheep would exclude the risk of cross-contamination of the hide at slaughter or when processing fallen stock or culled risk animals;

- The degree of purity of an ingredient. F*or example:* careful purification / filtration or crystallisation may exclude the risk that certain substances are contaminated with foreign proteins or long peptides;

- Whether or not cross-contamination was avoided/eliminated. F*or example:* gelatine from bones from countries where a BSE risk is low is safe if an appropriate production process was used, provided the specified risk materials were removed (e.g. skull bones).

4. A detailed discussion of cosmetic ingredients and products would take advantage of a regrouping of the products / substances / ingredients into classes according to the ruminant tissue of origin. To facilitate and scientifically underpin the evaluation of their safety with regard to BSE risks, the products/substances/ingredients can be broadly classified into 3 categories:

   - Products derived from tissues that are proven potential carriers of BSE infectivity and that are in the EU prohibited as "specified risk materials - SRMs". Such products are, for example: brain extract, brain lipids and hydrolysed spinal protein, which should under **no circumstances** be sourced from ruminants from countries where the BSE risk is *not highly unlikely* (GBR I);

   - Products derived from tissues that are proven not to be potential carriers of BSE infectivity. This concerns, for example, wool from live sheep and (probably) hides from healthy animals. Such products are, for example: lanolin and its derivatives, keratin and collagen. These products should in principle be safe, provided cross-contamination with SRMs is avoided.

   - Products derived from various other tissues or mixtures of tissues, where it is not always clear what these tissues are, whether cross-contamination is possible, etc. The safety assessment of such products would require inputs from technicians from the industry on the ruminant tissues that are used for the preparation of certain products/substances/ingredient, on the level of processing and on the level of purification.

5. **Note on products derived from small ruminants (sheep and goats)**

BSE has not been found in domestic flocks of small ruminants nor is there other evidence that BSE is present in small ruminants under field conditions or any indications pointing to an increased likelihood of such being the case. *Scrapie* on the other hand has been recognised for more than 200 years but has not been recognised as contributing to the epidemiology of human TSEs. Therefore sourcing raw materials from small ruminants for the production of cosmetics or ingredients should not result in a human exposure risk to BSE.

On the other hand, the number of small ruminants investigated for the presence of BSE is relatively small, BSE has been transmitted experimentally to TSE-susceptible sheep and natural sheep populations have been exposed to the same feed sources as cattle (albeit probably to a lower extent). The SSC therefore, throughout its opinions, considers it justified that sourcing and processing of tissues from sheep should comply with the same criteria as for cattle. The infectivity distribution in sheep that are susceptible to TSEs is, however, different as compared to cattle (see **Part II.A.**). Therefore, should BSE in sheep be found in small ruminant populations under field conditions, the frame hereafter may need revision for products derived from certain sheep tissues that may be infectious in sheep but not in cattle.

Relevant SSC opinions See *Summary overview of SSC opinions on Product Safety* by M. Vanbelle (**Part II.C**)

THE SAFETY OF PHARMACEUTICALS

By Keith H. Jones and Johannes Löwer

Preamble

The SSC opinions relevant to bovine derived materials used in the manufacture of pharmaceuticals, although they are substantial, are not comprehensive. A comprehensive review has been made and is available in the form of a guideline for the manufacture of medicinal products from the EMEA[32]. This guideline is updated on a regular basis in the light of the most recent scientific and technological evolutions, including the most recent SSC advice. The guideline as a consequence includes reference to all of the relevant SSC opinions. Once adopted, it carries the full force of community law, and will be mandatory for the pharmaceutical manufacturing industry.

A partial discussion of the safety of pharmaceuticals based only on the opinions of SSC would therefore be of limited value. This section is therefore limited to a statement of the scientific principles involved in assessing the risk for transmission of BSE as a result of using bovine derived materials in the manufacture of pharmaceuticals, but reference to the comprehensive guideline (EMEA/410/01 Rev. 1) is given:
http://www.emea.eu.int/
http://www.emea.eu.int/index/indexh1.htm
http://www.emea.eu.int/pdfs/vet/regaffair/041001en.pdf

A discussion of the safety of pharmaceuticals with respect to spongiform encephalopathies would not be complete if it would not include the risk posed by human forms of TSEs may they be linked to BSE or not. Therefore, SSC's TSE/BSE ad hoc working party and the Scientific Committee for Medicinal Products and Medical Devices (SCMPMD) analysed in great detail the possibility that Creutzfeldt-Jakob disease (CJD) or its variant (vCJD) might be transmitted by blood, blood products or human organs or tissues.

The safety of pharmaceuticals

The transmission of spongiform encephalopathy by medicinal products has been a matter of concern since before the recent epidemic of BSE. The effectiveness of transmission of spongiform encephalopathy by pharmaceuticals has been clearly demonstrated in veterinary medicine by the transmission of scrapie by looping ill vaccine prepared from

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32 EMEA: The European Agency for the Evcaluation of Medicial Products.

ovine spleen and brain; and in human medicine by human growth hormone prepared from human cadaveric pituitary glands and by human dura mater preparations.

The potential for transmission of spongiform encephalopathy by pharmaceuticals is substantial because more than 95% of medicinal products used in human and veterinary medicine are manufactured using materials of bovine origin. These include gelatine used for capsules or as a carrier or stabilising agent; tallow and tallow derivatives - particularly stearates used as filling agents; bovine derived wetting agents; bovine serum albumin and calf serum used as stabilising agents and as components of cell culture media in the manufacture of vaccines and other 'biologically derived' medicines; rennin used in the production of lactose; amino acids derived from hair and skin.

The principles which apply to limiting the risk of transmitting TSEs via medicinal products are those already recommended by the SSC for all other areas, namely:
safe sourcing
tissue selection
rigorous processing
limiting use to specified applications.

These principles and SSC opinions based on them have been used to develop recommendations for the manufacture of bovine derived materials used in the manufacture of medicinal products. Accordingly, specific SSC opinions have been delivered on the manufacture of gelatine, tallow and tallow derivatives, rennet and amino acids. These recommendations propose conditions and precautions to be used during the manufacture of each of these materials of bovine origin so that they can be further used in the manufacture of medicinal products.

A detailed analysis of experimental and epidemiological data lead to the conclusion that classical forms of CJD, although, on several occasions, transmitted by pharmaceuticals derived from tissues of the central nervous system or adjacent tissues, are not transmitted by blood components or blood products. However, as the experience with vCJD which differs in tissue distribution from CJD is limited the advice was given to follow a cautionary approach with respect to the possibility of vCJD transmission by blood or blood products. Quite a number of possible measures following the precautionary principle were discussed in a series of Opinions. They include the exclusion of plasma from donors who lived in countries with a high risk for vCJD or the introduction of general leucoreduction.

Risk/benefit considerations

Special considerations apply to the risk assessment of medicinal products as a result of the benefit that should be derived from their use and whether that benefit relates solely to the individual exposed or more widely to the population.

Medicines are most frequently administered for the benefit of individuals suffering from the effects of disease where they might be expected to bring direct benefit to the individual exposed to the risk. They are also used for the prevention of disease in otherwise healthy subjects, often of young age, where an important objective might be the achievement of a population benefit as well as protection of the individual. Under the latter circumstances a much greater benefit or lower degree of risk is required.

These considerations may make additional or lesser degrees of risk acceptable or contribute to a more rigorous approach to the risk assessment of medicinal products indicated for prophylactic use. Other factors such as route of administration, dose, age, presence or absence of concurrent disease, frequency and duration of treatment are also important considerations. For these reasons the risk assessment process for pharmaceuticals must be made on a product by product, or case by case basis. Furthermore they are often based on rapidly evolving science and need to be updated regularly as the knowledge base moves forward.

Relevant opinions: See *Summary overview of SSC opinions on Product Safety* by M. Vanbelle (**Part II.C**); **78**

Relevant opinions of the Scientific Committee for Medicinal Products and Medical Devices:

Opinion and report on the equivalency of alternative products to intestines of animal origin for use as surgical sutures, adopted on 16 September 1998

Opinion on the risk quantification for CJD transmission via substances of human origin, adopted on 21 October 1998

Opinion on the Safety of Hides and Skins, adopted on 24 March 1999

Opinion on the Policy Regarding the Use of Blood and Blood Products adopted by Written Procedure on 24 March 1999

Opinion on update of the opinion on the Risk Quantification for CJD Transmission via Substances of Human Origin, adopted on 16 February 2000

Opinion on the safety of Human-Derived Products with regard TSEs adopted on 18 January 2002

THE SAFETY OF RUMINANT BLOOD

By H.Budka

There is concern that animal TSEs might be spread by blood that has been used as food or feed, as fertiliser on pasture, or through specific blood components or blood based products that are still permitted to enter the market, including medicinal products and biologicals. While normally TSE risks are controlled by a combination of factors including production processes that are likely to contribute to some reduction of prion infectivity, the situation here is different: usually ruminant blood is used without any treatment that is able to decontaminate prions. Thus only sourcing, type of use and potential for contamination remain the key factors to control for TSE safety of ruminant blood.

Experimental studies on TSE infectivity in blood and its components

The majority of bioassays on infectivity in blood have been carried out in animals with clinically overt TSE. In consequence there is substantial ignorance about the early pathogenetic involvement of blood, especially in naturally occurring diseases. In BSE, transmission has not been achieved in natural disease, but blood has been shown to be infectious in experimental BSE in genotypically susceptible sheep and in sheep with naturally occurring scrapie after transfusion of large blood volumes. In experimental scrapie, blood components obtained during both the pre-clinical and clinical stages of disease from rodents, have revealed the presence of the infectious agent. In sum, while epidemiological evidence has so far failed to identify any blood-related cases of TSEs, data from both experimentally induced and natural TSEs suggest that blood has at least the potential to transmit disease.

Use and Sourcing

Slaughtered cattle, sheep, goats and deer could supply blood for food, feed and other purposes. All these species are susceptible to TSEs both naturally and experimentally. BSE as a natural disease has only been reported in cattle. The possibility of BSE being in sheep and goats cannot be excluded. No validated tests exist to detect TSE in live cattle, sheep, goats or deer. Close surveillance for the disease and effective ante mortem clinical inspection of all slaughter animals therefore remain essential.

Risk Assessment

Apart from the potential risk that ruminant blood might contain very low levels of endogenous infectivity, the question of contamination of blood from external sources must be addressed, in particular the possibility of brain tissue contamination at slaughter. Thus the most important aspect of risk relates here to such contamination.

1. the amount of brain material actually entering the bloodstream following the use of invasive stunning devices. Neither its volume range nor the range of particle size is known. Likewise, no quantitative estimates are available on contamination of blood with SRM materials during the slaughtering process other than by stunning the animals.
2. dilution of CNS material resulting from the emboli, and
3. the efficacy of the various processing steps in respect to inactivating the BSE agent.

There is little doubt that under certain circumstances, humans or animals could be exposed to the BSE agent by consuming blood products.

The SSC proposes a general approach for the risk assessment for blood within a given area, which basically involves 3 aspects:

Slaughterhouse

At the level of each slaughterhouse, the following risk factors should be in particular evaluated in respect to:

1. number, species and age of slaughtered animals;
2. number of potentially infected cows being killed and their brain material entering the bloodstream related to the stunning method used (non-penetrative vs. captive bolt, pneumatic devices, pithing);
3. the average amount of blood collected per animal;
4. the dilution by pooling blood from several animals;
5. the amount of such collected blood going to the industry to be processed for human or animal consumption.

The TSE risk derives in particular from the following factors:

– The highest risk of producing CNS emboli follows captive bolt stunning with compressed air into the cranial cavity.

– Cartridge operated captive bolt stunning followed by pithing presents the next highest risk.

There is insufficient knowledge to advise on the degree of risk from the use of penetrative cartridge-operated stuns without pithing, free bullets or non-penetrative guns.

There are no published papers on the effect of various stunning methods on sheep and goats and in regard to the generation of CNS emboli.

More information is required on the possible dissemination of CNS emboli into the systemic circulation.

TSE risks may exist as a result of the source of animals for slaughter.

TSE risks may occur independently of the stunning procedure, as result of TSE-infected material from SRM entering the blood after exit from the body.

Geographical BSE risk and surveillance

For the geographical BSE risk and surveillance reference is made to the GBR opinions adopted by the SSC.

The use of blood

At present, blood collected hygienically in licensed EU abattoirs can be used for food, feed and a variety of other purposes with or without any form of processing, including cosmetics, pharmaceuticals and technical use as fertiliser. For example, it is permissible to incorporate fresh untreated plasma into the materials used for the production of sausages, and it can be spread on land as a fertiliser. However, there could be a risk of the occasional presence of low levels of TSE infectivity in blood collected in abattoirs. Levels of infectivity that might represent a risk to animal or human health are not known. Control measures and/or decontamination standards thus might need to be developed to potentially TSE-infected blood collected in abattoirs.

Conclusions

For ruminant blood, the best approach to protect public health at present seems to assume that it could contain low levels of infectivity. However, even if this is true, it becomes almost irrelevant compared with the level of contamination that could occur as a result of the methods of stunning used in abattoirs. These procedures have been recognised to release particles of brain tissue (potentially containing high titres of TSE infectivity) into the bloodstream. The frequency at which this occurs appears to increase with the severity of the stunning process, and this is an area requiring further research. There are also opportunities for the contamination of pooled blood as a consequence of the release of brain tissue from the hole left by stunning, or with spinal cord during its removal (if a production-line process is not used). Nevertheless, given the low frequency at which

apparently healthy animals testing negative in a rapid post-mortem TSE test would have TSE infectivity in the CNS at the time of slaughter, it is considered that the overall potential level of infectivity in pooled blood will be low.

A summary of the SSC opinion on the safety of ruminant blood is given in the **Table** at the end of this contribution.

Recommendations

The SSC recommends that its opinion on the safety of ruminant blood is considered in conjunction with its opinions on "Fallen stock" (June 1999), "Intra-species Recycling" (June 1999), and “Stunning methods and BSE risks” (January 2002).

Consideration should further be given to avoid methods of captive bolt stunning with compressed air or followed by pithing ruminant food animals that increase the risk of CNS material entering the blood stream at slaughter wherever there is a significant risk from TSE[33]. In addition, sourcing from young[34] animals would further reduce the risk.

Improved methods for reducing the risk of cross contaminating blood with CNS or other SRM post-collection need to be developed or put in place where necessary. Brain spilling from the bullet hole into the blood tank should be prevented; surveys should check the absence of brain material in the blood tanks.

Where an element of risk is perceived, this may be reduced or eliminated by (a combination of) various strategies, as follows:

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33 Changing from pneumatic stunning or pithing, to stunning methods that avoid severe brain damage could go along with an increased risk of physical injury to slaughtermen (particularly during shackling and bleeding out) if the new methods or building facilities are not properly designed.

34 First infectivity in CNS of cattle is detected in most cases in the last quarter of the incubation period. Defining young animals could be done on the basis of the probability of occurrence of BSE according to the age. (See for example the annexes 3 and 4 of the Opinion of 28-29 October 1999 of the Scientific Steering Committee on the *Scientific Grounds of the Advice of 30 September 1999 of the French Food Safety Agency (the Agence Française de Sécurité Sanitaire des Aliments, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom*.

Source bovine blood from BSE-free areas or closed herds or other schemes that reduce to a minimum the probability of an animal being infected;

Subject the product to a "133^{o}C/3bar/20'" autoclaving or equivalent validated process.

Pharmaceuticals including vaccines are regulated products, and the use of bovine derived blood products in their manufacture is controlled on a case by case basis. The basic principles reviewed here should of course be respected.

Relevant SSC opinions (see annex II): **13,20, 21, 26, 28.**

Table: **Summary of the SSC opinion on the safety of ruminant blood.**

GBR*	HUMAN FOOD	ANIMAL FEED	COSMETIC	PHARMACEUTICAL	TECHNICAL (FERTILISER)
I	No risk with regard to BSE				
II **III** **IV**	As for meat: animals fit for human consumption, appropriate slaughtering process (without pithing/contamination by SRMs) and blood collection technique, avoidance of cross-contamination, etc.	As for food and: Avoidance of intra-species (ruminant) recycling	As for food	For oral and limited topical administration: as for food. Otherwise: a case-by-case risk assessment for: – parenteral and ophthalmic administration; – topical administration to large skin areas of open wounds; – vaccines; – implantable devices	As for feed, i.e. blood recuperated from animals at risk or part of an eradication programme, should not be disposed of as a fertiliser.

* Presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a region or country: Unlikely but not excluded (GBR II); Likely but not confirmed or confirmed, at a lower level (GBR III); Confirmed, at a higher level (GBR IV)

Note: The situation with blood differs from all other materials as processing normally does not reduce any infectivity. The SSC thus stated that "*Sourcing from young animals would further reduce the risk*" and "*Where an element of risk is perceived (this would apply to GBR II-IV), this may be reduced or eliminated by:*

– *sourcing from BSE-free areas or closed herds or other schemes....; and:*
– *subject to 133°/3 bar/20'".*

THE SAFETY OF NATURAL CASINGS DERIVED FROM THE SMALL INTESTINE OF SMALL RUMINANTS (SHEEP AND GOATS)

By R. Bradley

Definitions

The term casing refers to the envelope enclosing an animal product, principally containing meat, offals (like liver) or blood for human consumption, the whole being termed a sausage. This report refers only to the TSE risks in natural casings derived from the small intestine (duodenum to ileo-caecal junction inclusive) of small ruminants (sheep and goats), only to the casing and not to the contents. It cannot be excluded that small local enterprises harvest large intestines for local use.

Intestines used to produce natural casings are only sourced from animals destined for human consumption, slaughtered in licensed abattoirs following official *ante* and *post mortem* examination and passed fit for human consumption. The whole process of slaughter and subsequent procedures in the abattoir are in principle subject to official control.

General statements on the report

Consultation with members of the Scientific Working Group of the International Natural Casing Associations resulted in the following comments:

Desliming is the most important factor that influences the quality of natural casings with respect to marketing aspects and can be achieved by machine processing or manual processing. Machine processing is achieved by passing runners through a series of cleaning machines and tanks of hot water during which both the inner and outer layers of the small intestine are removed. (The inner layer, or mucosa, is that part which is believed to contain most of the infectivity in an infected intestine). Finally, the casings are passed through a finishing machine. Quality control is continual, and additional checks are made, such as measuring the gauge of the casings and making sure there are no holes. Any faulty casings are discarded. The runners are collected into hanks of 50, salted and placed in barrels of salt (sodium chloride) for a minimum of 30 days, prior to dispatch.

There are no commercially detectable differences in casings processed by hand or machinery.

It is known that sheep and goats can be experimentally infected with the agent that causes BSE and develop fatal spongiform encephalopathy. However, natural cases of BSE in sheep have not been reported in any country to date. If BSE is found in sheep or goats in the future there is a clear potential risk for humans as BSE is a zoonosis. The agent causing the experimental disease 'BSE in sheep', or more accurately 'scrapie caused by the BSE agent' (because the clinical and pathological features are closely similar to those of scrapie) is biologically indistinguishable from the agent that causes variant Creutzfeldt-Jakob disease of man. Precautionary risk reduction measures have been applied to certain risk sheep tissues (such as the skull, brain, eyes, spinal cord and spleen which are designated specified risk materials (SRM) that must be destroyed) in the EU and some other countries.

Small ruminants can be naturally affected by scrapie, a TSE naturally confined to these species, caused by various strains of scrapie agent (that is biologically and molecularly different from the BSE agent) and which are not regarded as human pathogens. Much of the risk assessment for BSE in sheep is based on knowledge from natural or experimental scrapie in sheep and goats. No formal action is taken against scrapie in regard to public health except that scrapie is a notifiable disease in the EU and animals suspected clinically to have scrapie are prohibited to enter any food or feed chain. Removal of SRM from sheep and goats reduces exposure of man and animals to scrapie (and to BSE if it occurs) even though the driving force for the legislation was the fear of BSE being found in sheep in the future.

Tissue distribution of infectivity in sheep and goats with natural TSE

What the tissue distribution of the BSE agent would be in natural BSE in small ruminants is unknown, because the disease is hypothetical and speculative. However, it is more likely to be similar to the distribution of scrapie agent in natural scrapie in sheep and goats than to natural BSE in cattle. That is, it would have a wide distribution in lymphoreticular tissues and nervous tissues. The brain, eye, spinal cord, associated ganglia, intestine, lymph nodes, and possibly other tissues come into consideration. Like scrapie, genetic resistance can occur and immunohistochemical detection of PrP can determine the precise sites of accumulation of prion protein in infected organs. Some studies have identified PrP not only in the gut-associated lymphoid tissue (GALT) but also in the enteric nerve plexuses (Auerbach's and Meissner's plexus). Thus any TSE-risk reduction resulting from the process of making a natural casing will be related to the completeness of the removal of the GALT and the two nerve plexuses,

Infectivity titres in intestinal and other tissues

An important missing component at the time of writing is the absence of data on the amount (titre) of infectivity in any infected tissues of experimentally BSE-infected sheep or goats. This is because there are no reports of infectivity titrations, including for the intestine. It is assumed that any titres that are present may be closely similar to those published for goats, in the clinical phase of scrapie and Suffolk and other breeds of sheep in the pre-clinical and clinical phase of natural disease. Unfortunately even these detailed studies did not investigate the titre of infectivity in parts of the small intestine other than the distal ileum which is rich in lymphatic tissue in the form of Peyer's patches. Infectivity in the distal ileum was consistent from at least 10 months of age when detectable infectivity was also present in spleen and lymph nodes. In some breeds, individual sheep with natural scrapie, confirmed by microscopic examination of the brain, had no detectable infectivity at all in the ileum, and in one case in a Montadale sheep, none in any tissue, except the CNS.

When significant levels of infectivity were found in the ileum they were of the same order of magnitude as in lymph nodes from a wide range of body sites and in spleen and tonsil. Thus it would seem logical that if a TSE risk were perceived for the intestine, then lymph nodes also would present a risk. Lymph nodes are present in some cuts of bone-in meat. The highest risk part of the intestine is presumed to be the ileum since it is the part with a consistently high level of GALT and usually has (scrapie) infectivity if other lymphoreticular tissues are infected.

In regard to natural casings, as distinct from intestine, if the presumed infected lymphatic tissue is removed before sale to the public, the TSE risk in the lymphatic tissue would be removed along with it, disregarding for the moment risks from cross-contamination. Partial removal would result in a risk reduction, though not elimination of infectivity in the GALT. Even if GALT were completely removed any infectivity in Meissner's plexus would remain as this is within the sub-mucosa that forms the casing. It therefore becomes important to determine:

If infectivity (as distinct from PrP) is present in the sub-mucosal nerve plexus

How much this contributes to the infectivity of the intestine as a whole.

It is noted that random-bred, female Swiss mice were used for the original bioassays of scrapie infectivity, which are likely to underestimate the real infectivity by some unknown factor because of the species barrier between sheep and mice. Thus the 'real' titres determined by i/c inoculation of sheep of the same susceptible *PrP* genotype may be higher than those reported. Therefore, any estimate of the reduction in risk by processing any material from infected sheep, including natural casings, might be

correspondingly larger than currently envisaged (for example, 80% reduction of 6 logs of infectivity is more efficient than 80% reduction of 2 logs of infectivity).

Parts of the intestine in which TSE infectivity may reside

Collectively, research studies show that tissue exists in the intestine of sheep that is able to harbour, and possibly replicate, TSE agents including BSE. These studies show that tissue exists in the intestine of sheep that is able to harbour/replicate TSE, including BSE, infectivity. These tissues are GALT, nerve cells and glia within the two nerve plexuses of the gut. In regard to GALT, FDC probably contribute the highest amount of PrP within the Peyer's patch. Intestinal dendritic cells and tingible body macrophages (both of which are mobile cells) and M cells probably contribute less.

The age of source animals and age at which intestine is infected

Casings are estimated to be collected from about 85% of slaughtered sheep. The age range might be estimated to be as follows in the UK: < 6 months 8.6 millions, (only in this group could infectivity (if present) be assumed to be at a low titre or absent) 6-12 months 5.5 millions and > 12 months, 1.9 millions.

If sheep were infected with BSE *via* feed in most instances this exposure is more likely to occur later (e.g. after weaning) than if infection came from other sheep (including the dam) or the environment when exposure would likely be higher immediately following birth than later. Nevertheless, evidence from research in experimentally challenged sheep shows that prion protein can be detected in the Peyer's patches of the intestine in some *PrP* genotypes of sheep at a relatively young age (5 months) but not in the enteric nervous system until 10 months. Guarantees cannot be given of freedom from infectivity by age.

Part of the small intestine used for natural casings

For some years, the European natural casings industry has been advised to, and in practice does, remove the whole of the ileum and a short part of terminal jejunum before preparing the intestine for processing into casings. This is a HACCP procedure in the European industry.

Risks from cross contamination in the abattoir

Currently electrical stunning, which is the most common method used for stunning small ruminants, is regarded as presenting a negligible risk of *embolic spread of brain tissue* to the blood stream. In some abattoirs (particularly those with a low throughput), may stun sheep by methods that penetrate the skull and damage the brain. A cartridge operated captive bolt pistol can cause brain emboli to enter the venous system in sheep and is still

permitted in the EU but more research has been advised to confirm the observation. Other risk methods of stunning food animals are banned in the EU.

A wide range of tissues (including the current SRM) could carry BSE infectivity and in theory might be a *source of infection for cross-contamination of intestine*. However, in practice these theoretical risks can be eliminated by careful application of meat hygiene rules.

The risks linked to *meat-and-bone-meal or BSE-contaminated bovine fat feeding* can now be considered as negligible.

Factors to be taken into account when making an assessment of the risk to the consumer from natural casings and bone-in meat

The absolute amount of infectivity remaining in a prepared casing is the important criterion in determining the TSE-risk for the consumer. In this regard it is important to also take account of the dose of infectivity that a consumer might consume at one meal. Casings are only eaten as an envelope of sausages rather than as a commodity on its own. Casings therefore contribute a relatively small amount by weight to a meal of sausages. Thus the dose of infectivity that might be consumed (if residual infectivity was present) will be calculated as a product of the weight of the casing multiplied by the absolute residual infectivity titre per unit mass of the casing. This contrasts with the higher theoretical TSE-risk in bone-in meat from the same infected animal (because of its content of possibly infected lymph nodes, peripheral nerves and bone marrow).

The risk analysis

The BSE agent has not been isolated from any sheep or goat with a scrapie-like disease or indeed any sheep under natural conditions. At the present time (2003) the hazard is therefore a hypothetical one.

From all the above data and if BSE were to occur in sheep or goats the following can be stated:

<u>Source</u>

Intestines harvested for casing manufacture come only from animals passed fit for human consumption in a licensed abattoir.

Small intestines from sheep could harbour the BSE agent. In regard to age, no exclusion can be made but small intestines from animals under six months of age are likely to present, on average, a lower risk than intestines from older animals. The *PrP* genotype could have a greater bearing on the infectivity at different ages of exposed sheep. The

degree of infectivity in the intestine at any particular age is likely to be very similar to that in the spleen (SRM) an in lymph nodes (not SRM) and form part of the sheep sold to the public.

Within the intestine, the ileum (notably the distal ileum) is likely to have a clearly detectable level of infectivity. Infectivity in other parts cannot be excluded.

Process

In the EU, Switzerland and in some other countries no part of the large intestine or the whole ileum and short piece of terminal jejunum of inconsistent length is used for casing manufacture and trade. Any TSE risk in these tissues is removed. The risk of cross contamination of the remaining part of intestine that is used (duodenum and most of the jejunum) by SRM or other BSE-infected material is negligible provided the EC meat hygiene and other regulations are complied with and enforced.

The cleaning operation removes half of any infectivity present in the enteric nervous tissue. In addition, removal of the mucosa and Peyer's patches is efficient but could not be guaranteed to be complete in all parts of all casings. In some parts it will be perfectly removed and in others not. Overall on average over 80% of this tissue is removed and it could be almost 100%. There are no data on starting or finishing titres so it is not possible to be precise about the amount of infectivity removed but it is estimated to be at least 2 logs and possibly over 3 logs. It is noted that no risk reduction is achieved on consumer sales of bone-in meat like a leg of lamb from the same animal. Some structures in the leg could contain infectivity.

Use

Natural sheep and goat casings have only one use, namely as an outer envelope for sausages. No TSE risk reduction occurs during the filling process. When sausages are cooked the outer surface (the casing) reaches a temperature of about 175ºC for some minutes. This may have some inactivating effect on TSE agents present in the casing but it could not be guaranteed to sterilise the casing.

In regard to human consumption the contents of a sausage contributes by far the greatest mass to a sausage meal with the casing contributing only a few grams (< 4.0g).

Conclusion

A significant BSE risk-reduction is achieved by the EU and Swiss sheep and goat natural casings industry. This is secured by eliminating at source those parts of the intestine with the highest risk and by removal of most of the infectious material in the remainder. Both processes are easy to audit for enforcement purposes. It is not possible to remove all

infectivity but what remains is likely to be lower at the point of consumption in sausages enveloped in natural small ruminant casings than it would be from an equivalent weight of bone-in sheep meat form the same animal.

TSE risk reduction from the small intestine

This is an achievable objective but it is not possible to accurately quantify what the level of reduction would be, not least because no natural cases of BSE exist in small ruminants and because there is no knowledge of the titre of infectivity in any part of the intestine. Any estimates of the risk reduction are therefore speculative and subjective. Some guidance can be given from the starting titres in scrapie but there is no knowledge of the infectivity left in natural casings from scrapie-infected sheep. The best judgements can come from studies showing the intestinal tissues that are removed when intestines are processed into casings. These are as follows:

Complete removal of the whole ileum and part of the jejunum.

Complete removal of the serosa, outer longitudinal muscle, Auerbach's nerve plexus and inner circular muscle.

Removal of manure.

Removal of the mucous membrane (epithelium and lamina propria and its contents).

Research

There are a number of serious deficits in knowledge that do not allow a quantitative risk analysis to be undertaken.

There are no reported data on the titres of infectivity in the different parts of small ruminant intestines or in the casings made from them (or indeed any tissues) including in experimental BSE.

There is insufficient knowledge about the contribution made by GALT on the one hand and enteric nervous tissue on the other to the total infectivity.

No studies have been reported about the deposition of PrP in neurones of the autonomic nervous system in other body organs such as the heart for example, which are still allowed for human consumption.

There is also insufficient knowledge about the contribution that M cells, intestinal dendritic cells and tingible body macrophages (as distinct from FDC) make to the total infectivity in the intestine.

Following processing of an intestine to make a casing it is not known what is the reduction in FDC which is a critical point as these are the cells in the intestine known to contain prion protein in an infected animal.

There are insufficient data on the effect of stunning methods in small ruminants and the generation of brain emboli and whether or not they can enter into the systemic circulation.

There are no current data available to show that hand versus machine processing of small ruminant casings produces an equivalent result. This deficit is being corrected by an industry funded study at the University of Utrecht.

Conclusions

Natural casings prepared by the European and international natural casings industry are products that have had a TSE risk-reduction process applied to them.

It is impossible to completely remove TSE infectivity from a TSE-infected intestine during the processing to make a natural casing.

It is possible to reduce the intestinal infectivity considerably by removal of the ileum and by the normal methods of natural casing manufacture. Approximately 50% of any infectivity in the nervous tissue is likely to be removed by the latter process and an unquantifiable amount of the intestine in other cell types (e.g. of lymphatic tissues) in the rest of the casing. Nevertheless this amount is likely to be substantial, perhaps estimated overall to be in the region of > 80%. Some parts of the length of the casing may be completely decontaminated and others not.

Cooking may reduce infectivity still further also by an unquantifiable amount.

By contrast to natural casings, meat sold on the bone has no risk reduction process applied to it and heat applied to it during cooking would only secure the same temperature as achieved in the sausage casing in the superficial layers. TSE infectivity would exist in the carcass lymph nodes at levels comparable to those in intestines from the same age and genotype of animal. Infectivity might also exist in peripheral nerves and in bone marrow.

Collectively this report indicates that if there is a TSE risk in intestines the risk would be lower from natural casings than from the intestine.

In the EU, in 2002 if all the rules are enforced there should be a virtual absence of any infectivity in the gut contributed by feed. Risks from this source could be regarded as negligible.

There are reasons to be concerned that the continuing use of penetrative stunning of sheep might contaminate other tissues (with brain material) sold legally to the consumer in certain circumstances. These risks could be important if BSE occurred in sheep.

It is accepted that there are likely to be other ways to protect the consumer from exposure to the BSE agent in sheep which is not part of this report. For example, by permitting only tissues including bone-in meat and natural casings from certain ages of animal or from certain *PrP* genotypes into the human food chain.

At its meeting of 12-13 September 2002, the Scientific Steering Committee (SSC) examined the most recent data on the safety with regard of BSE, of sheep casings (including that presented in the above report) and concluded that there is no additional or new evidence justifying the possible inclusion of sheep casings in the list of specified risk materials.

Relevant SSC opinions (see annex II):**12, 15.**

THE SAFETY OF RUMINANT MILK

By R. Bradley and D. Heim

Introduction

Milk, in the context of this report, refers to the unadulterated and untreated product derived from cattle in conformity with current milk hygiene regulations. The risk of cross-contamination with TSE-infected material is virtually impossible.

Colostrum is the first milk derived from a cow after calving. This is not normally permitted in the human food supply.

Milk is a source material for preparing other food like cream and cheese, and starting materials like lactose and galactose that are used in medicinal and biological products.

Epidemiological studies

If colostrum and/or milk were infected then it would be most likely represented as a form of maternal transmission.

– In Human TSE: no mother to child transmissions of kuru, sporadic CJD or vCJD have been reported.

– Animal TSE

  The collective evidence points toward minimal involvement of any form of vertical/maternal transmission of BSE in cattle. Virtually all calves receive colostrum but only beef suckler calves generally receive milk from their dam so the widely differing incidences of BSE in dairy and beef herds does not support either colostrum or milk being a vehicle of transmission of BSE. No cases of BSE have been reported in the offspring of over 2000 cases of BSE outside the UK. The incidence of BSE in the offspring of confirmed cases of BSE in the UK is the same as the incidence in the epidemic as a whole. Data on the expected number of cases of BSE from feed exposure alone and the observed number of cases shows no excess in the latter in any year. No cow to calf disease transmissions has been reported in association with BSE infected beef suckler cows from any country though a specific study in Great Britain has only been able to follow a relatively small number of animals to conclusion. In a cohort study there is no evidence that BSE can occur in the absence of a feed-borne source of infection.

There is no firm epidemiological evidence to support the view that colostrum or milk carry infectivity in sheep and goats with scrapie.

In TME of farmed mink there are no indications for maternal transmission. Maternal transmission of Chronic Wasting Disease (CWD) may occur but if it does it is relatively uncommon and the mechanism is unknown.

Transmission studies

To date, no parent to offspring transmissions have been reported in any animal species (including primates) used in experimental studies.

– Human TSE

Milk from humans affected with kuru has been inoculated into chimpanzees by multiple parenteral routes but no disease has resulted in over 30 years.

In 1992 in Japan, colostrum from a 38-year-old pregnant woman with sporadic CJD was reported to be infected when injected i/c into mice. However, this original report is not supported following subsequent study.

– Animal TSE

Oral, intraperitoneal and intracerebral challenge of mice with mammary gland and pooled milk from cows with confirmed BSE resulted in no transmission. Milk pools from cows with confirmed BSE , collected at early, middle and late lactation, were inoculated or fed to susceptible mice. No neurological illness or TSE-like pathology resulted in any mouse.

No detectable infectivity has been found in the mammary gland of non-pregnant ewes with scrapie, or in the colostrum of high-risk ewes at parturition following i/c inoculation of susceptible mice. Similarly no infectivity was detected in the mammary gland or milk from three goats with clinical scrapie kept in contact with sheep from the same farm.

In a separate study no detectable infectivity was found in colostrum or milk from sheep or goats naturally affected with scrapie following i/c or sub-cutaneous, or oral challenge of mice, but experimental detail is lacking.

No detectable infectivity was found in the mammary gland of farmed mink with TME following bioassay by the i/c route in mink.

Critical comments of some of the above studies

The Scientific Steering Committee noted that, in the absence of any infectivity studies on certain tissues including milk and colostrum by i/c inoculation of the homologous species in bovines, ovines and caprines, and in the absence of all the necessary experimental and epidemiological data as detailed in the report, precise estimates of the TSE risks cannot be made.

Research

Currently there seem not to exist plans to inoculate bovine milk i/c into cattle. However, a new study is in progress that seeks to determine if prion protein can be detected in fractions of milk derived from cattle experimentally challenged orally with high (100g) or low (1g) amounts of brain from affected cattle. The earliest that interim experimental results could be available would be the spring of 2003.

In regard to sheep milk a small-scale study is presently running at the NPU (N Hunter, personal communication, March, 2001**)** with scrapie susceptible lambs removed from their mothers and hand reared. No results are yet reported.

Recent reports of the experimental transmission of scrapie and of BSE to susceptible genotypes of TSE-free sheep following i/v transfusion of about 400ml of blood (or buffy coat) from sheep incubating experimental BSE or natural scrapie raises concern that low levels of infectivity could exist in white cells that also form a component part of milk in this species. The experiment is not yet concluded.

Conclusions

The evidence available to date does not point at milk or colostrum representing a possible TSE risk.

However, the SSC supports the recommendation that for precautionary reasons the milk, colostrum or milk products from suspect BSE cases should not be offered for consumption.

Should BSE become probable or be found in small ruminants then a reassessment has to be undertaken.

Relevant SSC opinion (see annex II): **25.**

PART II D

QUANTITATIVE RISK ASSESSMENT

A FRAME FOR THE QUANTITATIVE ASSESSMENT OF THE RESIDUAL BSE RISK IN CATTLE-DERIVED FOOD PRODUCTS

By D. Dormont, M. Doherr and Ph.Verger

Whether a product is used as food/feed, a cosmetic product, or a medicinal product or medical device will determine the route and the length of possible exposure. The routes can be oral, intravenous, topical, and/or inhalatory and the nature of the product will also determine whether or not there may be a repeated exposure.

The human exposure risk will depend on the following main factors:

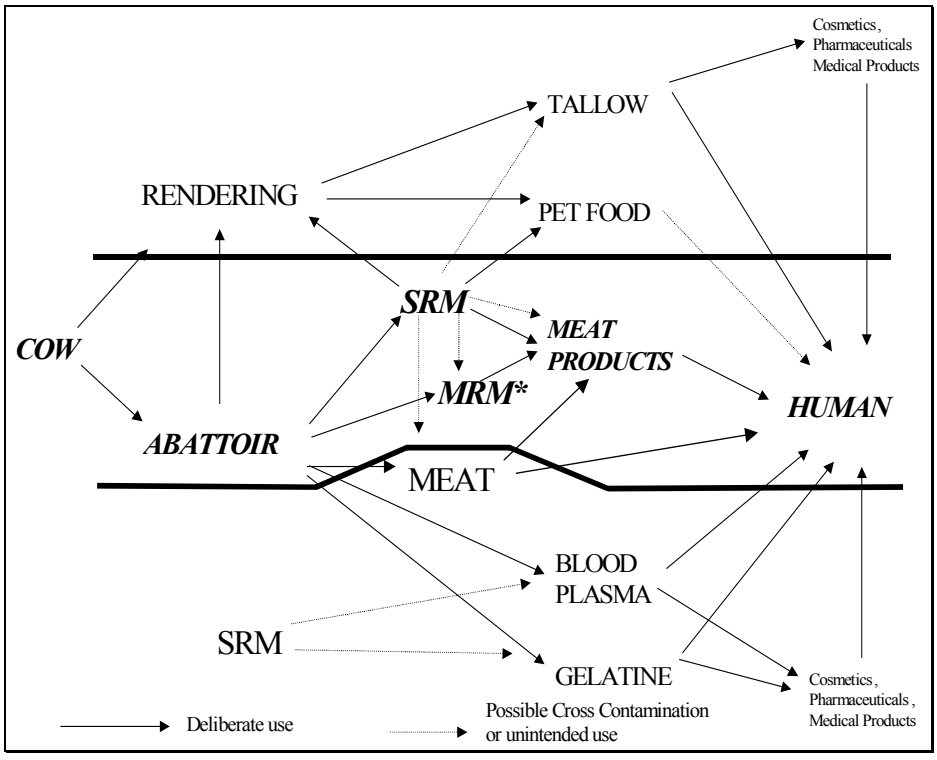
– the likelihood that an animal infected with BSE enters the human food chain;

– the amount and distribution of infectivity in that animal;

– the ways in which the various tissues that could contain infectivity are used in the food chain;

The possible routes of exposure of humans to BSE infectivity are multiple (**Figure**).

Figure: Potential Routes of Exposure to Infective Cattle Tissues



Deterministic and stochastic approaches towards BSE risk quantification

For the assessment of the residual BSE risk in ruminant-derived products entering the food chain, two approaches can basically be followed to quantify the residual BSE risk of animal derived products:

a. In the ***deterministic approach,*** a single value is attributed to each parameter in the assessment. This value corresponds to the most likely value this parameter (commonly) takes or is assumed to take.

   The deterministic modelling approach permits the risk manager to rapidly estimate (by linear extrapolation) the risk under alternative conditions (e.g. higher/lower tissue infectivity levels; larger/smaller batch sizes; complete/incomplete specified risk materials removed; etc.). However, the rigidity of the deterministic approach may result in unrealistic scenarios as, for example, the likelihood is almost zero that all values, assumptions and scenarios combined will at once be "average", best" or worst".

b. In the ***probabilistic approach***, the above problem is at least partly avoided. The model is run many times and for each of the model runs, *combinations* of risk values for each parameter are selected at random.

   A major advantage of the probabilistic approach is that it helps to understand interactions between parameters whilst taking into account uncertainties and that therefore it is a most useful tool in decision-making. One should however be aware that the assumed probability distributions in reality reflect at once both the scientific uncertainties in certain areas[35] and the fact that certain field conditions follow a statistical distribution[36].

When using the results of quantitative BSE risk assessment it should be kept in mind that not only a range of values may have been attributed to a given parameter but also that other uncertainties still accompany the BSE problem. By its nature quantitative assessments of the residual risk in consumer products lead to numerical figures which are indicative of the extent of expected events and should be understood more like orders of magnitude ranges than exact predictions. In addition, the level of uncertainty increases as the number of scientific unknowns increase.

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35 For example: is the species barrier 1, 10, 100, 1000 or 10000? Are the minimal infective doses 1000, 100, 10 or 1 mg?

36 For example: the risk reduction during production will not always be identical for all plants and within a given factory, but is likely to be distributed around this value. In this respect, it should be noted that the

Summary overview of the input data needed for quantitative BSE risk assessments.

A comprehensive quantitative assessment of residual BSE risk posed by cattle derived products requires information for the following input variables:

1. The **processing risk**.

   The probability that an infective bovine is slaughtered for food (or the "Processing risk") is the most relevant parameter for the human exposure risk.

   It is the **prevalence** of BSE positive animals that become slaughtered for food and determines the probabilities that at least once per year a BSE+ animal is slaughtered but not detected and that a production batch contains material from an infected animal.

2. **The possible infectious load of the cattle by-products**

   The tissue infectivity distribution and typical tissue titres of the BSE agent will determine the infectious load of the animal materials used for the production of a food or derived product. Details are provided in the contribution from G. Wells and H.A. Kretzschmar on *Pathogenesis, tissue infectivity distribution and specified risk materials* **(Part II.A).**

   The age of the infected animal that is slaughtered and processed influences the infective load and its distribution between the tissues of the animal. On the basis of the available knowledge, it is possible to define three age categories of cattle, which have different potential levels of infectivity, mainly as a function of their age at slaughter. Depending on the category, the infectivity that could enter the food chain will differ, both in quantity and with regard to the specified risk tissues:

   Veal Calves (less than 1 year). The level of infectivity in the CNS tissues of these animals can be considered to be negligible. However, there may be infectivity in the intestines, in particular the ileum.

   Cattle older than 1 year, but less than 30 months. These animals could, if infected at birth, show some level of infectivity, though it would be very unlikely to be the same as in a clinical case of BSE. The CNS is not necessarily highly infective, even if the animal was infected very early in life.

   Cattle older than 30 months. If infected early in their life, these animals may show infectivity levels close to those of clinical BSE-cases, even if no clinical signs are

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TSE validation studies carried out by GME involved only processes that could be considered to apply generically to all of the GME member companies that produce gelatine.

apparent. It is clearly evident from the Swiss surveillance of fallen stock and the UK surveillance of cattle over 30 months i.e. those excluded from the food chain under the Over Thirty Months Scheme (OTMS), that apparently healthy but nonetheless infected animals do enter the human food chain in countries where BSE is prevalent in the cattle population. In this category of bovines, the level of infectivity will be high and the CNS is certain to be highly infective.

3. **The typical size of the batch of raw materials** entering the production chain

The **typical batch size** of the raw materials is an important parameter, because it determines the probability that a given batch is BSE+. The number of animals included in such a batch depends on the amount of materials obtained from each slaughtered bovine and the proportion of raw products from other sources than cattle bones (i.e. gelatine from cattle or pig hides).

Defining the batch size for risk assessments purposes depends upon whether the production process is continuous or in batches. For certain products (e.g. melted fats) one can assume that during the production process or during the storage afterwards, a thorough mixing takes place and that infectivity (if any) present in parts of the raw material entering a process is diluted over the end-batch as a whole. But for other processes the raw materials may remain in the production process as discrete amounts or are only partly mixed and the possible presence of residual infectivity (if any) may be limited to a given fraction of the end-batch. In the latter case, the dilution effect is lower and limited to the size of the discrete amounts of raw material that entered the production chain.

Infected animals per batch. As long as the BSE-cases remain geographically scattered, the number of exposed consumers would be proportional to the number of processed BSE-infected animals and the average exposure dose would remain rather constant. If the BSE-density is so high that more than one infective animal could enter a single batch of production, the number of consumers exposed would remain stable while the dose per exposed individual would increase proportional to the number of infected animals entering the batch.

4. **Processing conditions.** The effects of processing determine whether or not any of the risks present are reduced significantly.

Processing conditions influence the level of infectivity in the product. For example, certain production processes for gelatine reduce the infective load at least a 50,000-fold. However, normal cooking and industrial food processing are unlikely to significantly affect the level of infectivity.

5. Serving size or the **amount**[37] **consumed per intake**. Together with the batch size, the serving size influences the dose of exposure and the number of persons exposed. This value also determines the number of possibly infectious doses that may be included in a batch of raw materials.

6. The **modalities of use / application**. Whether a product is used as food/feed, a cosmetic product or a medicinal product or a medical device will determine **the route of possible infection** which can be oral, intravenous, topical, etc. They will also determine whether or not and over how long a period there may be **repeated exposure**.

7. The **condition and genotype of the user**. It may be assumed that certain population groups including for example children, elderly and immuno-compromised people, are more susceptible to possible infection than others. For TSEs, immuno-compromised people may be more susceptible because there is clear evidence of the involvement of the lymphoreticular system in the pathogenesis of **vCJD** (and other **TSEs**). With regard to the genotype of **vCJD**-affected individuals, all of those tested so far have been shown to express methionine homozygously at codon 129 of the **PrP** gene. Considering that this allelic frequency occurs in only around 38% of the human population, it has been listed as one of the risk-factors for developing **vCJD**. However, insufficient time has elapsed to know whether or not other codon 129 genotypes will be equally (or even more) susceptible but have longer incubation periods. Experimental data from animal studies on TSEs also show that genes other than **PrP** have an influence on susceptibility and incubation periods.

   In practice only items 1) through 5) can be taken into account and the route of exposure is assumed to be oral. For the inclusion of items 6) and 7) additional information may be needed which is currently unavailable. Moreover, they relate to fields of application for which special grade products are needed anyway (e.g. for the production of certain special grade gelatines).

Worst case assumptions, risk assessment and risk management.

A comment that is frequently made when it concerns the choice of scenarios for risk assessment is that they (almost always) have the tendency of being based exclusively on worst case scenarios and do not adequately reflect normal field practice. It needs, however, to be pointed out that risk assessment exercises are in the first instance intended to facilitate risk management decisions. For example, a risk scenario assuming that all

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37 The term “amount” is preferred above “dose”. (It is easy to determine the amount consumed but the dose requires a knowledge of titre too which is more difficult. In general the range of variation in amount is likely to be small whereas the variation in titre could be large in the worst scenario.)

good manufacturing practices and the legislation are correctly implemented will/may assure the producer and consumer that a product is safe provided these conditions are fulfilled, but may be of little help for the risk manager who has to propose risk reducing scenarios on the basis of "what if a certain condition is not fulfilled" scenarios.

Numerical combinations of the various worst case values (for example: dose + species barrier) for risk assessment purposes into one "multi-worst case figure" would eventually result in an unrealistic scenario. It is therefore recommended that *realistic* worst case scenarios are used to assess the human exposure risk.

Infectious dose and species barrier.

Quantitative assessment of the risks must take into account the size of the species barrier between cattle and humans, the infection source, the dose of agent, the estimation of the minimum infectious dose and the potential effects of cumulative exposure to low doses of infected materials, the route of exposure and its efficacy in establishing TSE agent infection, the pathogenesis of TSEs and the genetic susceptibility of humans. In this context the following should be mentioned:

The size of this cattle-to-humans species barrier is not known, neither is the dose response relationship for humans. A practical approach is therefore to present the level of exposure in terms of consumption of defined amounts of the BSE agent, measured in Cattle Oral Infective Doses (CoID) and to assume, as a worst cases scenario, the absence of a species barrier. (It needs to be emphasised that the $CoID_{50}$ is only an indicator and should not be confused with the Human Oral Infective Dose ($HoID_{50}$), which is not known.)

Population risk

Risks from exposure to amounts of infection below the minimum infectious dose cannot be determined in the current state of the scientific knowledge. Whether the dose/response relationship in the low dose range (for low levels of potential residual infectivity in products after appropriate processing and handling, i.e. after appropriate sourcing, removal of SRMs, processing, avoidance of cross-contamination, etc.) is linear, or follows for example a sub-linear dose-response relationship, or a Poisson distribution, does not immediately affect the outcome of the assessment as such (in terms of absolute numbers of people at risk), but may affect the perception of the risk in management terms. In the first case, a whole (sub-)population is theoretically exposed to a same, but low level of residual infectivity. In the second case, a major part of a population will not be exposed at all to any infectivity, because it is concentrated (aggregated) and localised in a smaller number of consumption units. However, the part of the population that is exposed, will more likely get infected because the infectivity level is relatively higher.

In terms of risk assessment, the risks resulting from (low) residual infectivity should at present - until further evidence is available - be calculated as if a population as a whole is exposed and assuming a linear dose-response curve down to the low dose range[38] This is a conservative assumption and would mean, *as an example*, that a product containing an evenly distributed residual infectivity of 10^{-3} ID50/g and given to each of 1 million individuals, may result in 500 individuals being infected[39]. The accumulative infectivity scenario has to be considered as valid for risk assessment of the effects of repeated sub-infectious doses, provided the interval of administration is not too long (see further).

Multiple exposure

The dose response relation is not known and little if anything is known about the (cumulative) risk resulting from a repetitive use - possibly during a prolonged period - of a product such as a food, feed, cosmetic, medicinal product or medical device, containing sub-lethal infectivity levels.

An accumulative infectivity scenario may also be valid, provided the interval of administration is not too long (probably less than about 2 to 3 days) and that the repeated doses are sufficiently high so that an infective dose is reached in steady state (the repeated individual doses must be higher than the capacity of an individual to inactivate the infectivity during the interval of administration. From laboratory results it appears that the clearance period is approximately 24-48 hours in rodent models; beyond that, macrophages are again capable to take up their clearance function. Further research in this area is needed.

In terms of risk assessment, repeated exposure would thus increase the risk both in absolute number of cases and the likelihood that exposure would result in effective infection.

Routes of exposure to specified risk materials in food

The routes of exposure to SRMs can be summarised as follows:

1. Consumption of Specified Risk Materials as such

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38 The dose response relation is not known. Whether the dose/response relationship in the low dose range (for low levels of potential residual infectivity in products after appropriate processing and handling, i.e., after appropriate sourcing, removal of SRMs, processing, avoidance of cross-contamination, etc.) is linear or follows for example a sublinear dose-response relationship, does not immediately affect the outcome of the assessment as such (in terms of absolute numbers of people at risk) but may affect the perception of the risk in management terms.(See report)

39 This is just an example; elements such as the weight of the inoculum and of the infectious material or the weight of the bovine material to which the individual was exposed to., are not taken into account.

SRMs are consumed as such. It is known that brain and spinal cord (amourette in French) were consumed in this way, as well as ileum and all the small intestine (andouillette in French) from young veal (< 6months). Even spleen and eyes might occasionally have been eaten. Trigeminal ganglia and dorsal root ganglia are not consumed as such, although there will be some direct consumption of DRG (and possibly spinal cord) from cuts of meats served on the bone and including part of the vertebral column (e.g. T-bone steak, rib of beef).

2. Consumption of Specified Risk Materials after transformation

SRM are transformed and integrated into food products in such a way that it is not detectable by the consumer. The inclusion of materials derived from SRM into food products may happen intentionally or by contamination.

Intentional inclusion of SRM. The use of brain or spinal cord in "paté" or sausages is an example of the intentional use of SRM. Other SRM may also be included into food products as direct ingredients.

Contamination of edible products with materials derived from SRM. Contamination is always possible if the inclusion of SRM is technically possible and does not create quality problems.

3. Estimation of the Exposure Level and of the number of persons exposed.

In order to estimate the expected number of people that would be exposed to an infected dose, several critical factors have to be considered. Some of them are related to the sources, others to the routes.

Relevant SSC opinions (see annex II): **3,5, 59, 60**.

ANNEX I: CHRONIC WASTING DISEASE
AND TISSUES THAT MIGHT CARRY A RISK FOR HUMAN FOOD AND ANIMAL FEED CHAINS

By J. Brugère-Picoux

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) or "prion disease" of certain species of native North American deer: mule deer (*Odocoileus hemionus)* and white-tailed deer (*Odocoileus virginianus)* and Rocky Mountain elk (*Cervus elaphus nelsoni*), occurring in captive, farmed and free living populations. This is the only TSE known to affect free-ranging wildlife species. Its reported occurrence other than in North America (NA) is confined to a single case in an elk imported into Korea from NA.

Historical perspective of occurrence

The clinical syndrome of wasting and death in **captive cervids** was first recognised in the late 1960's in mule deer, in wildlife facilities in Colorado and Wyoming, USA. Neuropathological studies showed that it was a spongiform encephalopathy. By 1982 the disease had also been described in elk in the same facilities. The first case of CWD in Canada was diagnosed in farmed elk in1996 and is thought to have been introduced with elk imported from the USA in the late 1980s.

CWD in **free-ranging cervids** in the USA was first diagnosed in an elk in 1981 and in a mule deer in 1984. Since 2001, surveillance of wildlife in Canada has detected 7 cases of CWD in wild deer.

Host range & transmissibility

Only three species of Cervidae are known to be naturally susceptible to CWD. Experimental transmission studies have not yet been able to show transmission of CWD from deer to cattle. Genetic studies show phylogenetic differences in PrP sequence between Cervidae, Bovidae and humans suggesting an appreciable species barrier for possible transmission of CWD to cattle and humans. Molecular studies and epidemiological observations support this apparent resistance of certain phylogenetic families.

Experimental studies to transmit CWD have been conducted, mostly by intra-cerebral (IC) inoculation, providing information on susceptibility by the most efficient means of interspecies transmission, but not on interspecies susceptibility by natural routes of transmission (e.g. oral exposure).

Epidemiology

There is considerable evidence that CWD is both infectious and contagious but specific details of natural transmission remain to be determined. Epidemiological data have shown that CWD is readily spread by lateral transmission in cervid populations. Indirect transmission via environmental contamination may also play a role in the natural dynamics of CWD and rapid increase in prevalence within captive herds suggests this form of transmission may be locally, quite efficient. There is less evidence for the existence of maternal transmission and alone it is unlikely to sustain epidemics of CWD. The occurrence of CWD is not explained by feed-borne exposure associated with rendered ruminant meat and bone meal (MBM) as has been shown for BSE.

Although hypotheses as to a possible origin of CWD have been suggested (e.g. scrapie), there is no evidence in support of any of the theoretical sources.

Pathogenesis

In CWD affected deer and elk there is a very wide tissue distribution of PrP^{CWD}, resembling that of scrapie and BSE agents in tissues in TSE-susceptible sheep and this is in contrast to the distribution pattern of agent in BSE of cattle. However, tissue distribution of PrP^{CWD} is not identical for deer and elk. In the latter species detectable levels in peripheral tissues are apparent later in the incubation period. This widespread peripheral distribution of PrP^{CWD} early in the incubation period (at least in deer) presents significant, if not insurmountable, difficulty with respect to the potential for the removal of specified risk materials (SRM) in CWD. The significant involvement of the alimentary tract suggests a potential for progressive shedding of PrP^{CWD} and the agent through the disease course.

Diagnosis

Clinical signs of CWD in deer and elk are not specific but consistently include progressive weight loss. Behavioural changes include decreased interactions with other animals, listlessness, lowering of the head, drooping ears, blank facial expression and repetitive walking in set patterns. In elk, behavioural changes may also include hyper-excitability, nervousness, ataxia and head banging. Free ranging CWD affected elk may loose fear of humans. In deer and elk polydipsia and polyuria also commonly occur. The clinical disease is progressive and always fatal. Caretakers familiar with individual animals often recognise subtle changes in behaviour well before serious weight loss occurs. The clinical course of CWD varies from a few days to approximately a year, with most affected animals surviving from a few weeks to three or four months.

The **incubation period** range in naturally occurring CWD is not known. Evidence of pre-clinical infection has been found in deer fawns and elk calves from about 6 months of

age. The youngest naturally infected mule deer diagnosed with clinical disease suggests 16 to 17 months as an approximate minimum incubation, whereas the earliest diagnosis of CWD in elk was in a 24 months old animal.

Differential diagnosis include mineral deficiencies leading to neurological signs listeriosis, copper deficiency, etc. and disorders leading to loss of body weight e.g. fading elk syndrome. Aspiration pneumonia, presumably caused by ptyalism and difficulty in swallowing, may lead to misdiagnosis of the condition if histological and/or immunohistochemical examinations of nervous or/and lymphoid tissues are not carried out.

The **post-mortem diagnostic examination** often reveals gross lesions of emaciation, reflecting the clinical signs. Aspiration pneumonia, which may be the actual cause of death, is also a common post-mortem finding in animals affected with CWD. On **microscopic examination** of clinically affected animals, spongiform lesions are observed in the central nervous system. The earliest detectable lesions in the brain, in the parasympathetic vagal nucleus in the medulla oblongata at the obex, suggests that this is the most important site to examine for surveillance of clinically normal animals. Immunohistochemical (IHC) detection of the disease specific protein marker PrP^{CWD} is used to test brain tissue. PrP^{CWD} also accumulates in tonsillar and other lymphoid tissues at an early stage of the pre-clinical disease (in deer but not in elk). Tonsillar biopsies may be used in pre-clinical diagnosis for detecting CWD in live deer.

Surveillance

In both USA and Canada surveillance programmes include passive and active surveillance strategies. Target animals include those showing clinical signs, particularly, emaciation, discovered by, or reported to, wildlife agencies and those obtained from surveys of normal deer and elk killed by hunters or agency personnel, or road kills.

Surveillance of **free-ranging cervids** for CWD in USA has been ongoing in Colorado and Wyoming since the late 1970s and has since been extended to additional states. CWD has been diagnosed in free-ranging mule deer in Wyoming, Colorado, Nebraska, South Dakota and New Mexico and in free-ranging elk in Wyoming, Colorado, and South Dakota. It has been found in free-ranging white-tailed deer in Wyoming, Colorado, South Dakota, Nebraska, Wisconsin, and Illinois. In Canada, surveillance for CWD since April 2001 has found CWD in wild deer in Saskatchewan.

Surveillance in USA for CWD in **farmed Cervidae** began in 1996 and CWD positive herds have been diagnosed in South Dakota, Nebraska, Colorado, Oklahoma, Kansas, Montana and Minnesota. Also, CWD has been diagnosed in farmed white-tailed deer in Wisconsin. In Canada, surveillance of farmed cervids for CWD since 1997 has detected CWD-infected farms in Saskatchewan (elk) and in Alberta (elk and white tailed deer).

Control strategies

Control measures include prevention of introduction, notification of the disease, control or ban on movements, quarantine, screening/testing, eradication of affected herds, cleansing and disinfection of farms before re-population, compensation and measures to prevent/stop spread from free range to farmed cervids(or vice versa). Because of the commercial aspect of game ranching, animals have frequently been moved across the US and Canada and there is also natural movement of deer and elk across state lines. However, recently, laws have been made to prevent the movement of captive animals across state lines. Surveillance data do not as yet provide information on accurate figures of the prevalence of the disease in NA and the risk factors are not well understood. Some control measures for farmed deer are in place. However, movement of free-ranging deer provides a major difficulty for control strategies.

Following screening, herd certification may be an option. However, given limited knowledge on the incubation of the disease and its variation in clinical presentation it is likely to take a period as long as 5 years of surveillance of all juvenile and adult mortality before a farmed herd may be certified free of CWD.

There has been significant **economic impact** on the NA farmed cervid industry but the total effect is difficult to quantify. A high cost has been involved in compensation of Canadian farmers after eradication on CWD positive farms in addition to the cost of quarantine of farm and grassland following CWD. In Canada, elk are raised for the production of antler velvet and meat and for trophy/hunting and about 70% of velvet antler was formerly exported to South Korea. Some trading partners closed their markets to Canadian cervids and cervid products, including semen, embryos and velvet.

Surveillance for TSEs in cervids in Europe

There is no published information on the possible occurrence for TSEs in cervid species on the European continent available. Research and surveillance programs on CWD in farmed or wild Cervidae have not existed until recently and therefore there are no data on which to draw conclusions about CWD in the Cervidae populations of Europe.

Food and feed safety and human and animal risk

Although CWD is not similar to BSE in terms of epidemiology, in that there is no evidence of natural spread of the disease to phylogenetic families other than the Cervidae, it still may pose a theoretical risk for animal and human food safety.

Food safety and human health

There is no evidence that CWD can be transmitted to humans consuming meat or handling infected cervids or their products, however this possibility cannot be ruled out.

Phylogenetic differences in PrP sequence suggest an appreciable species barrier for possible transmission of CWD to humans. However, since the basis of the transmission barrier in relation to the TSE is complex and not solely a function of PrP sequence of donor and recipient it remains theoretically possible that the CWD-agent could infect humans.

The World Health Organisation recommends that people not consume animal products from any animal infected with a TSE disease and public health policies in Canada and the US are consistent with this direction.

In NA health officials advise hunters not to consume meat from animals known to be infected with CWD. In addition, they suggest hunters take simple precautions when field dressing deer or elk taken in areas where the disease is found.

Feed safety and animal health

The FDA has recently provided new guidance to state public health and agriculture officials throughout the US in which is stated that material from CWD positive animals or animals at high risk for CWD is not permitted to be used as an ingredient in feed for any animal.

In Canada there are no mandatory controls on rendering carcasses and offal from cervids other than those tested positive for CWD or animals that have been exposed to test positive animals. However, the Canadian Renderers' Association has a voluntary ban on the rendering of cervids. Canada prohibits the feeding of ruminant derived proteins to ruminants.

Risk of spread to Europe

Available information indicates that there is negligible trade in live cervids originating in NA to the EU but there are indications of imports of small annual tonnage of edible products from game. Some licences for exports to EU-countries were granted to private persons for hunter-related trophies (skin, antlers). Data provided by Eurostat confirm data provided by the USA and Canada. It is unclear what, if any, trade exists in antler, embryos or semen from cervids between NA and EU countries.

Current surveillance activities in Europe should be encouraged in order to provide more detailed base line data as the current studies are assessed as insufficient to detect a CWD infection in cervids should it be present.

Conclusions

A theoretical risk for prion transmission to humans consuming products of CWD affected-cervids of all ages in countries where CWD exists cannot be excluded. Similarly

also, such transmission risk to domestic animals cannot be excluded. There is therefore a scientific basis on which to exclude tissues from animals that carry a CWD risk, from human or animal feed chains.

However, the early and widespread involvement of tissues in CWD infected animals practically prevents exclusion of any tissues in defining a SRM list, or setting any lower age cut-off as has been defined for cattle in relation to BSE.

Available information indicates that imports of live cervidae from NA to EU and trade in meat products from cervid species are negligible, but it is important to ensure that no transfer of risk takes place through trade of live cervids or derived products.

At present, there are no scientific data that CWD is occurring in Cervidae elsewhere than in those countries from which it has been previously reported. However, systematic TSE surveillance of cervid populations has either been absent or has only just started in European countries. Until results of such surveillance become available no conclusion can be drawn with regard to the occurrence of CWD or similar TSE in the cervid population of Europe.

Recommendations

Given that the possible risks of exposure relate to the tissues of cervids from NA, reinforced protection of the cervid population and animal and public health in Europe could be considered.

Moreover, systematic surveillance is essential to establish the probability of occurrence and incidence of CWD in the cervid populations of Europe. Because of the complexity of conducting such surveillance on a statistical basis throughout the EU, initial research should address the susceptibility of European cervid species to TSEs. Furthermore, a surveillance programme, which might initially target the examination of cervids dying in or culled from zoological collections and fallen stock in farmed cervid populations, prior to decisions on the screening of free-ranging cervids, is recommended.

Relevant SSC opinions (see annex II): **39.**

ANNEX II

LIST OF OPINIONS AND REPORTS RELATED TO TSE/BSE, ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE BETWEEN NOVEMBER 1997 AND APRIL 2003.

Web Address: http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html

General aspects

1. **Opinion on** Organophosphate (OP) poisoning **and hypothetical involvement in the origin of BSE. Adopted on 10-11 April 2003.**
2. Opinion on hypotheses on the **origin and transmission** of BSE. Adopted on 29-30 November 2001.
3. Opinion on **oral exposure** of humans to the BSE agent: infective dose and species barrier. Adopted on 13-14 April 2000.
4. Preliminary opinion on **oral exposure** of humans to the BSE agent: Infective dose and species barrier. Adopted on 2-3 March 2000.
5. Opinion on the **Human Exposure Risk (HER)** via food with respect to BSE. Adopted on 10 December 1999.
6. Opinion on the possible **vertical transmission** of Bovine spongiform encephalopathy (BSE). Adopted on18-19 March 1999.
7. Report on the possible **vertical transmission** of Bovine Spongiform Encephalopathy (BSE) - Submitted on 18-19 march 1999.
8. Opinion on possible links between BSE and **Organophosphates** used as pesticides against ecto- and endoparasites in cattle. Report and opinion adopted on 25-26 June 1998.
9. Opinions adopted on **19-20 February 1998**.

Tissue infectivity distribution and Specified Risk Materials

10. Opinion on BSE risk of the **bovine autonomic nervous system. A**dopted by the on 6-7 March 2003).
11. Update of the Opinion on TSE **Infectivity distribution in ruminant tissues.** Initially adopted on 10-11 January 2002 and amended on 7-8 November 2002.
12. Complement to the SSC opinion of 4-5 April 2002 on **safe sourcing of small ruminant materials** (with special reference to the safety with regard to BSE risks of sheep intestines and casings). Adopted on 12-13 September 2002.
13. Opinion on the implications of the recent papers on **transmission of BSE by blood** transfusion in sheep. Adopted on 12-13 September 2002.

14. Amendment on opinion on the safety of **bovine embryos**: Amendment to opinion of 18-19 march 1999 on the possible vertical transmission of Bovine Spongiform Encephalopathy (BSE). Adopted on 16 may 2002.
15. Report on the safety of **sheep intestine and natural casings** derived therefrom in regard to risks from animal TSE and BSE in particular. Report prepared for 7 may 2002.
16. Opinion and report assessment of the human BSE risk posed by **bovine vertebral column** including dorsal root ganglia. Adopted on 16 may 2002.
17. Opinion on the safety of **animal rennet** in regard to risks from animal TSE and BSE in particular. Adopted on 16 may 2002.
18. Opinion on the safety of **calf-derived rennet.** Adopted on 04-05 April 2002.
19. Statement on prions in **muscle.** Adopted on 04-05 April 2002.
20. Opinion on stunning methods and BSE risks (the risk of dissemination of **brain particles into the blood** and carcass when applying certain stunning methods). Adopted on 10-11 January 2002.
21. Report on stunning methods and BSE risks (the risk of dissemination of **brain particles into the blood** and carcass when applying certain stunning methods). Prepared for 13 December 2001.
22. Opinion on TSE **infectivity distribution in ruminant tissues** (state of knowledge, December 2001). Adopted on 10-11 January 2002.
23. Preliminary opinion and report on stunning methods and BSE risks (the risk of dissemination of **brain particles into the blood** and carcass when applying certain stunning methods). Adopted on 6-7 September 2001.
24. Opinion on adipose tissue associated with the **digestive tract** of cattle, sheep and goats: an appreciation of possible TSE risks. Adopted on 28-29 June 2001.
25. Safety of **milk** with regard to TSE: State of affairs. Adopted on 28-29 June 2001.
26. Opinion on the Implications of the Houston *et al* paper in The Lancet of 16 September 2000 on the **Transmission of BSE by blood transfusion in sheep**. (The Lancet, Vol. 356, pp 999-1000; 955-956; 1013). Adopted on 26-27 October 2000.
27. Opinion on **specified risk materials of small ruminants** (Follow-up to the SSC opinion of 24-25 September 1998 on the Risk of Infection of Sheep and Goats with BSE agent). Adopted on 13-14 April 2000.
28. Opinion on the safety of **ruminant blood** with respect to TSE risks. Adopted on13-14 April 2000.
29. Listing of **Specified Risk Materials**: a scheme for assessing relative risks to man - Opinion of the Scientific Steering Committee adopted on 9 December 1997. Re-edited version adopted on 22-23 January 1998.

BSE in sheep

30. Opinion on **safe sourcing** of small ruminant materials. Adopted on 04-05 April 2002.
31. Suggested strategy to **investigate the presence of BSE in small ruminants.** Adopted on 04-05 April 2002.
32. Opinion on the safety of small ruminant products should **BSE in small ruminants** become probable / confirmed. Adopted on 18-19 October 2001.
33. Opinion on **pre-emptive risk assessment** should BSE in small ruminants be found under domestic conditions. Adopted by the Scientific Steering Committee at its meeting of 8-9 February 2001.
34. Report and Opinion on the Criteria for **diagnosis** of clinical and pre-clinical TSE disease in sheep and for differential biochemical diagnosis of TSE agent strains. Adopted on 13-14 April 2000.
35. The policy of **breeding and genotyping** of sheep, i.e. The issue whether sheep should be bred to be resistant to scrapie. Adopted on 22-23 July 1999.
36. **Surveillance** of TSEs in sheep and goat in relation to the risk of infection with bovine spongiform encephalopathy agent and related actions to be taken at EU level. Actions to be taken on the basis of (1) the September 1998 SSC Opinion on the Risk of infection of sheep and goats with the BSE agent and (2) the April 1999 SEAC Subgroup report on Research and surveillance for TSEs in sheep. Adopted on 27-28 May 1999.
37. Statement on the SEAC Subgroup report on **Research and surveillance** for TSEs in sheep, released in April 1999. Adopted on 22-23 April 1999.
38. Opinion on **the risk of infection** of sheep and goats with Bovine Spongiform Encephalopathy agent. Adopted on 24-25 September 1998.

TSEs and BSE in food animal species other than ruminants

39. Opinion on **Chronic Wasting Disease** and tissues that might carry a risk for human and animal feed chains. Adopted on 6 – 7 March 2003.
40. Report on **Chronic Wasting Disease** and tissues that might carry a risk for human food and animal feed chains. Adopted on 6-7 March 2003.
41. Opinion on the feeding of wild **fishmeal** to farmed fish and recycling of fish with regard to the risk of TSE. Adopted by the Scientific Steering Committee at its meeting of 6-7 March 2003.
42. Opinion on the potential requirement for designation of specified risk materials in **pigs.** Adopted on 6-7 March 2003.
43. Opinion on **necrophagous birds** as possible transmitters of TSE/BSE. Adopted on 7-8 November 2002.

Safety of products

44. Opinion on the safety of **tallow derivatives** from cattle tallow. Adopted on10-11 April 2003.
45. Updated opinion and report on the safety of **dicalcium phosphate (DCP) and tricalcium phosphate (TCP)** from bovine bones, used as an animal feed additive or as fertiliser. Submitted on 6-7 March 2003.
46. Updated Opinion on the safety with regard to TSE risks of **gelatine** derived from ruminant bones or hides. Adopted on 6-7 March 2003.
47. Updated opinion on the safety with regard to TSE risks of **gelatine** derived from ruminant bones or hides. Adopted on 5-6 December 2002.
48. Updated opinion on the safety with regard to TSE risks of **gelatine** derived from ruminant bones or hides. Adopted on 12-13 September 2002.
49. Opinion on **peptides from pig mucosa**: risks with respect to TSEs. Adopted on 21-22 February 2002.
50. Updated opinion on the safety with regard to TSE risks of **gelatine** derived from ruminant bones or hides from cattle, sheep or goats. Adopted on 28-29 June 2001, editorial changes adopted on 6-7 September 2001.
51. Updated opinion on the safety with regard to TSE risks of **gelatine** derived from ruminant bones or hides from cattle, sheep or goats (Including amendments to the scientific report attached to the opinion of 21 January 2000). Adopted on 28-29 June 2001.
52. Revised opinion and report on: The safety of **tallow** obtained from ruminant slaughter by-products. Adopted on 28-29 June 2001.
53. Opinion and report on safety with respect to the TSE risks of **collagen** produced from ruminants hides. Adopted on 10-11 May 2001.
54. Opinion on the safety of **organic fertilisers** derived from ruminants animals. Adopted on 10-11 May 2001.
55. Opinion and report on the safety of **dicalcium phosphate** precipitated from ruminant bones and used as an animal feed additive – Update adopted 27-27 October 2000.
56. Statement of the Scientific Steering Committee on its Report and Scientific Opinion on mammalian derived **meat and bone meal** forming a cross-contaminant of animal feedstuffs. Adopted on 26-27 October 2000.
57. Considerations on the safety of **amino acids from human hair hydrolysate** used in cosmetic products for topical application, with regard to Transmissible Spongiform Encephalopathy risks. Adopted on 25-26 May 2000.
58. Updated Report and Scientific Opinion on the safety of **hydrolysed proteins** produced from bovine hides. Adopted on 25-26 May 2000.

59. Preliminary report on **Quantitative Risk Assessment** on the Use of the Vertebral Column for the production of Gelatine and Tallow. Submitted on13-14 April 2000.

60. Opinion on **Quantitative Risk Assessment** on the Use of the Vertebral Column for the production of Gelatine and Tallow. Adopted on 13-14 April 2000.

61. Scientific report and opinion on the safety of **gelatine** - Updated on 20-21 January 2000.

62. Report and opinion on the evaluation of the "133°/20'/3 bars heat/pressure conditions" for the production of **gelatine** regarding its equivalency with commonly used industrial gelatine production processes in terms of its capacity of inactivating/eliminating possible TSE infectivity in the raw material. Adopted on 21-22 January 1999.

63. Report and Opinion on the safety of **gelatine**. Adopted on 21-22 January 1999.

64. Evaluation of the *ELLCO-FOOD* process for the production of **gelatine** regarding its equivalency with commonly used industrial gelatine production processes in terms of its capacity of inactivating/eliminating possible TSE infectivity in the raw material. Report adopted on 22-23 October 1998 and updated on 10-11 December 1998.

65. Report and Scientific Opinion on the safety of **hydrolysed proteins** produced from bovine hides. Adopted on 22-23 October 1998.

66. Scientific Opinion on the safety of **organic fertilisers** derived from mammalian animals. Adopted on 24-25 September 1998.

67. Report and Scientific Opinion on mammalian derived **meat and bone meal** forming a cross-contaminant of animal feedstuffs. Adopted on 24-25 September 1998.

68. Updated scientific report on the safety of **meat-and-bone meal** derived from mammalian animals fed to non-ruminant food producing farm animals. Submitted on 24-25 September 1998.

69. Report and opinion on the safety of **dicalcium phosphate** precipitated from ruminant bones and used as an animal feed additive. Adopted on 25-26 June 1998.

70. Opinion on the safety of **tallow** derived from ruminant tissues. Adopted on 26-27 March 1998.

71. Opinion on the safety of **meat and bone meal** from mammalian animals, naturally or experimentally susceptible to Transmissible Spongiform Encephalopathies. Amended version adopted on 26-27 March 1998 (and updated on 3 April 1998).

72. Opinion on the Safety of **Gelatine.** Amended version adopted on 26-27 March 1998 (and updated on 3 April 1998).

73. Preliminary Opinion on the safety of **gelatine**. Adopted on 19-20 February 1998.

74. Preliminary Opinion on the safety of **tallow**. Adopted on 19-20 February 1998.

75. Preliminary Opinion on the safety of **meat-and-bone meal**. Adopted on 19-20 February 1998.

Risk reduction strategies

76. Opinion on the additional safeguard provided by different **culling schemes** under the current conditions in the UK and DE. Adopted on 11 January 2002.
77. Report on the additional safeguard provided by different **culling schemes** under the current conditions in the United Kingdom and Germany. Prepared on 10-11 January 2002.
78. Updated opinion on **sourcing of ruminant materials from GBR I** countries for medical devices. Adopted on 29-30 November 2001.
79. Opinion on **Sourcing of from GBR I Countries** (Sourcing of Ruminant Materials from GBR I Countries for Medical Devices). Adopted on 6-7 September 2001.
80. Opinion on the scientific basis for **import bans** proposed by Austria with regard to BSE risks in Germany and France. Adopted on 29-30 March 2001.
81. Opinion on the questions submitted by EC services following a request of 4 December 2000 by the **EU Council of Agricultural Ministers** regarding the safety with regard to BSE of certain bovine tissues and certain animal-derived products. Adopted on 12 January 2001.
82. Opinion of the Scientific Steering Committee (1) on the scientific basis for **import bans** proposed by 3 Member States with regard to BSE risks in France and the Republic of Ireland; (2) on the scientific basis for several measures proposed by France with regard to BSE risks; (3) and on the scientific basis for banning animal protein from the feed for all farmed animals, including pig, poultry, fish and pet animals. Adopted on 27-28 November 2000.
83. Opinion on BSE-related **culling in Cattle.** Adopted on 14/15 September 2000.
84. Scientific Opinion on the conditions related to **"BSE Negligible risk (closed) bovine herds"**. Adopted on 22-23 July 1999.

Surveillance and rapid tests

85. Opinion on the **field trial evaluation** of two new rapid BSE post mortem tests Results achieved using the LIA Test (Prionics) and the aCDI Test (InPro) in the field trial. Adopted on 6 March 2003.
86. Opinion on a programme for the **evaluation of rapid post mortem tests** to detect TSE in small ruminants. Adopted on 7-8 November 2002.
87. Opinion on design of a **field trial** for the evaluation of new rapid BSE post mortem tests. Adopted on 22 February 2002.
88. Opinion on requirements for **statistically authoritative BSE/TSE surveys.** Adopted on 29-30 November 2001.

Recycling of animal by-products and animal waste

89. Opinion on "The proposal for controlled use of ruminant **SRMs as feed for fur animals** in Finland". Adopted on 27 October 2000.

90. Opinion on the risk born by **recycling animal by-products as feed** with regard to propagating TSE in non-ruminant farmed animals. Adopted on 17 September 1999.

91. Report on the risk born by **recycling animal by-products as feed** with regard to propagating TSE in non-ruminant farmed animals. Adopted on 16-17 September 1999.

Disposal of animal by-products and animal waste

92. Opinion on **six alternative methods for safe disposal** of animal by-products. Adopted on 10-11 April 2003.

93. Opinion and report on a treatment of animal waste by means of high temperature (150°C, 3 Hours) and high pressure **alkaline hydrolysis**. Adopted on 10-11 April 2003.

94. A framework for the **assessment of the risk** from different options for the safe disposal or use of animal by-products which might be contaminated with microbiological agents including TSE. Adopted on 10-11 April 2003.

95. Opinion on the use of **small incinerators** for BSE risk reduction. Adopted on 16-17 January 2003.

96. Opinion on **open burning** of potentially TSE-infected animal materials. Adopted on 16-17 January 2003.

97. Opinion on the use of **burial** for dealing with animal carcasses and other animal materials that might contain BSE/TSE. Adopted by the Scientific Steering Committee Meeting of 16-17 January 2003.

98. Updated opinion and report on a treatment of animal waste by means of high temperature (150°c, 3 hours) and high pressure **alkaline hydrolysis.** Adopted on 16 may 2002 and revised on 7-8 November 2002.

99. Opinion and report on the treatment of animal waste by means of high temperature (150°c, 3 hours) and corresponding high pressure **alkaline hydrolysis.** Adopted on 16 may 2002.

100. A framework for the **assessment of the risk** from different options for the safe disposal or use of meat and bone meal (MBM) and other products which might be contaminated with TSE's and other materials. Adopted on 28-29 June 2001.

101. Note on the safe **handling, transport and temporary storage of meat-and-bone meal** which may be contaminated with a BSE agent or other pathogens. Adopted on 26-27 October 2000.

102. Preliminary and incomplete notes on the **safe handling, transport and storage of MBM** and other bovine derived materials which may be contaminated with the BSE agent or other pathogens - Comments compiled on 25-26 May 2000.

103. Opinion on the **risks of non conventional transmissible agents**, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, rats, laboratory animals and fish) or via condemned materials. Adopted by the Scientific Steering Committee at its meeting of 24-25 June 1999.

104. Report on the **risks of non conventional Transmissible agents** conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals (including also: ruminants, pigs, poultry, fish, wild/exotic/zoo animals, fur animals, cats, laboratory animals and fish) or via condemned materials. Submitted on 24-25 June 1999 (Containing updates, 13 July 1999).

BSE epidemic in the United Kingdom

105. Opinion and report on BSE in United Kingdom's **cattle born after 31 July 1996** [BARBs]. Adopted on 10-11 April 2003.

106. Opinion on the six **BARB BSE cases in the UK since 1 August 1996** (the six BARB BSE cases born and confirmed in the UK after 1 August 1996: Is there a need to review the opinions of the Scientific Steering Committee with regard to the UK date-based export scheme and other TSE-related risks?). Adopted on 29-30 November 2001.

107. Opinion on Bovine Spongiform Encephalopathy in a second UK animal **born after 1 August 1996** (Case confirmed in Northern Ireland). Adopted on 29-30 March 2001.

108. Opinion on monitoring some important aspects of **the evolution of the Epidemic of BSE** in Great-Britain. Update providing an epidemiological commentary on BSE projections for Great Britain (GB) and on surveillance, as well as on the occurrence of "Born After the Real Ban - BARB" cases. Adopted on 7-8 December 2000.

109. Report on monitoring Some Important aspects of **the evolution of the Epidemic of BSE** in Great-Britain, Update providing an epidemiological commentary on BSE projections for Great Britain (GB) and on surveillance, as well as on the occurrence of "Born After the Real Ban - BARB" cases. Submitted on7-8 December 2000.

110. Report and Scientific Opinion on **export from the UK of bone-in veal**. Adopted on 14-15 September 2000.

111. Opinion on the UK decision to **lift the ban on the consumption of meat on the bone.** Adopted on 13-14 April 2000.

112. Opinion on the Scientific Grounds of the Advice of 30 September 1999 of the **French Food Safety Agency** (the Agence Française de Sécurité Sanitaire des Aliments, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom. Adopted on 28-29 October 1999 (edited following a written procedure (30.10 - 15.11.99) and re-edited on 9-10 December 1999.

113. Summary report based on the meetings of 14 and 25 October 1999 of the TSE/BSE *ad hoc* group of the Scientific Steering Committee on the Scientific Grounds of the advice of 30 September 1999 of the **French Food Safety Agency** (the Agence Française de Sécurité Sanitaire des Aliments, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom. Adopted and edited following on 26.10-16.11.99.

114. Opinion on Monitoring some important aspects of the **evolution of the Epidemic of BSE in Great-Britain** (Status, April 1999). Adopted on 27-28 May 1999.

115. Opinion on the safety of bones produced as by-product of the **Date Based Export Scheme.** Adopted on 22-23 October 1998.

116. Report on the UK **Date Based Export Scheme** and the UK proposal on Compulsory Slaughter of the Offspring of BSE Cases. Adopted on 8-9 December 1997 (Re-edited version adopted on 22-23 January 1998.

117. Final Opinion on the **UK-Date Based Export Scheme**. Adopted on 19-20 February 1998.

Geographical BSE risk: methodological aspects.

118. Opinion on the Geographical BSE risk for sheep and goats (GBR-S): adaptation of the cattle GBR methodology to small ruminants, in case BSE in small ruminants would become probable or evident under field conditions. Adopted on 7-8 November 2002.

119. Update of the opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR). Adopted on 7 November 2002.

120. Opinion on the geographical BSE-risk (GBR) and its evolution over time in the European Union Member States (adopted by the SSC at its plenary meeting of 21/22 February 2002. Endorsed on 7 July 2002.

121. Updated opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR). Adopted on 11 January 2002.

122. Final opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR). Adopted on 6 July 2000.

123. Opinion of the Scientific Steering Committee on a method for assessing the Geographical BSE-Risk (GBR) of a country or region. Up-dated on January 2000.
124. Opinion on a method to assess the Geographical BSE-Risk (GBR) of Countries or Regions. Revision adopted on 22-23 April 1999.
125. Preliminary-opinion on a method to assess the geographical BSE-Risk of Countries or Regions. Adopted on 10 December 1998.
126. Opinion on BSE risk. Adopted on 26-27 March 1998.
127. Opinion on defining the BSE risk for specified geographical areas. Adopted on 23 January 1998.
128. Preliminary Opinion on BSE risk. Adopted on 19-20 February 1998.
129. Final Opinion on the contents of a "Complete dossier of the **epidemiological status** with respect to TSEs". Adopted on 19-20 February 1998. Adopted on 19-20 February 1998.

Geographical BSE risk (GBR): Opinions on the GBR of countries

130. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Paraguay**. Adopted on 10-11 April 2003.
131. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Uruguay**. Adopted on 10-11 April 2003.
132. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Brazil**. Adopted on 10-11 April 2003.
133. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Argentina**. Adopted on 10-11 April 2003.
134. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Chile**. Adopted on 10-11 April 2003.
135. Opinion and report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Costa Rica.** Adopted on 10-11 April 2003.
136. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Belarus**. Adopted on 10-11 April 2003.
137. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **FYR Macedonia**. Adopted on 10-11 April 2003.
138. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Estonia**. Adopted on 10-11 April 2003.
139. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Lithuania**. Adopted on 10-11 April 2003.
140. Opinion on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Cyprus**. Adopted on 10-11 April 2003.

141. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Singapore**. Adopted on 06 March 2003.
142. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **New Caledonia**. Adopted on 06 March 2003.
143. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Greece**. Adopted on 06 December 2002.
144. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in the **Principality of Andorra**. Adopted on 06 December 2002.
145. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **New Zealand**. Adopted on 07 November 2002.
146. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Israel**. Adopted on 13 September 2002.
147. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Malta**. Adopted on 13 September 2002.
148. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Slovenia**. Adopted on 13 September 2002.
149. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Vanuatu**. Adopted on 27 June 2002.
150. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Turkey**. Adopted on 27 June 2002.
151. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in the **Republic of San Marino**. Adopted on 27 June 2002.
152. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Latvia**. Adopted on 27 June 2002.
153. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Iceland**. Adopted on 27 June 2002.
154. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Croatia**. Adopted on 27 June 2002.
155. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Bulgaria**. Adopted on 27 June 2002.
156. Opinion on the geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Finland**. Update adopted on 16 May 2002.
157. Opinion on the geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Austria**. Update adopted on 16 May 2002.
158. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **El Salvador**. Adopted on 29 June 2001.

159. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Nigeria**. Adopted on 29 June 2001.
160. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Panama**. Adopted on 29 June 2001.
161. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Costa Rica**. Adopted on 11 May 2001.
162. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Kenya**. Adopted on 11 May 2001.
163. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Romania**. Adopted on 11 May 2001.
164. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Slovenia**. Adopted on 11 May 2001.
165. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Albania**. Adopted on 30 March 2001.
166. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Brazil**. Adopted on 30 March 2001.
167. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Colombia**. Adopted on 30 March 2001.
168. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Cyprus**. Adopted on 30 March 2001.
169. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in the **Czech Republic.** Adopted on 30 March 2001.
170. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Estonia**. Adopted on 30 March 2001.
171. Opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Hungary**. Adopted on 30 March 2001.
172. Opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **India**. Adopted on 30 March 2001.
173. Opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Mauritius**. Adopted on 30 March 2001.
174. Opinion the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Pakistan**. Adopted on 30 March 2001.
175. Opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Poland**. Adopted on 30 March 2001.
176. Opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in **Singapore**. Adopted on 30/03/2001.

177. Opinion on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR) in the **Slovak Republic**. Adopted on 30 March 2001.
178. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Botswana**. Adopted on 09 February 2001.
179. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Lithuania**. Adopted on 09 February 2001.
180. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Namibia**. Adopted on 09 February 2001.
181. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Nicaragua**. Adopted on 09 February 2001.
182. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Swaziland**. Adopted on 09 February 2001.
183. Opinion on the Geographical risk of Bovine Spongiform Encephalopathy (GBR) in **Uruguay**. Adopted on 12 January 2001.

Geographical BSE risk (GBR): Reports on the GBR of countries

184. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Paraguay**. (April 2003).
185. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Uruguay**. (April 2003).
186. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Brazil**. (April 2003).
187. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Argentina**. (April 2003).
188. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Chile**. (April 2003).
189. Report and report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Costa Rica**. (April 2003).
190. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Belarus**. (April 2003).
191. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in FYR **Macedonia**. (April 2003).
192. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Estonia**. (April 2003).
193. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Lithuania**. (April 2003).
194. Report on the Geographical risk of bovine spongiform encephalopathy (GBR) in **Cyprus**. (April 2003).

195. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Singapore** – 2003 (March 2003)
196. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **New Caledonia** – 2003 (March 2003)
197. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Greece** – 2002 (December 2002)
198. Final Report on the assessment of the Geographical BSE-Risk (GBR) of the Principality of **Andorra** – 2002 (December 2002).
199. Final report on the updated assessment of the Geographical BSE-Risk (GBR) of **New Zealand** – 2002 (November 2002).
200. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Israel** – 2002 (September 2002).
201. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Malta** – 2002 (September 2002).
202. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Slovenia** – 2002 (September 2002).
203. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Vanuatu** – 2002 (June 2002).
204. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Turkey** – 2002 (June 2002).
205. Final Report on the assessment of the Geographical BSE-Risk (GBR) of the **Republic of San Marino** – 2002 (June 2002).
206. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Latvia** – 2002 (June 2002).
207. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Iceland** – 2002 (June 2002).
208. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Croatia** – 2002 (June 2002).
209. Final Report on the assessment of the Geographical BSE-Risk (GBR) of **Bulgaria** – 2002 (June 2002).
210. Final report on the updated assessment of the Geographical BSE-Risk (GBR) of **Finland** - 2002 (May 2002).
211. Final report on the updated assessment of the Geographical BSE-Risk (GBR) of **Austria** - 2002 (May 2002).
212. Final report on the Assessment of the Geographical BSE - Risk of **El Salvador** (June 2001).

213. Final report on the Assessment of the Geographical BSE - Risk of **Nigeria** (June 2001).
214. Final report on the Assessment of the Geographical BSE - Risk of **Panama** (June 2001).
215. Final report on the Assessment of the Geographical BSE - Risk of **Costa Rica** (May 2001).
216. Final report on the Assessment of the Geographical BSE - Risk of **Kenya** (May 2001).
217. Final report on the Assessment of the Geographical BSE - Risk of **Romania** (May 2001).
218. Final report on the Assessment of the Geographical BSE - Risk of **Slovenia** (May 2001) .
219. Report on the Assessment of the Geographical BSE - Risk of **Albania** (March 2001).
220. Report on the Assessment of the Geographical BSE - Risk of **Brazil** (March 2001).
221. Report on the Assessment of the Geographical BSE - Risk of **Colombia** (March 2001).
222. Report on the Assessment of the Geographical BSE - Risk of **Cyprus** (March 2001).
223. Report on the Assessment of the Geographical BSE - Risk of the **Czech** Republic (March 2001).
224. Report on the Assessment of the Geographical BSE - Risk of **Estonia** (March 2001).
225. Report on the Assessment of the Geographical BSE - Risk of **Hungary** (March 2001).
226. Report on the Assessment of the Geographical BSE - Risk of **India** (March 2001).
227. Report on the Assessment of the Geographical BSE - Risk of **Mauritius** (March 2001).
228. Report on the Assessment of the Geographical BSE - Risk of **Pakistan** (March 2001).
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230. Report on the Assessment of the Geographical BSE - Risk of **Singapore** (March 2001).
231. Report on the Assessment of the Geographical BSE - Risk of the **Slovak Republic** (March 2001).
232. Report on the Assessment of the Geographical BSE - Risk of **Botswana**.

233. Report on the Assessment of the Geographical BSE - Risk of **Lithuania**.
234. Report on the Assessment of the Geographical BSE - Risk of **Namibia**.
235. Report on the Assessment of the Geographical BSE - Risk of **Nicaragua**.
236. Report on the Assessment of the Geographical BSE - Risk of **Swaziland**.
237. Report on the Assessment of the Geographical BSE - Risk of **Uruguay**.
238. Report on the Assessment of the Geographical BSE - Risk of **Austria** (July 2000).
239. Report on the Assessment of the Geographical BSE - Risk of **Belgium** (July 2000).
240. Report on the Assessment of the Geographical BSE - Risk of **Denmark** (July 2000).
241. Report on the Assessment of the Geographical BSE - Risk of **Finland** (July 2000).
242. Report on the Assessment of the Geographical BSE - Risk of **France** (July 2000).
243. Report on the Assessment of the Geographical BSE - Risk of **Germany** (July 2000).
244. Report on the Assessment of the Geographical BSE - Risk of **Ireland** (July 2000).
245. Report on the Assessment of the Geographical BSE - Risk of **Italy** (July 2000).
246. Report on the Assessment of the Geographical BSE - Risk of **Luxembourg** (July 2000).
247. Report on the Assessment of the Geographical BSE - Risk of The **Netherlands** (July 2000).
248. Report on the Assessment of the Geographical BSE - Risk of **Portugal** (July 2000).
249. Report on the Assessment of the Geographical BSE - Risk of **Spain** (July 2000).
250. Report on the Assessment of the Geographical BSE - Risk of **Sweden** (July 2000).
251. Report on the Assessment of the Geographical BSE - Risk of **United Kingdom** (July 2000).
252. Report on the Assessment of the Geographical BSE - Risk of **Argentina** (July 2000).
253. Report on the Assessment of the Geographical BSE - Risk of **Australia** (July 2000).
254. Report on the Assessment of the Geographical BSE - Risk of **Canada** (July 2000).
255. Report on the Assessment of the Geographical BSE - Risk of **Chile** (July 2000).
256. Report on the Assessment of the Geographical BSE - Risk of **New Zealand** (July 2000).
257. Report on the Assessment of the Geographical BSE - Risk of **Norway** (July 2000).
258. Report on the Assessment of the Geographical BSE - Risk of **Paraguay** (July 2000).

259. Report on the Assessment of the Geographical BSE - Risk of **Switzerland** (July 2000).

260. Report on the Assessment of the Geographical BSE - Risk of **USA** (July 2000).

ANNEX III

COMMUNITY LEGISLATION ON BSE SINCE MID-1997, REFERRING TO SCIENTIFIC OPINIONS

Legal text *	Contents
D 97/534/EC of 30 July 1997	Prohibition of the use of SRM (mainly brain, eyes and spinal cord)
D 97/866/EC of 16 December 1997	Postponement to 1/4/1998 of the date of application of D 97/534/EC (SRM)
D 98/272/EC of 23 April 1998	Epidemio-surveillance for all animal TSEs
Rec. 98/477/EC of 22 July 1998	Information necessary to support applications for the evaluation of TSE status
D 98/653/EC of 18 November 1998	Total ban on dispatch of live cattle and all cattle products from Portugal (Portugal embargo)
D 98/692/EC of 25 November 1998	Amendment of the UK embargo - Principles of the second step towards lifting the ban under the Date-based Export Scheme applicable in the entire UK
D 1999/129/EC of 29 January 1999	Amendment of D 94/381/EC – Hydrolysed proteins
D 1999/534/EC of 19 July 1999	Conditions for the production of MBM and tallow (Repeals D 96/449/EC)
D 1999/881/EC of 14 December 1999	Postponement to 30 June 2000 of the date of application of D 97/534/EC(SRM)
D 1999/724/EC of 28 October 1999	Health rules on gelatine
D 2000/374/EC of 5 June 2000	Amendment of D 98/272/EC – Introduction of rapid post-mortem test in monitoring for BSE
D 2000/418/EC of 29 June 2000	Prohibition of the use of SRM (Repeals D 97/534/EC)
D 2000/764/EC of 29 November 2000	Amendment of D 98/272/EC – Reinforcement of the surveillance
D 2000/766/EC of 4 December 2000	Temporary ban on use of MBM
D 2001/2/EC of 27 December 2000	Amendment of D 2000/418/EC – Extension of the list of SRM (bovine intestines)

Legal text *	Contents
D 2001/9/EC of 29 December 2000	Conditions for feeding certain animal proteins
D 2001/25/EC of 27 December 2000	Prohibition of the use of dead animals in the production of animal feed
D 2001/165/EC of 27 February 2001	Amendment of D 2001/9/EC – Hydrolysed proteins
D 2001/233/EC of 14 March 2001	Amendment of D 2000/418/EC – Extension of the list of SRM (vertebral column)
D 2001/270/EC of 29 March 2001	Amendment of D 2000/418/EC – Imports from third countries
D 2001/384/EC of 3 May 2001	Amendment of D 2000/418/EC – Extension of the list of derogating third countries
R 999/2001 of 22 May 2001	Prevention, control and eradication of certain TSE
R 1248/2001 of 22 June 2001	Amendment of R 999/2001 – Surveillance and testing
R 1326/2001 of 29 June 2001	Amendment of R 999/2001 – Transitional measures
R 270/2002 of 14 February 2002	Amendments of R 999/2001 – SRM, surveillance, animal feeding and placing on the market of ovine and caprine animals and products thereof
D 2002/670/EC of 20 August 2002	Amendment off D 98/256/EC – Adaptation of some DBES conditions
R 1494/2002 of 21 August 2002	Amendment of R 999/2001 – continuing of BSE testing in fallen stock Deletion of the restrictions on trade of bovine embryos – Clarifications of some SRM rules
R 1774/2002 of 3 October 2002	Health rules concerning animal by-products not intended for human consumption
D 2002/1003/EC of 18 December 2002	Survey of prion protein genotypes in sheep breeds
R 260/2003 of 12 February 2003	Culling rules for scrapie
D 2003/100/EC of 13 February 2003	Breeding programmes for sheep