Variant CJD (vCJD) and Bovine Spongiform Encephalopathy (BSE): 10 and 20 years on: part 2

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Abstract

Up until February 2006, variant CJD (vCJD), the human disease associated with transmission of BSE from cattle, has been confirmed in 160 patients resident in the UK and 28 elsewhere, some of whom have never visited the UK. Cases have been reported in France (16 cases), Ireland (3), USA (2), Canada, Italy, Japan, the Netherlands, Portugal, Saudi Arabia and Spain (1 each). The presumed main period of hazard for ingestion of the BSE agent in bovine products in the UK is 1984-89, or perhaps up to 1995-6 but at a reduced level. Debated incubation periods for vCJD are discussed, with special reference to the wide, but currently reducing, range of predicted further primary cases in the UK. The primary disease seems to be preferentially acquired by, and expressed in, relatively young people. All but one of the British cases examined so far were homozygous for methionine at the polymorphic codon 129 of the prion protein PRNP gene. Tests of appendix specimens from large numbers of otherwise normal subjects at the time of appendicectomy have revealed lymphoreticular accumulations of PrP^{Sc} in a few samples. Furthermore, three patients who died of vCJD had appendices removed by appendicectomy whilst healthy. Two of these appendices were retrospectively shown to be positive for PrP^{Sc} and one removed 10 years before clinical onset was negative. This has led to worries regarding the possibility of pre-clinical or sub-clinical prion-associated disease in an unknown proportion of the population. To date, there has been no known association of primary vCJD with occupation, medicines, immunising agents, gelatine, or surgery (including the use of catgut sutures), or exposure to bovine products other than by ingestion. There is much concern that human-to-human (secondary) vCJD infection is transferred by blood transfusion. A possible risk is also perceived from infected blood products, human organs and tissues, or via contaminated surgical instruments or devices though, so far as is known, no cases have yet arisen in this way. Steps have been taken to reduce the risks and much research is in hand in this field. Continued TSE surveillance, the maintenance of adequate preventive controls, attention to possible parenteral challenges, and further research studies are of paramount importance.

Key words: BSE, vCJD, active surveillance, rapid testing for PrP^{Sc}, TSE Roadmap

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Introduction

In our preceding paper [3], we summarised current issues in relation to BSE in cattle and the possible further impact on public health. Here, we consider uncertainties in regard to the vCJD epidemic that was initiated by the consumption of food produced from BSE-infected cattle and now threatens to be extended by human-to-human transmission.

Transmission of BSE to man

Is the link between bovine BSE and human vCJD clearly established? Biological and molecular strain typing studies have demonstrated clearly that for practical purposes the BSE agent and the vCJD agent are one and the same, or at least have a similar pathogenicity for man and for macaque monkeys to which

both agents have been transmitted. The association between BSE and vCJD is further supported by the epidemic curves, the presumed response to the various bans, the time-scales of the two diseases and their geographical occurrence (Fig. 1, Table I).

Following a recommendation from the Southwood Working Party on BSE, from 8 Aug 1988 all cattle suspected to have BSE were removed from all food and feed chains and destroyed. Subsequently, a specified bovine offal (SBO) and finally a specified risk material (SRM) ban was introduced and controls were put in place on the use of the vertebral column and restrictions on the manufacture and sale of mechanically recovered meat (MRM) (Fig. 1). Epidemiological enquiry into vCJD has revealed no association with occupation, medicines, immunisation, gelatine, surgery (including
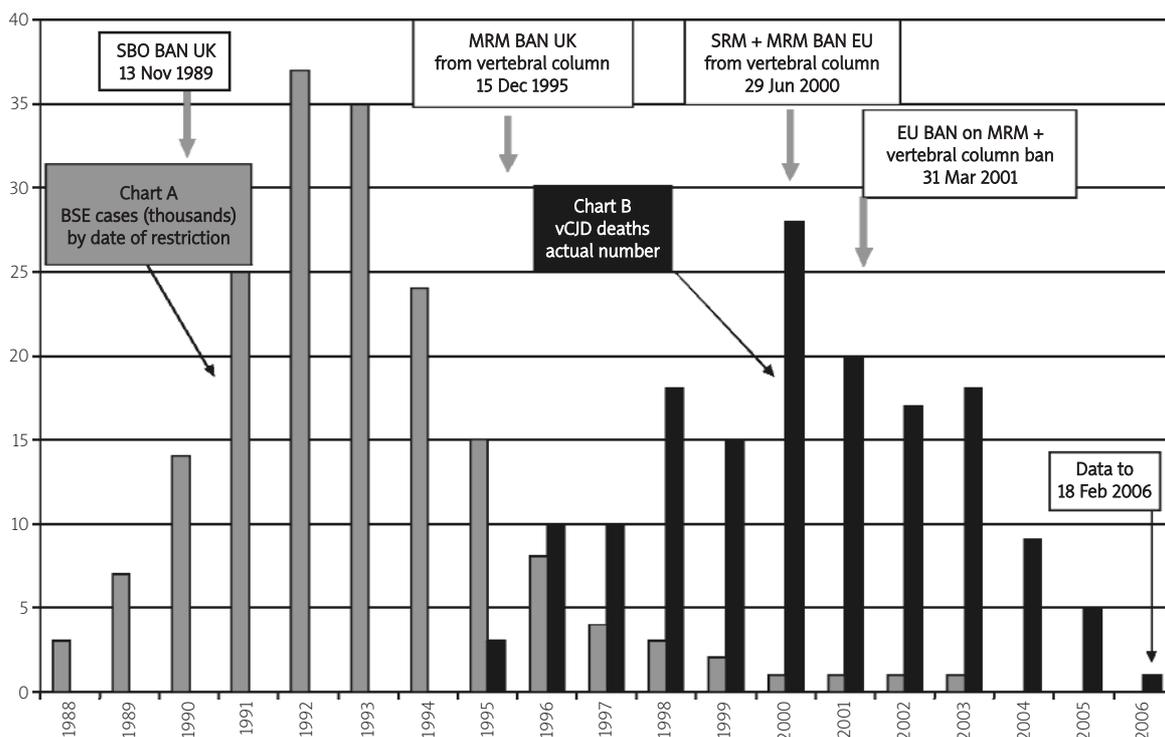

Fig. 1. Epidemic progress of BSE (Chart A) and vCJD (Chart B) in the UK and dates of the main bans imposed to protect public health in the UK and EU

Source: Ban data DEFRA [14]; BSE data OIE [34]; vCJD data National CJD Surveillance Unit [33].

Note: The 1989 SBO ban for Scotland and Northern Ireland did not become law until Jan. 1990. It was extended on 25 Sep. 1990 to prohibit use of SBO in any animal feed and prohibited exports to other EU Member States. On 10 July 1991 export to third countries was prohibited by Department of Trade and Industry legislation. The EC legislation is complex and individual documents should be consulted for more precise information. Although it appears that harmonized legislation for the EU was much delayed, formal proposals for it commenced in 1997. Furthermore some Member States with BSE had implemented some form of national SBO ban in advance.

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Table I. Definite or probable cases of vCJD worldwide and associated cases of BSE (and date of first report) in countries where both diseases occur

Country	vCJD cases*	Total BSE cases	First BSE report**
Great Britain	UK – 160	180,892	1986
Northern Ireland	154 + 6 alive	2,160	1988
France	16	969	1991
Republic of Ireland	3	1,558	1989/1989
USA	2	2	2004/2005
Portugal	1	988	1990/1994
Spain	1	590	2000
Italy	1	131	1994/2001
Netherlands	1	78	1997
Japan	1	22	2001
Canada	1	6	1993/2003
Saudi Arabia	1	0	

Sources: Cattle data OIE [35], DEFRA [13]; vCJD data UK, National CJD Surveillance Unit [33], vCJD data other countries, SEAC [39].

Note: Figures vary slightly depending on the source of information and the date of central reporting.
*Deaths are ascribed to countries on the basis of the country in which clinical signs commenced. The country of exposure is rarely known with certainty (except for cases of secondary human to human transmission). If exposure was from food, it could be from imported contaminated products. Nevertheless it is most likely that at least one case in Ireland, both cases in the USA and 1 case each in Canada and Japan were exposed in the UK.
**In countries where BSE has occurred in imported and native-born cattle and the first case of the former preceded the latter, two dates are given. The first is the date of report of the first imported case and the second the date of the first native-born case.

the use of catgut derived from cattle intestine) or exposure to bovine materials or by-products by routes other than orally. Even brain consumption (as brain) has not been incriminated though this last cannot be completely ruled out because brain might have been included in processed food products before the 1989 SBO ban was in place or complete. A theoretical source that has caused great concern (to the extent that it is now completely banned from use in the EU) is mechanically recovered (or separated) meat (MRM) from ruminant animal bones. Historically, skulls were not used to prepare MRM because the teeth were destructive to the machinery. The specific risk would be from the vertebral column because it is virtually impossible to remove all vestiges of spinal cord, dorsal root ganglia (DRG) and associated autonomic and spinal nerves. Thus, there is a global restriction recommended by the OIE that from countries with a controlled or undetermined BSE risk, MRM from skulls and vertebral column from cattle >30 months old should not be traded. As European law prohibits any use or trade of ruminant MRM, any risk from this source is now eliminated completely in the EU.

All of these considerations generate confidence that new exposure of humans born and resident in the EU and Switzerland is most unlikely from cattle. No new challenges for the European population are apparent from cattle even though a variant form of BSE (bovine amyloidotic spongiform encephalopathy, (BASE)) has been detected in a small number of animals in Italy [6]. However, scrapie is presently receiving considerable attention in the EC. Although it is apparently not a risk for humans and has not been shown to be transmitted even to sheep and goats via feed, there are several plans to eradicate it. This is partly because there is no easy way to distinguish the various scrapie agents from the BSE agent in a rapid and convincing way, especially in the live animal, and partly because the presence of scrapie severely restricts international trade in sheep and goats and some of their products. One case of BSE in a goat in France has been reported [3].

Incubation periods for vCJD

In regard to the primary transmission from cattle to man, if the most likely period for transmission of BSE to humans was indeed 1984-89, the incubation periods for the early cases of human BSE infection (vCJD) seem to be of the order of 10 years. If the danger period is extended forward to 1996, shorter human incubation periods are a possibility. On the other hand, if the risk of human infection extended back before the 1980s, much longer incubation

periods might be postulated. Since the date of exposure for no individual is known, the actual length of the incubation period is also unknown, though analysis of the epidemiological data enables a reasoned judgment to be made at least for patients with the *PRNP* MM polymorphism.

By contrast, and in regard to secondary transmission from man to man as a result of blood transfusion, for two patients (see below) the incubation period is known with a greater degree of precision, namely 6.5 years [31] and 8 years [10]. Assuming transfusion to be the cause in these cases, there would be no species barrier. Furthermore, the intravenous route of exposure is orders of magnitude more efficient than the oral route and the amount of blood transfused is likely to be greater than the amount of infected cattle product consumed at one meal. These considerations seem to be consistent with the (presently unproven) assumption that a shorter incubation period is likely in secondary (man-to-man) transfused infections than in primary transmission from cattle by the oral route (*c.*10 years or so). Caution should be exercised however, because we do not know the titre of infectivity in human blood from an infected donor (estimated to be 0-60 i/v ID_{50}/g) [15]. This is likely to be orders of magnitude less than in brain material from affected cattle (10^6 cattle i/c ID_{50}/g) [5] or humans (estimated to be 10^8 i/c ID_{50}/g [15]) and bearing in mind that the i/v route is about 10 times less efficient than the i/c route [15].

Longer incubation periods could arise in patients with codon 129 MV and VV polymorphism (see below). Parallels have been drawn between vCJD and kuru, a TSE associated with endocannibalism and funeral rites in the eastern highlands of Papua New Guinea where the range of incubation periods extends from 4 to more than 40 years. Thus, it has been postulated [9] that some early cases of vCJD seen in the UK may have received their infections earlier than the mid-1980s and could represent infection acquired before we were even aware of BSE in cows. This is worrying, but a note of caution is justified on at least two counts. It seems that a factor, or several factors, operated when BSE transmitted to human patients. Perhaps this is related to the size of the challenge dose, or to repeated (cumulative) challenges, though evidence for the latter is unconvincing [16,21,28]. It may be that a coincident infection acted to make a victim susceptible to the BSE (vCJD) agent at a particular

time (sometimes perhaps even early in childhood, in the case of teenagers who have died). Indeed, various models indicate that vCJD infection seems to be preferentially acquired by young people, and older subjects may be more resistant [11,41]. We do not yet know if they resist infection or become infected but do not develop clinical disease, either at all, or after extended incubation.

In contrast to the situation with BSE in cattle [3], since 1996 the average age of vCJD patients has not changed significantly (median age at onset 26 and at death 28 years) [32] despite the fact that the major exposures from food were probably confined to a relatively short period. As noted above, teenagers and young adults might be more susceptible to infection and develop clinical signs more readily [11,41], such as may have occurred in the kuru epidemic where some young children succumbed and some adults exposed around the same time had very long incubations indeed. However, it is important to note that the agent that causes vCJD is unique among human TSE agents and is biologically indistinguishable from the BSE agent [3], so kuru might not be a good model to guide us. Another consideration is that, in the UK, young people may have selectively consumed food containing mechanically recovered meat (MRM) with a potentially high TSE-risk [3].

BSE/vCJD agent, PrP and its associated gene

Prusiner and colleagues suggested that the infectious agent in BSE, and thus in vCJD, is a prion - a small proteinaceous infectious particle that resists inactivation by procedures which modify nucleic acids and thus may be devoid of detectable DNA [37]. To date, the prion is known to be very small, passing through small-pore filters, and to be remarkably heat-stable and resistant to a wide range of antimicrobial chemicals including formaldehyde when tested in crude mixtures of infective brain homogenates. Despite this, chemical and physical methods have been devised that are effective and widely used in hospitals, autopsy rooms and industrial suites to secure safety [40]. The importance of thorough cleaning is stressed, with careful removal of all traces of protein from surfaces before disinfection and from surgical instruments prior to sterilization.

The particle has not yet been visualized as such, but some structures called scrapie-associated fibrils

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and prion rods, seen by electron-microscopy after chemical extraction and treatment of infective material, may be aggregates of prions. The terminology has evolved in a confusing manner. A host-encoded glycoprotein that is a normal constituent of cell membranes in man and animals is designated PrP^C. The letter C denotes the normal form of the protein expressed in many cell types and notably in the brain. The abnormal form PrP^{Sc} was so called because it was first associated with scrapie (Sc) in sheep but is commonly used to relate to any TSE. (Various other symbols are used to differentiate between the normal and abnormal forms of PrP.)

Attention has been drawn to codon 129 of the gene that codes for PrP^C in man, the prion protein (*PRNP*) gene. Homozygous individuals may have two methionine alleles (MM) or two valine (VV) alleles. Heterozygous individuals have one of each allele (MV) at codon 129. All of the patients who have died of vCJD and have been genetically investigated so far are homozygous for methionine (MM). A possible, but uncertain, exception was a heterozygous (MV) patient apparently infected by blood transfusion from a donor who subsequently developed vCJD [36]. The heterozygote recipient developed no neurological symptoms prior to death and showed no evidence of vCJD-related neuropathology or PrP^{Sc} accumulation in the central nervous system. This case is therefore provisionally considered to be an asymptomatic vCJD infection that may (preclinical case) or may not (subclinical case) have gone on to be expressed as vCJD. Present evidence indicates that MM homozygosity at codon 129 is associated with susceptibility to vCJD. Alternatively, it may be that MM is a factor that predisposes to a shorter incubation period, whilst MV heterozygosity or VV homozygosity may be considered as factors conferring relative resistance or longer incubation periods. If this is the case, then we might expect to see another wave or waves of cases of vCJD representing infections that were incubated in MV or VV patients. Yet another worrying possibility is that the disease produced in the latter patients (if any) might differ clinically from vCJD in patients with the MM allele. These points are part of the reason for caution when the experts are pressed to predict the future course of events in this difficult field.

Studies of the *PrP* gene of cows (which, like most other animal species, are homozygous for methionine at the equivalent codon) have revealed polymorphisms in the octa-repeat region but these show no association with disease occurrence. It is believed that cattle are uniformly susceptible to BSE. By contrast, the sheep *PrP* gene is polymorphic at several codons and notably at codons 136, 154 and 171. Some genotypes are highly susceptible to scrapie, others are much more resistant and some are of intermediate 'resistance'[20]. Use has been made of this variability by breeding rams homozygous for 'resistance' to increase the desirable alleles in the national flock in various European and other countries [12].

vCJD: Concepts and trends

The change from normal PrP^C to misfolded PrP^{Sc} is post-translational. The misfolding results in the conversion of an α helix-rich protein to a form rich in β sheet, for example by a process of dimerisation in a type of chain reaction. The abnormal form is partially protease-resistant whereas PrP^C is denatured by proteases and this enables a distinction to be made between the two. Results of experimental studies in rodents suggest that, in natural human infection by the oral route, it is likely that the agent is initially taken up by migrating intestinal dendritic cells. These transport it to follicular dendritic cells (FDC) resident in lymphoreticular sites in the gut and elsewhere such as in Peyer's patches of the jejunum and ileum and lymphoid tissue of the appendix and large intestine. FDC maturation is assisted by lymphocytes, suggesting an important role for these cells in TSE, including increasing the risk of infection in chronically inflamed tissues [30]. Accumulation and/or replication of infectivity in the FDC is a necessary prelude to neuroinvasion effected through peripheral nerves of the autonomic nervous system to the spinal cord and then the brain [1,8,38]. PrP^{Sc} accumulates in the brain, infectivity titres rise and there is progressive neurodegeneration leading to death. Patients with symptoms and signs of the disease that we now recognise as vCJD were first observed in the UK in 1995. The number of definite or probable deaths from vCJD in the UK and worldwide are given in table I and the UK data are presented graphically in figure 1. Annual numbers of deaths from vCJD rose thereafter to a peak of 28 in 2000 and have then fallen progressively (at least, up to Feb. 2006). Once again, however, a note of caution is needed. The number of onsets of vCJD (new cases each year) increased in 2004 (9 cases) compared with 2003 (5 cases). This

may be a more reliable indication of the current trend and must moderate our optimism.

vCJD: The uncertain future

The ominous interpretation suggested by Collinge [9] is that the graph of the UK figures might include some cases of infection acquired before the overt infection in British cattle, so that an unknown number of human cases relating to infection acquired in the 1980s and later have still to be accounted for. It is tempting to draw premature but more reassuring conclusions from the shape of the graph of these mortality figures. If the patients with vCJD seen in the last decade were not attributable to infections acquired from 1984 or later (when the BSE outbreak started), they would be part of a cohort of patients who had long incubation times of 12-22 years. If such cases were going on to represent the extremes of long incubation, as in kuru, their numbers should not be so strikingly affected by the controls that were introduced from 1988-1996. Perhaps, however, there might be a strange biphasic character in their presentation, and a possible explanation could rest on the influence on incubation times of MV heterozygosity or VV homozygosity at the polymorphic codon 129. Even without this influence of host genetics on possible susceptibility, Cooper and Bird [11] have noted that subjects in the birth cohorts studied by them had different dietary exposure intensities to BSE that prevailed before and after significant preventive actions in 1989. Their calculations indicate that, for presently unknown reasons, significant numbers of later (primary) cases of vCJD may yet be seen in the UK in the present decade as a second wave of patients who presumably ingested the agent in cattle meat products containing or contaminated with SRM.

The shape of the graph of vCJD incidence in the UK in the last decade seems to be compatible with the view that we are experiencing the effects of infection transmitted to human patients when the BSE outbreak occurred in cows in the mid-1980s or 1990s, or just a few years before this, say, in the early 1980s. In the light of the worst predictions on this premise, we have been fortunate so far. Most of these early extrapolations were based on the assumption that all of the deaths from vCJD recorded to date in the UK were attributable to infections resulting from the overt outbreak of BSE in cattle. This is a reasonable basis for prediction, though some of the earlier published estimates were alarming and present estimates are more reassuring (but see below). In the present paper, we have taken account of alternative suggestions that there may be another (later) human sequel to the bovine outbreak. We also bear in mind that BSE and cases of vCJD in other countries may have longer courses to run.

Most of the literature on BSE and vCJD to date implies that vCJD in man is acquired as a primary disease from cattle. The probable (secondary) human-to-human transmission of vCJD infection via transfusion of infected blood now appears to be almost certain, but doubt remains (as noted above) about the patient originally reported by Peden et al.[36], who may be an example of an asymptomatic infection in an MV heterozygote recipient. A death has certainly been reported in a methionine homozygote recipient who received blood from a donor who was healthy at the time but who developed and died from vCJD subsequently [31]. The most recent report in Feb. 2006 is of another patient who is symptomatic but presently alive [10]. There is much concern that further cases of vCJD may arise in this way. The worry extends beyond the UK to other countries across the world and it has led to major alerts and product withdrawal, with restrictions on donors of blood, organs and tissues. There are on-going urgent reviews of the safety of human blood and plasma and derived products, and similar reconsiderations of the safety of human tissues and medical and surgical instruments that may have become contaminated with the vCJD agent. In the context of transfusion, the use of leucodepletion is a recognised step to safeguard blood for transfusion, but it is not considered to be an absolute protection as tests (with blood from scrapie-infected hamsters) showed that about half of the potential infectivity may remain after the treatment [22].

Awareness

There is another area of concern relating to the widespread distribution of PrP^{Sc} that is known to occur in lymphatic tissues of vCJD patients [26] when tests are done based on immunohistochemical (IHC) techniques and/or sensitive Western Blot technology [26,29]. There is evidence that, at least in the spleen and the tonsil, infectivity can also be demonstrated [4].

A worrying development has been the finding of PrP^{Sc} in an appendix removed from an otherwise

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healthy patient who went on to develop vCJD 8 months later [23]. In 2004, a report mentioned another incident where appendix tissue removed at appendicectomy was positive for PrP^{Sc} two years before the symptoms and signs of vCJD appeared and four years before death [25]. Appendix tissue removed from a third patient at appendicectomy 10 years before vCJD was diagnosed, was negative for PrP^{Sc} [4].

Three anonymous surveys have been done to determine the prevalence of PrP^{Sc} in appendices removed at routine appendicectomy. In the first, no positives were found in 3075 samples [27]. In the second survey, 1 positive appendix was found out of 8318 specimens examined by IHC [24] and the distribution of PrP^{Sc} was similar to that found in the two preclinical samples noted above. In the third study, 3 out of 12,674 appendix samples were positive for PrP^{Sc} by IHC [25]. The latter figure gives an estimated prevalence of 237 vCJD infections per million in the UK (95% CI 49-692 per million) [25]. However, although the immunocytochemical method performed to detect PrP^{Sc} in this study appears to be specific, it is unlikely to be fully sensitive for all phases of the (unknown) incubation period, and this causes further uncertainty in the estimation of the numbers of vCJD infections that have occurred. Similar anonymous studies have been done on tonsils removed at tonsillectomy. In the first of these studies [27], all 95 tonsils examined by IHC were negative for PrP^{Sc}; and, in the second tonsil survey, no positives were found in 2,000 tonsils examined [18].

The positive findings with appendicectomy specimens have led to the speculation that pre-clinical or sub-clinical vCJD may be present in a proportion of the population. There is an understandable call for large-scale prospective screening to provide evidence for estimations of prevalence and to increase awareness of possible secondary transmission in surgery, particularly in relation to young people who may have been exposed to BSE before it was brought under control, though the magnitude of any hidden pre/subclinical population is likely to be underestimated [29].

All of these considerations increase the urgent need for tests that would reliably and speedily determine whether a patient may be in danger of developing vCJD, ideally by a minimally invasive procedure. Promising results are being reported, for example by Castilla, Saá and Sato [7] who have employed protein misfolding cyclic amplification (PMCA) technology to amplify very small amounts of PrP^{Sc} (that might be present in blood) by over 6,500 times and thus substantially increase the sensitivity of the test. A number of other potentially useful tests for detecting tiny amounts of the misfolded protein are under development and are being presented at recent and future meetings on TSE and blood. For a review of diagnostic methods in animals see Gavier-Widén et al. [19]. Technology and research is also progressing in the elimination of TSE infectivity and decontamination and the evaluation and control of instrument- and device-borne prion infection [2,17].

Concluding remarks

Our analysis of the currently available data allows us to be optimistic in foreseeing the elimination of BSE in the EU and countries that follow the EU plan. The outlook is necessarily less assured for the control of BSE in other countries adopting less stringent remedies. In consequence, in regard to vCJD in 'Europe', we are content that there has been effective and virtually complete elimination of food-borne BSE from cattle to man, but we are more cautious for some countries elsewhere. The current trends in the human epidemic of vCJD in the UK are reassuring, but we note that the present reduction in numbers of these cases stems from measures adopted in the veterinary, rather than the medical field. We are presently much more uncertain about the future trends in the human epidemic that might result from a currently concealed, unknown number of infected people in the European population, due either to their *PRNP* genotype (MV or VV) or to existing pre-clinical or sub-clinical infection. If such concealed populations exist, are their numbers too small to permit maintenance of the epidemic, or are they already considerable and being added to by current practices? There is a need for continuing surveillance for BSE and all forms of CJD in the UK, EU and indeed globally, in view of the uncertainty of the occurrence and extent of BSE in many countries of the world, the absence of fully effective measures and their enforcement, and the uncertainty about future trends in the human epidemic of vCJD in the UK despite effective controls on BSE.

The hazard of vCJD has greatly complicated the careful vetting of blood donors and there is an urgent requirement for a test that might detect infected persons. We are convinced that blood transfusion is a means of transmission of vCJD but note that the recent UK infections occurred before leucodepletion of

blood was in place. Currently we know of no certain human-to-human transmissions by other routes or mechanisms, but it is probably too early to say that they will not occur. Whilst we are strongly encouraged by the actions taken to research and to reduce infectivity in blood and to determine effective ways to clean and decontaminate instruments and surfaces, we are aware that many problems remain to be solved if we are to deal effectively with this daunting challenge.

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Note added in Proof (June 2006)

The *PRNP* genotype of two of the three appendix tissue samples that tested positively for PrPSc in the large retrospective prevalence study referred to in this paper has now been determined [1]. The genotype in both is confirmed as homozygous for the valine allele (VV) at codon 129 of the *PRNP* gene. There was insufficient material to test the third sample. This is the first indication that valine homozygotes may be susceptible to vCJD infection and shows now that all *PRNP* codon 129 genotypes may be susceptible to vCJD infection, which was not known before.

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