4. The link between BSE and vCJD

4.1 Following the emergence of BSE in 1986, the potential for transmission of the disease to humans was of major concern to both scientists and government officials. The Southwood Working Party advised that, on the basis of experience with scrapie, the risk of transmission to humans was remote.560 Because the possibility that BSE could be transmitted orally to humans could not be entirely ruled out, the Working Party advised that known affected animals should not enter the human food chain, nor should their milk. Furthermore, they considered it a reasonable assumption that should BSE transmit to humans, the resultant clinical disorder would closely resemble CJD. In their recommendations for research, the Consultative Committee on Research chaired by Dr Tyrrell (the Tyrrell Committee) concurred that the risk to humans from BSE was remote and noted that epidemiological studies conducted around the world had not identified a causal link between scrapie and CJD.561 However, the need to be able to give the same assurance about the lack of effect of BSE on human health was recognised. The Committee therefore recommended the surveillance of cases of CJD with particular reference to overall incidence, geographical distribution, age and sex distribution, occupational history, association with medication and any atypical clinical features. They also recommended prospective monitoring of groups with high exposure to bovine materials. The work of the Southwood Working Party and the Tyrrell Committee is described in vol. 4: *The Southwood Working Party, 1988–89* and vol. 11: *Scientists after Southwood* respectively.

CJD surveillance

4.2 The establishment of the CJD Surveillance Unit (CJDSU) in 1990 in response to the Tyrrell recommendations is discussed in detail in vol. 8: *Variant CJD*. The main aim of the unit was to study the occurrence of CJD in the UK and to identify any change in the characteristics of the disease – clinical, pathological or epidemiological – that might be linked to the occurrence of BSE.562

4.3 From 1992 the CJDSU began to identify cases of CJD that could have been linked with BSE. Initially cases of CJD in farmers with a history of BSE on their farms were identified, though analysis did not support a link with BSE. In 1995 cases of CJD were reported in two young people. The occurrence of CJD in young people was an exceedingly rare event, and until this point only four such cases in teenagers had been recorded worldwide.563 By the end of 1995, the number of cases in young people reported to the CJDSU had increased to three,564 and by March 1996 this number had risen to 10. An account of the emergence and investigation of CJD in farmers and in young people can be found in vol. 8: *Variant CJD*.

560 IBD2
561 IBD4
562 S Will para. 8
4.4 Neuropathological findings in the brains of these ten cases revealed a consistent but unusual pattern with large amyloid plaques as seen in kuru but atypical of sporadic CJD. All cases tested were found to have a similar methionine/methionine genotype in codon 129 at the prion gene locus and did not have familial prion mutations. The unusual clinical features and novel pathology suggested a new variant of the disease, which is now termed ‘variant CJD’ (vCJD). BSE was considered to be the most likely origin of the new disease since exposure to the BSE agent in the UK would have been greatest in the mid-1980s, and the sudden emergence of cases in young people was consistent with an incubation period for the disease of 5 to 10 years.

4.5 The following paragraphs discuss experimental studies set up to investigate a link between the two diseases.

**Experimental studies into a BSE/CJD link**

4.6 Initial studies in 1988 aimed to test the likely transmissibility of BSE to humans by the inoculation of marmosets, a primate species. Two animals were inoculated intracerebrally and succumbed to disease in early 1992. However, it was known that scrapie was readily transmissible to marmosets (albeit with a shorter incubation period), so the results were not thought to be surprising. Thus, the study did not offer significant insights into the transmissibility of BSE to humans. Once vCJD had been recognised, a host of studies were initiated to investigate a possible link between BSE and vCJD. These studies were reported after March 1996. At that time, evidence for the link between the diseases was only circumstantial, and some doubted its existence. We have examined events occurring after 20 March in order to resolve these doubts and to consider the adequacy of the response to the emergence of vCJD prior to March 1996.

4.7 Strain-typing studies were undertaken in both mice and primates to characterise the pattern of disease in terms of the incubation period and disease pathology of different transmissible spongiform encephalopathies (TSEs). In the study carried out jointly by the Neuropathogenesis Unit (NPU) and CJDSU which began in early 1995, mice were inoculated intracerebrally and intraperitoneally with infected brain homogenate. The disease patterns of BSE, what later became known as vCJD, feline spongiform encephalopathy (FSE) and TSEs of exotic ruminants were shown to be extremely similar, while differing from those of scrapie and sporadic CJD.

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Another study in macaques by Lasmezas’ group showed that the clinical, neuropathological and molecular features of disease resulting from intracerebral inoculation of BSE brain homogenate were very similar to those observed in vCJD. These results provided positive evidence that vCJD and BSE were caused by the same strain of agent.

4.8 Independent confirmation of a link was provided by the western blotting studies of Professor Collinge’s group, published in 1996 and 1997. The western blotting technique is described in Chapter 1. Results showed that in samples of brain digested with proteolytic enzymes and subjected to gel electrophoresis, three bands of protease-resistant prion protein could be visualised using labelled antibody. The size of band depends on the number of sugar molecules bound to the protein molecule by a cellular reaction termed glycosylation. PrPSc may have one, two or no sugars bound to the molecule, and these variations lead to the appearance of three bands on the western blot; the fraction with two sugars migrates more slowly than the others during electrophoresis. The pattern of bands is characteristic of the strain of PrPSc and the DNA polymorphism at codon 129. There are four main patterns, depending on the relative proportion of each fraction and the conformation of the PrPSc molecule. Sporadic CJD was found to be type 1 or type 2, depending on the variant at codon 129. Most iatrogenic CJD cases have a type 3 pattern. Variant CJD and BSE have a type 4 pattern. Samples from cats with FSE and from zoo species affected with TSEs also have the type 4 pattern, consistent with an origin from the BSE strain of PrPSc. These data, together with the temporal and geographical association of vCJD and BSE, provide compelling evidence that the two diseases were caused by the same prion strain.

4.9 Recent work has identified additional human PrPSc types. Type 4t PrPSc has been discovered in lymphoreticular tissue obtained from vCJD patients but not from patients with other prion diseases. The pattern of type 4t was found to be consistent, but slightly different from that seen in vCJD brain (type 4). Further types (6, 7 and 8) are currently under investigation.

4.10 These additional types are not, however, universally accepted, since similar analysis by another group could only identify two patterns of prion glycosylation, with vCJD segregating with sporadic CJD. Variant CJD could be discriminated on the basis of its glycoform ratio, though the wisdom of doing so was questioned since glycosylation is a co- and post-translational event, and hence likely to be affected by the cell type of the species in which it occurs.

4.11 More recently, important work has shown that the discrepancies between these two studies could be due to the effect of metal ions on the prion protein. Both type 1 and type 2 PrPSc (ie, sporadic CJD) showed altered glycosylation patterns.

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569 The term 4t rather than 5 was used to reflect that it was similar to type 4 but from tonsils rather than brain.
following the removal of metal ions prior to protease digestion. Rather than producing their distinct patterns, both types gave indistinguishable and common fragment patterns. Similar treatment of PrPSc types 3 and 4 (iatrogenic and vCJD respectively) did not alter their characteristic cleavage pattern. These results showed that the respective conformations of type 1 and type 2 PrPSc are dependent on the presence of metal ions and that metal ion depletion induces a conformational change in the protein, exposing a new proteolytic cleavage site that is common to both metal-ion-depleted conformers. These findings not only explained the discrepancies between the two studies, but also provided a simple post-translational mechanism that could be involved in conferring strain-specific properties to distinct PrPSc conformers.

4.12 Transgenic mice have also provided a powerful tool for the comparison of BSE and vCJD. Replacement of the PrP gene in mice with that from another species effectively abolishes the species barrier, enabling the study of disease in an appropriate experimental model. Mice containing the human PrP gene inoculated with either BSE or vCJD showed similar clinical symptoms and histopathological patterns, which were distinct from sporadic or iatrogenic CJD. Western blot analysis of digested brain material again revealed similar glycoform patterns for both BSE and vCJD infected groups. More recent studies by Prusiner have shown that the incubation period and neuropathology of BSE in mice transgenic for the bovine PrP gene are almost exactly the same as those of vCJD in the same breed of mice, though different from scrapie.

4.13 A striking feature of vCJD has been the young age of its victims. The youngest patient recorded to date developed the disease at the age of 12 and the oldest at the age of 55. The average age of onset is about 29 years of age. Observations in patients who have developed CJD following treatment with contaminated growth hormone suggest a range of incubation periods from 5 to 15 years, with an average of 13 years. If the incubation period were similar in vCJD (and there are no data to support this assumption), the average age of infection in vCJD might be around 15 years of age.

4.14 A widely held view is that vCJD was transmitted in beef products in which parts of the animal containing high titres of BSE infection were included: especially brain, spinal cord and dorsal root ganglia. Such tissues might be expected to be found in mechanically recovered meat. It is therefore relevant to consider if processed beef products containing these materials were disproportionately consumed by children and young adults. Our attention was brought to a study in which the age-related consumption of these products was considered in a group of individuals aged 15 years and over. It was found that the consumption of beef, sausages, meat pies and corned beef was not correlated to the age of the individual. However, in the case of burgers, the consumption fell steeply with age and furthermore was correlated with the age distribution of the first 20 cases of vCJD. Burgers were the only meat product to show a steep decline in consumption with age. The study notes evidence which indicates that among all meat products, homogenates of cow brain have been used commonly to bind ground beef in

577 D.O. Morrison, private communication
burgers. The use of brain in food was banned in the UK in 1989, and we have received some contrary evidence that brain was seldom included in meat products even before that date. Nonetheless, the use of MRM in processed food remains a strong candidate as a possible route of infection in young vCJD patients. Other possibilities which remain to be tested and which might account for the age distribution of vCJD patients include:

i. Increased incidence of infections or ulcers of the nasopharynx, such as tonsillitis, or of the lower intestinal tract, such as gastroenteritis, in children compared with adults. There is evidence that TSEs may transmit through broken skin and mucous membranes, such as those that line the gastrointestinal tract.

ii. Infection through gum lesions associated with eruption of teeth.

iii. Iatrogenic transmission via vaccines prepared in cultures containing bovine constituents.

4.15 With regard to the possibility of genetic susceptibility, the only evidence of this comes from the observation that all vCJD patients (now numbering more than 50) are homozygous for the methionine variant at codon 129 of the PRNP gene. As this variant occurs in 38 per cent of the population, many other factors (including polymorphisms at other genetic loci) must also be involved in susceptibility to this disease. This is discussed further in vol. 8: Variant CJD.

Estimates of the size of the vCJD epidemic

4.16 In this Report we consider in depth the measures taken to address the possibility that BSE might be transmissible to humans. These measures were taken as precautions against a risk considered remote. Their importance can only be demonstrated with hindsight by reference to the extent to which BSE has in fact proved transmissible to humans. In the following sections we show that this is a matter on which it is too early to draw firm conclusions.

4.17 Attempts to estimate the possible size of a vCJD epidemic have been hampered by the many variables associated with the disease. Many important factors in determining the probability of BSE transmission to an individual, such as dose, route of exposure, incubation period, genetic susceptibility and scale of the species barrier between cattle and man, are unknown. Nevertheless, several groups of epidemiologists and statisticians have attempted to predict the possible number of cases, using complex mathematical models. Unsurprisingly, these groups have arrived at varying estimates, ranging from very few cases to many millions, as a result of the different methodologies adopted.

4.18 One of the first studies was published by Cousins and colleagues in 1997, and was based on the 14 cases of vCJD confirmed by the end of 1996. Their estimate was dependent on many variables and was highly sensitive to the distribution of average incubation periods. Thus, it was estimated that the incidence of vCJD

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579 Audit of Bovine and Ovine Slaughter and By-Products (Ruminant Products Audit), IBD5 tab 17 p.7
could be anything between 75 and 80,000. However, the authors recognised the limitations of basing their calculations on so few cases. Further analysis carried out by Ghani and colleagues from the Wellcome Trust Centre for the Epidemiology of Infectious Disease in 1998, extended the work of Cousens and colleagues, determining that with the information that was currently available, the uncertainty over the future size of the epidemic was likely to remain for the following three to five years.\textsuperscript{581} However, they concluded that the current data could have been consistent with epidemics ranging from less than 100 to several million cases.

4.19 In a study published in 1999, Professors Philip Thomas and Martin Newby of City University, London, estimated that, if BSE is indeed the cause of vCJD, not more than a few hundred cases would be expected and that most likely, the actual number would be 100 or less.\textsuperscript{582} The continued low incidence of vCJD since publication of their study was considered to be confirmation that the upper limit of their estimate was likely to be accurate.

4.20 However, the methodology adopted by Thomas and Newby has been criticised by Ferguson and colleagues from the Wellcome Trust Centre for the Epidemiology of Infectious Disease, who claim that they misused a test statistic and as a consequence arrived at an estimate for the upper bounds of disease incidence that was too low.\textsuperscript{583} Using the same data as Thomas and Newby, Ferguson et al. determined that only very wide bounds could be placed on future vCJD incidence. Furthermore, they questioned the suitability of a model fitting to only four years of case data for a disease that may have an incubation period of several decades. Rather, Ferguson et al. proposed extensive scenario analysis as a more cautious and thorough approach to determining the range of potential future epidemic patterns consistent with current data.

4.21 The conclusions of Thomas and Newby were also examined by the Epidemiology Subcommittee of Spongiform Encephalopathy Advisory Committee (SEAC), whose remit is to assess any information about the epidemiology of vCJD and develop, as far as possible, advice on trends in the disease.\textsuperscript{584} In a written statement to the Inquiry Professor Peter Smith, Chairman of the Subcommittee, emphasised the importance of knowing the future size of an epidemic in order to make policy decisions and to estimate the future burdens that might be put on the National Health Service (NHS). The Epidemiology Subcommittee felt that the analyses carried out by Thomas and Newby were flawed and therefore rejected their conclusions.

4.22 Following the discovery that abnormal PrP can be detected in certain tissues, such as tonsils and appendices, before the onset of clinical symptoms, two projects have recently been supported by the Department of Health (DH) and the Medical Research Council (MRC). The projects are based on the plan to test tonsil and appendix tissue anonymously for the prevalence of PrP\textsuperscript{Sc}. Information on the prevalence of incubating disease could then be used to reduce the current uncertainty about the future size of the epidemic.

\textsuperscript{584} S583 Smith para. 1
A recent study by Ghani and colleagues aimed to determine the age-group or groups that would provide the most information on the size of a potential epidemic from tissue samples.\textsuperscript{585} Ghani et al. predicted that anonymous testing would be most informative if undertaken on samples from 25–29-year-olds, but random samples of appendix tissue removed recently from individuals of all age-groups would be almost as informative. Screening of this nature could only exclude a large epidemic if a small number of infections were detected and if the test was able to detect infection early in the incubation period. The usefulness of anonymous testing of stored tissues has, however, been questioned, since it is unknown if the presence of PrP\textsuperscript{Sc} is necessarily indicative that an individual is destined to develop clinical disease.\textsuperscript{586}

4.23 Interim results of this study, which have recently become available, show that of 3,000 tonsil/appendix samples tested, none was found to contain PrP\textsuperscript{Sc}.\textsuperscript{587} This failure to detect a single positive test is consistent with previous estimates of an epidemic anywhere between 100 and a million cases, ie, the result neither increases the minimum number of cases expected nor reduces the maximum. However, this result is subject to a number of limitations. Firstly, the sample size was low. Modelling studies demonstrate that tests in tens of thousands of samples would be required to determine the future size of an epidemic. Indeed, these results are interim ones from a larger study examining around 18,000 samples, the results of which will be available by the end of 2000 and 2002. Secondly, the samples examined were all archived fixed material, which makes analysis more difficult. Studies examining fresh material are getting under way. Thirdly, the relationship between the presence of PrP\textsuperscript{Sc} in lymphoid tissue and the subsequent onset of clinical disease has not been proven, though it is highly probable. Moreover, the length of incubation period and the stage in the disease at which PrP\textsuperscript{Sc} is present in the lymphoid tissue are unknown. Thus the result of the analysis of any sample may only be indicative of the position at the time at which the sample was taken.

4.24 Thus the likely scale of an epidemic of vCJD is still uncertain and the subject of much debate. The emergence of vCJD cases to date has been consistent with both low and high estimates for future cases. It appears that the differences in opinion outlined above will not be resolved until such time as many more of the characteristics of vCJD are known and understood. In particular, the development of a robust blood test which could identify at least 75 per cent of those incubating the disease would allow more extensive screenings and more reliable estimates of the size of a future epidemic.

The effect of route of infection on disease phenotype

4.25 The different animal and human TSEs are known to vary in their clinical presentation and histopathological patterns. For example, behavioural signs herald the onset of disease in scrapie, BSE and sporadic CJD,\textsuperscript{588} whereas kuru and


\textsuperscript{585} Cooper, J.D., Gore, S.M. and De Angelis, D. (in press) Prevalence of Detectable Abnormal Prion Protein in Persons Incubating vCJD: Plausible Incubation Periods and Cautious Inference, Journal of Epidemiology and Biostatistics

\textsuperscript{586} Department of Health Press Release, 28 April 2000

iatrogenic CJD (from contaminated growth hormone injections) present as
cerebellar syndromes with prominent ataxia. Iatrogenic CJD due to intracerebral
or intraocular transplants manifests as classical CJD with progressive dementia. Familial CJD and Gerstmann-Sträussler-Scheinker (GSS) syndrome show a wide
range of phenotypes characteristic of the specific prion mutation and, in some cases,
depending on a polymorphic variation at the PrP gene locus. These observations
are consistent with the concept that disease pattern can be dependent on host
susceptibility factors such as genotype and on the strain of agent. However,
it is also conceivable that, given the mechanism of prion disorders whereby
neurodegeneration is caused by the aggregation of PrPSc followed by cell death, the
pattern of nerve cells targeted in the brain may also depend on the route of infection.

4.26 Indeed, the incubation period and clinical symptoms of iatrogenic CJD have
been found to differ depending on the mode of infection. When the infectious
agent is introduced into or near the brain, incubation is significantly shorter than
when the agent is introduced peripherally. Disease has been shown to develop
within around 20 months when the infective agent is introduced either by surgical
instrumentation or tissue transplantation. In contrast, infection through injection of
contaminated human growth hormone yields disease after an average of 13 years.
As mentioned above, clinical symptoms also differ: intracerebral inoculation
produces a demential clinical syndrome similar to sporadic CJD, whereas peripheral
inoculation and grafts of dura mater result in a syndrome similar to kuru.

4.27 Variant CJD has recently (1999) been found to differ from sporadic, familial
and iatrogenic CJD in that the lymphoreticular system in patients with vCJD
contains high levels of PrPSc. This finding may reflect a route of infection through
the alimentary canal, rather than spontaneous PrP mutation in somatic cells as
postulated in sporadic CJD, germ line mutation as in familial CJD, or parenteral
inoculation as in iatrogenic CJD.

4.28 Uncertainty remains on the question of how BSE might have been transmitted
to humans to cause vCJD. Oral exposure to the BSE agent through infected meat
products seems more likely than, for example, infection through parenteral
medicines containing contaminated bovine material, because of the high level of
infectivity invariably found in the LRS. Research aimed at correlating clinical
findings with the route of inoculation in experimental systems may allow the route
of infection in cases of vCJD to be inferred, and could lead to practical strategies for
disease prevention.

Summary

4.29 The evidence discussed above that vCJD is caused by BSE seems
overwhelming. Uncertainties exist about the cause of CJD in farmers, their wives
and in several abattoir workers. It seems that farmers at least might be at higher risk

590 Ibid.
591 Ibid.
The Lancet, 340, 24–7
593 Ibid.
594 This work also showed that LRS biopsy sampler may allow early diagnosis of vCJD by western blotting. Hill, A., Butterworth,
R., Joiner, S., Jackson, G., Rosser, M., Thomas, D., Frosh, A., Tolley, N., Bell, J., Spencer, M., King, A., Al-Sarroy, S.,
Ironside, J., Lantos, P. and Collinge, J. (1999) Investigation of Variant Creutzfeldt-Jakob Disease and Other Human Prion
Diseases with Tonsil Biopsy Samples, The Lancet, 353, 183–9
than others in the general population. Increased ascertainment (ie, increased identification of cases as a result of greater awareness of the condition) seems unlikely, as other groups exposed to risk, such as butchers and veterinarians, do not appear to have been affected. The CJD in farmers seems to be similar to other sporadic CJD in age of onset, in respect to glycosylation patterns, and in strain-typing in experimental mice. Some farmers are heterozygous for the methionine/valine variant at codon 129, and their lymphoreticular system (LRS) does not contain the high levels of PrPSc found in vCJD. It remains a remote possibility that when older people contract CJD from BSE the resulting phenotype is like sporadic CJD and is distinct from the vCJD phenotype in younger people.

4.30 Estimates of the likely scale of a possible epidemic of vCJD are wide-ranging and the subject of much debate. To know the likely number of cases is very important, not least to enable preparations to be made for the care of victims, as well as to be able to draw up guidelines to reduce the risk of transmission from infected but asymptomatic people. Preliminary results of the study examining tonsil and appendix material for signs of infection were not informative in this regard and full results are awaited. A blood test that would allow the widespread screening of the population by a simple method is still being sought.