



**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.

G rard 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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- 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 <sup>1</sup> (**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):	
<ul style="list-style-type: none"> <li>• Sporadic (idiopathic) CJD</li> </ul>	<p><b>Unknown</b>, probably spontaneous conformation change of PrP<sup>c</sup> or somatic mutation</p>
<ul style="list-style-type: none"> <li>• Familial CJD</li> </ul>	<p><b>Genetic</b> (<i>PRNP</i> mutations or insertions)</p>
<ul style="list-style-type: none"> <li>• Iatrogenic CJD</li> </ul>	<p><b>Infectious</b> [dural or corneal transplants, hormone treatment with preparations from cadaveric pituitary gland, intracerebral electrodes or neurosurgical instruments]</p>
<ul style="list-style-type: none"> <li>• Variant CJD (v-CJD)</li> </ul>	<p><b>Infectious</b> (presumed food-borne exposure to the BSE agent)</p>
Gerstmann-Sträussler-Scheinker disease (GSS)	<p><b>Genetic</b> (<i>PRNP</i> mutations, classically P102L)</p>
Familial fatal insomnia (FFI)	<p><b>Genetic</b> (<i>PRNP</i> 178 mutation, 129M)</p>
Sporadic fatal insomnia (SFI) (same phenotype as FFI)	<p><b>Unknown</b> (probable causation as in sporadic CJD)</p>
Kuru	<p><b>Infectious</b> (ritual cannibalism by Fore people in Papua-New Guinea)</p>

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

A conformationally abnormal, protease-resistant isoform (PrP<sup>res</sup> or PrP<sup>Sc</sup>, 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<sup>Sc</sup> 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<sup>Sc</sup> exists in a predominantly beta-pleated form in contrast to the alpha-helix dominant PrP<sup>C</sup><sup>18</sup>. Substantial evidence supports the notion that PrP<sup>Sc</sup> itself is the infectious agent<sup>18</sup>, albeit others argue for a viral or other microbial agent as the pathogen involved in either the transmission of PrP<sup>Sc</sup> or causation of the PrP<sup>C</sup>-PrP<sup>Sc</sup> change<sup>19,20</sup>. The PrP<sup>C</sup> to PrP<sup>Sc</sup> conversion is considered by many to be the basis for propagation of infectivity in an auto-catalytic refolding process. While PrP<sup>Sc</sup> is usually a good predictor of infectivity, failure to show its presence does not necessarily indicate absence of infectivity<sup>21</sup>. PrP<sup>Sc</sup> 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<sup>22</sup>.

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<sup>23</sup>. *PRNP* codon 129 is important as a genetic susceptibility factor in sporadic<sup>24</sup> and iatrogenic CJD<sup>25</sup> as well as determining clinico-pathological phenotypes in all human TSEs<sup>26,27</sup>. 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<sup>27,28</sup>. 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<sup>C</sup> *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 TSEs is unknown. Oxidative stress<sup>29,30</sup>, and apoptosis<sup>31,32</sup> contribute to the cell death process. As recently summarised<sup>33</sup>, the neural pathogenesis involves either the neurotoxic effect of PrP<sup>Sc</sup> or loss of function of PrP<sup>C</sup>. Toxic intermediates or alternative pathogenic forms of PrP, like the unusual transmembrane form (indicated as C<sup>tm</sup>PrP) might also have a role. Neuronal loss, which seems to be selective<sup>34</sup>, is accompanied by astrogliosis and microgliosis and cytokine production, but typical inflammatory responses and cellular infiltration are lacking<sup>35,36</sup>.

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<sup>37-39</sup>. The complement system as well as B-cells also have a role in peripheral prion pathogenesis<sup>40,41</sup>. 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<sup>42</sup> and/or via parasympathetic pathways to the brain stem along the vagus nerve<sup>43</sup>. 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<sup>44</sup>.

### **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<sup>C</sup> into PrP<sup>Sc</sup> underlies its development. An epidemiological case control study was unable to identify any specific risk factors for sporadic CJD<sup>45</sup>. 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<sup>46</sup> (**Table 1**), must be prevented by appropriate control measures in hospitals<sup>47</sup>. 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<sup>Sc</sup> glycoform 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<sup>27,28</sup>.

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<sup>Sc</sup> 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<sup>48</sup>. 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.euroid.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%<sup>48</sup>. The most important differential diagnoses comprise Alzheimer's disease, Lewy body dementia, vascular disorders, and rare conditions like Hashimoto encephalopathy<sup>49</sup>.

**Table 3: Clinical diagnostic criteria for CJD surveillance purposes** <sup>50</sup>

<b>Sporadic CJD Definite</b>	Diagnosed by standard neuropathological techniques; and/or immunocytochemically and/or western blot confirmed protease resistant PRP and/or presence of scrapie associated fibrils.
<b>Sporadic CJD Probable</b> (in the absence of an alternative diagnosis from routine investigation).  <u>And</u>	Progressive dementia; <u>and</u> at least two out of four of the following four clinical features: <ul style="list-style-type: none"> <li>• Myoclonus</li> <li>• Visual or cerebellar disturbance</li> <li>• Pyramidal/extrapyramidal dysfunction</li> <li>• Akinetic mutism</li> </ul> <ul style="list-style-type: none"> <li>• a typical EEG during an illness of any duration <u>and/or</u></li> <li>• a positive 14-3-3 CSF assay and a clinical duration to death &lt; 2 years</li> </ul>
<b>Sporadic CJD Possible</b>  <u>And</u>	Progressive dementia; <u>and</u> at least two out of four of the following four clinical features: <ul style="list-style-type: none"> <li>• myoclonus</li> <li>• visual or cerebellar disturbance</li> <li>• pyramidal/extrapyramidal dysfunction</li> <li>• akinetic mutism</li> </ul> <ul style="list-style-type: none"> <li>• no EEG or atypical EEG; <u>and</u></li> <li>• duration &lt; 2 years</li> </ul>
<b>Iatrogenic CJD</b>	Progressive cerebellar syndrome in a recipient of human cadaveric-derived pituitary hormone; <u>or</u> sporadic CJD with a recognised exposure risk, e.g. antecedent neurosurgery with dura mater graft.
<b>Familial CJD</b>	NB. For the purpose of surveillance this includes GSS disease and FFI.  Definite or probable CJD <u>plus</u> definite or probable CJD in a first degree relative; <u>and/or</u> neuropsychiatric disorder <u>plus</u> disease-specific <i>PRNP</i> mutation.

In a consensus report <sup>51</sup>, 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 <sup>52</sup>, 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<sup>50</sup>**

- |  |
|--|
| <p>1. <b>Creutzfeldt-Jakob disease (CJD)</b></p> <p>1.1. <b>Sporadic, iatrogenic</b> (recognised risk) or <b>familial</b> (same disease in 1st degree relative or disease-associated <i>PRNP</i> mutation):</p> <p>Spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical grey matter; and/or</p> <p>Encephalopathy with prion protein (PrP) immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivacuolar types).</p> <p>1.2. <b>Variante CJD.</b> 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.</p> <p>2. <b>Gerstmann-Sträussler-Scheinker disease (GSS)</b> (in family with dominantly inherited progressive ataxia and/or dementia and one of a variety of <i>PRNP</i> mutations): Encephalo(myelo)pathy with multicentric PrP plaques.</p> <p>3. <b>Familial fatal insomnia (FFI)</b> (in member of a family with <i>PRNP</i><sup>178</sup> mutation): Thalamic degeneration, variably spongiform change in cerebrum.</p> <p>4. <b>Kuru:</b> Spongiform encephalopathy with cerebellar atrophy and presence of Kuru plaques.</p> |
|--|

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<sup>53</sup>. 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<sup>54</sup>.

There is very strong evidence that the origin of vCJD is from BSE<sup>55</sup>. Typing of different TSEs has been performed on PrPr<sup>Sc</sup> Western blots that show “signature” PrPr<sup>Sc</sup> patterns<sup>56</sup>, and by experimental inoculation of inbred<sup>57</sup> or transgenic<sup>56,58,59</sup> 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<sup>60,61</sup>. 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) <sup>62</sup>.

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<sup>Sc</sup> 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 <sup>64,65</sup>. 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<sup>Sc</sup> and infectivity, biopsy examination of the tonsil has been used to support a vCJD diagnosis <sup>66</sup>, and anonymous mass screening of surgical specimens <sup>67</sup> 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<sup>Sc</sup> 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 <sup>59</sup>. 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 <sup>68</sup>. 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 <sup>63</sup>**

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
<b>Definite:</b>		IA (progressive neuropsychiatric disorder) <u>and</u> 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<sup>1</sup>. 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<sup>2</sup>, 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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<sup>1</sup> 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)

<sup>2</sup> 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:

	<b>See section:</b>
– 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 not 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<sup>3</sup> 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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<sup>3</sup> 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*.<sup>4</sup>

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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<sup>4</sup> 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<sup>5</sup>, 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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<sup>5</sup> Representing more than 97 % of all BSE cases recorded so far

present in what are currently listed as the “sheep specified risk materials”<sup>6</sup>, but also in other tissues, particularly intestines and lymph nodes, and also enteric nervous system and associated autonomic nerves and blood<sup>7</sup>. 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<sup>8</sup> 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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<sup>6</sup> 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**)

<sup>7</sup> 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)

<sup>8</sup> 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 breakdown 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<sup>9</sup>, 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

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<sup>9</sup> 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 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<sup>C</sup> to PrP<sup>Sc</sup>; 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

### **Preamble: 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	31876
Deutschland	0	0	0	0	0	1 <sup>(a)</sup>	0	3 <sup>(a)</sup>	0	0	2 <sup>(a)</sup>	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 <sup>(a)</sup>	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 <sup>(b)</sup>	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 <sup>(b)</sup>	14 <sup>(b)</sup>	17 <sup>(b)</sup>	18 <sup>(b)</sup>	16	19 <sup>(b)</sup>	16 <sup>(b)</sup>	74	80	83	95	149	246	333	1175
Italia	0	0	0	0	0	0	0	2 <sup>(a)</sup>	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 <sup>(a)</sup>	1 <sup>(a)</sup>	1 <sup>(a)</sup>	3 <sup>(a)</sup>	12	15	31	30	127	159	150 <sup>(b)</sup>	113	86	729
<b>Total min UK</b>	0	0	15	15	23	21	20	40	34	117	122	236	290	482	968	1001	3384
<b>Total EU/UE</b>	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 <sup>(b)</sup>	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 <sup>(a)</sup>	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	4
<b>Total</b>	<b>4</b>	<b>41</b>	<b>65</b>	<b>115</b>	<b>165</b>	<b>239</b>	<b>291</b>	<b>210</b>	<b>155</b>	<b>104</b>	<b>96</b>	<b>54</b>	<b>70</b>	<b>46</b>	<b>55</b>	<b>41</b>	<b>1751</b>
<b>World</b>	<b>446</b>	<b>2514</b>	<b>7246</b>	<b>14424</b>	<b>25390</b>	<b>37316</b>	<b>35140</b>	<b>24540</b>	<b>14664</b>	<b>8311</b>	<b>4553</b>	<b>3487</b>	<b>2641</b>	<b>1956</b>	<b>2217</b>	<b>2166</b>	<b>187011</b>

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 <sup>1</sup> (in million)	BSE Suspect Animals <sup>2</sup> Positive		Risk Animals <sup>3</sup>			Healthy Animals <sup>4</sup>			BSE Eradication <sup>5</sup>	
		Nr	Positive	Nr	Positive	Ratio <sup>7</sup>	Nr	Positive	Ratio <sup>7</sup>	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 <sup>6</sup>	5.0	872	467	221,089	635	28.7.1	171,585	14	0.82	945	0
<b>Total</b>	<b>40.4</b>	<b>2,558</b>	<b>673</b>	<b>1,235,340</b>	<b>1,142</b>	<b>9.2</b>	<b>9,093,284</b>	<b>287</b>	<b>0.32</b>	<b>57,601</b>	<b>15</b>

<sup>1</sup> Source: Eurostat

<sup>2</sup> Animals reported as BSE clinical suspects

<sup>3</sup> Dead-on-farm animals, emergency slaughtered animals, animals sent for normal slaughter but found sick at ante mortem inspection

<sup>4</sup> Healthy animals subject to normal slaughter

<sup>5</sup> Birth and rearing cohorts, feed cohorts, offspring of BSE cases, animals from herds with BSE

<sup>6</sup> GB & Northern Ireland

<sup>7</sup> Positives per 10,000 tested animals

**TSE Testing in Goats**  
Cumul January-December 2002

	Adult goats <sup>1</sup> (in million)	TSE Suspect Animals <sup>4</sup>		Risk Animals <sup>2</sup>			Healthy Animals <sup>3</sup>			TSE Eradication		Total	
		Nr	Positive	Nr	Positive	Ratio <sup>6</sup>	Nr	Positive	Ratio <sup>6</sup>	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 <sup>5</sup>	0.04	0	0	6	0	0.0	9	0	0.0	0	0	15	0
<b>Total</b>	<b>8.53</b>	<b>56</b>	<b>4</b>	<b>17,164</b>	<b>15</b>	<b>8.9</b>	<b>35,026</b>	<b>11</b>	<b>3.2</b>	<b>1,580</b>	<b>9</b>	<b>53,826</b>	<b>39</b>

<sup>1</sup> Source: Eurostat December 2001

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

<sup>3</sup> Healthy animals subject to normal slaughter

<sup>4</sup> Animals reported as TSE clinical suspect

<sup>5</sup> GB & Northern Ireland

<sup>6</sup> Positives per 10,000 tested animals

**TSE Testing in Sheep**  
Cumul January-December 2002

	Adult sheep <sup>1</sup> (in million)	TSE Suspect Animals <sup>4</sup>		Risk Animals <sup>2</sup>			Healthy Animals <sup>3</sup>			TSE Eradication		Total	
		Nr	Positive	Nr	Positive	Ratio <sup>6</sup>	Nr	Positive	Ratio <sup>6</sup>	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 <sup>5</sup>	16.08	536	421	1,348	6	44.5	31,145	33	10.6	0	0	33,039	461
<b>Total</b>	<b>66.07</b>	<b>2,749</b>	<b>656</b>	<b>73,147</b>	<b>217</b>	<b>29.7</b>	<b>238,673</b>	<b>193</b>	<b>8.1</b>	<b>39,702</b>	<b>463</b>	<b>354,271</b>	<b>1,529</b>

<sup>1</sup> Source: Eurostat December 2001

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

<sup>3</sup> Healthy animals subject to normal slaughter

<sup>4</sup> Animals reported as TSE clinical suspect

<sup>5</sup> GB & Northern Ireland

<sup>6</sup> 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<sup>Sc</sup> 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<sup>Sc</sup> 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<sup>Sc</sup> 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 ruminant 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<sup>†</sup> 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 course 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 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.

**Table: Overview of current knowledge with regard to possible TSE infectivity<sup>†</sup> 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.

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

<sup>10</sup> LN = Lymph node MP = Mesenteric/portal; PF = Prefemoral; PS = Prescapular; RP = Retropharyngeal; BM = Bronchomediastinal

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

<sup>11</sup> Research results in print.

<b>MATERIAL:</b>	<b>Cattle</b>	<b>Small ruminants</b>
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
<b>REPRODUCTIVE TISSUES</b>		
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	

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

<b>MATERIAL:</b>	<b>Cattle</b>	<b>Small ruminants</b>
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<sup>Sc</sup> 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<sup>3.5</sup> mouse i.c/i.p ID<sub>50</sub>/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<sub>50</sub> 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<sub>50</sub>'s in 1g could be approximately 0.55 to 4.2, although with higher titres of BSE affected brain (e.g 10<sup>5</sup> mouse i.c./i.p. ID<sub>50</sub>/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<sup>12</sup> 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

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<sup>12</sup> 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<sup>C</sup>) is soluble and can be fully degraded by proteolytic enzymes, the abnormal form of the protein (often referred to as PrP<sup>Sc</sup> (for scrapie), PrP<sup>BSE</sup>, PrP<sup>CJD</sup> according to the disease and species in which it occurs, or PrP<sup>d</sup> 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<sup>res</sup> when the identification of this protein has involved a preliminary treatment demonstrating its protease resistance). This PrP<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup>. 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<sup>C</sup>. Some methods allow steps for the concentration of the protein based on its abnormal solubility. Following this extraction procedure, PrP<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> can also be 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<sup>d</sup> 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<sup>d</sup> in fluids, such as cerebrospinal fluid, using fluorescence spectroscopy.

Immunohistochemistry can also be used to identify PrP<sup>d</sup> 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<sup>d</sup> using antibodies recognising the prion protein. These pretreatments increase the specific antibody labelling of PrP<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> that would probably not be detected by some of the biochemical methods, and enable precise identification of the specific brain regions in which PrP<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup>, suggested from recent observations that amplification of misfolded PrP<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> accumulation could be detected in the lymphoid tissues, despite the occurrence of clinical signs of scrapie and the presence of detectable accumulation of PrP<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup>; 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<sup>13</sup>

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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<sup>13</sup> 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.**

	<b>Enfer</b>	<b>CEA</b>	<b>Prionics</b>	<b>Wallac</b>
<b>Specificity</b>	100%	100%	100%	89.8%
<b>Sensitivity</b>	100%	100%	100%	69.8%
<b>Dilution</b>	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<sup>Sc</sup> : Analysis of the distribution of the quantitative signals identified gradients of PrP<sup>Sc</sup> 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<sup>Sc</sup> 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  $-70^{\circ}\text{C}$ ) 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.

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## 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  $(1 - \alpha)$  – least one positive animal if the true prevalence is  $p_0$  or higher can be calculated as

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

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

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 [1] 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  $\alpha\%$  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:

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

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  $B = 0, 1, \dots, 9$ . 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  $B = 0, 1, \dots, 9$ .**

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<sup>14</sup>, but prudently initial surveillance targeted the age-group in which TSE test positivity was most likely, namely adults (above 12 months)<sup>15</sup>.

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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<sup>14</sup> See the SSC Preliminary opinion of 6-7 September 2001 on Stunning methods.

<sup>15</sup> 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.