Chapter 5
Environmentally Acquired Transmissible Spongiform Encephalopathy

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Abstract From the ritual cannibalism of kuru to the modern "cannibalism" of iatrogenic and variant forms of Creutzfeldt–Jakob disease, the history of environmentally acquired spongiform encephalopathy is reviewed. Sources, original recognitions, inter-relationships, and distinctive characteristics of the various forms of disease are discussed, credits (and debits) are acknowledged, and failures and victories recalled as the era of acquired CJD draws to a close.

Keywords Kuru • Iatrogenic Creutzfeldt–Jakob disease • Variant Creutzfeldt–Jakob disease • Bovine spongiform encephalopathy • Human growth hormone • Dura mater grafts • Neurosurgery • Blood-borne infection

5.1 Kuru

The prototype of human transmissible spongiform encephalopathy (TSE), kuru was almost certainly spread through the practice of ritual cannibalism, and was proven to be experimentally transmissible to primates in 1966 (Gajdusek et al. 1966). It is now mainly of historical interest, but certain epidemiological and clinical features are relevant to the later occurrences of iatrogenic and variant forms of Creutzfeldt–Jakob disease (CJD). From oral accounts by elders in the afflicted Foré-speaking peoples in the Eastern Highlands of Papua New Guinea, the disease first appeared early in the twentieth century and rapidly achieved epidemic proportions. The best guess as

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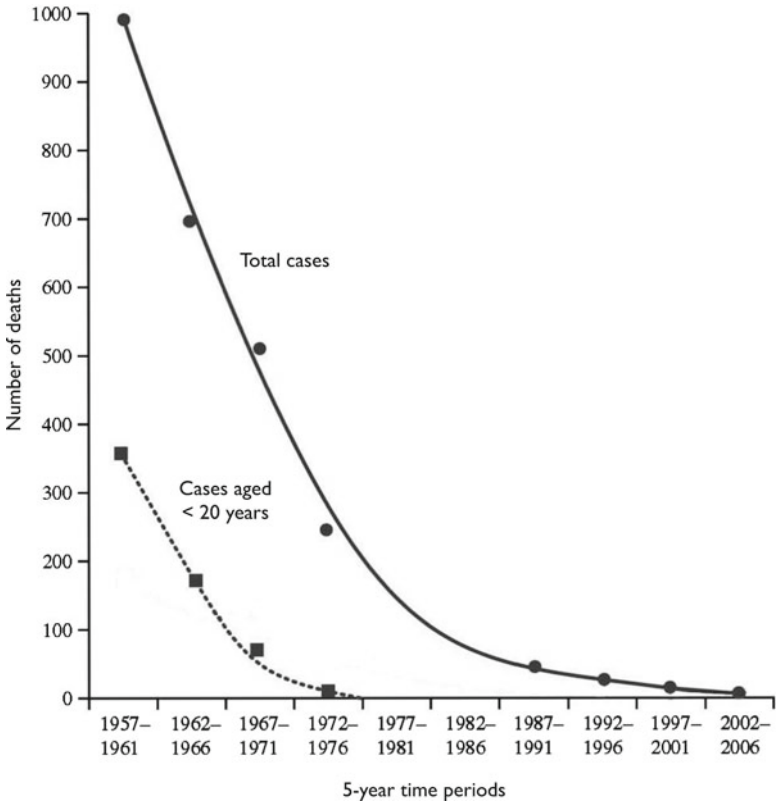

Fig. 5.1 Kuru mortality 1957–2006. Only one case has been identified during the most recent period (2007–2011). (Modified with permission from Alpers MP (2008) The epidemiology of kuru: monitoring the epidemic from its peak to its end. Philos Trans R soc B 363:3707–3713)

to its origin is the cannibalistic consumption of a random case of sporadic CJD among the Foré, which then spread via the continued practice of ritual cannibalism through the 1950s, when missionaries and the Australian colonial administration used a "carrot and stick" approach to eliminate the practice (fines or jail versus trade goods). The average incubation period is estimated to have been 12 years, and the age-specific "dieback" of the disease began with the youngest individuals—i.e., those who had been most recently exposed (Fig. 5.1) (Alpers 2008). Since the turn of the century, there have been only eight deaths: three in 2000, two in 2001, one in 2003, one in 2005, and one (the latest, and possibly the last) in 2009. Four were male and four were female, all in older adults between 55 and 62 years of age (personal communication, Dr. Michael Alpers).

It is ironic that the high incidence of kuru in children and young women was not, as originally thought, due to hormonal or genetic factors, but a much more prosaic reason: women, surrounded by their infants and young children, prepared the bodies

for cooking and were also the principle consumers of brains and viscera. It is also ironic that "morality" rather than medicine brought an end to the disease.

Two further features of kuru are interesting in the context of environmentally acquired CJD. The clinical syndrome was predominantly cerebellar, with little or no dementia, a feature that would also characterize peripheral infection from contaminated cadaveric human growth hormone, but not oral infection from bovine spongiform encephalopathy (BSE). Also, the age at onset of disease (a rough indication of the incubation period) was, on average, considerably shorter in codon 129 homozygotes than heterozygotes, but with a significant early overlap between the two, which may yet bear on questions about the future incidence of variant CJD (vCJD) due to infection by the agent of Bovine Spongiform Encephalopathy (BSE) (Cervenáková et al. 1998).

5.2 Creutzfeldt–Jakob Disease

Environmentally acquired forms of CJD occupy a far more important niche in the ensemble of TSE than their numbers would suggest. This importance lies in two facts: they can be prevented (if their cause is recognized), and they stimulate public concern, which translates to public funding of the whole field of TSE, without which research shrinks to the level accorded the category of "orphan diseases". We are seeing this phenomenon today as iatrogenic CJD, BSE, and vCJD recede into the background of public and government consciousness. Their chronology falls conveniently into four successive decades.

5.3 Iatrogenic CJD

Iatrogenic CJD has very recently been reviewed and brought up to date through the end of 2011 (Tables 5.1 and 5.2) (Brown et al. 2012). Selected historical references are included in the following account, together with a few more recent references; however, most national and regional surveillance teams have either used internet web sites to provide updated numbers, or have not made the information available to the general public.

5.4 The 1970s: Cornea and EEG Depth Electrodes

Somewhat more than a year after publication of the experimental transmission of CJD to a chimpanzee in 1968, a 55-year-old man died of pneumonia following a 2-month history of "incoordination, memory deficit, involuntary movements and myoclonia" (Duffy et al. 1974). At autopsy, a cornea was removed and transplanted

Table 5.1 Global distribution of cases of iatrogenic CreutzfeldtJakob disease

	Surgical procedures				Medical procedures		
	Dura Mater Grafts	Surgical instruments	EEG needle	Corneal transplants[a]	Growth hormone[b]	Gonadotropin	Packed red cells[c]
Argentina	1						
Austria	3				1		
Australia	5					4	
Brazil					2		
Canada	4						
Croatia	1						
France	13	1			119		
Germany	10			1			
Ireland					1		
Italy	9						
Japan	142						
Netherlands	5				2		
New Zealand	2				6		
South Korea	2						
Qatar					1		
South Africa	1						
Spain	14						
Switzerland	3		2				
Thailand	1						
UK	8	3			65		3
USA	4			1	29		
Totals	228	4	2	2	226	4	3

[a]Additional possible single cases following corneal transplant or keratoplasty (not included in table) in Japan, the UK, and the USA

[b]Brazil and New Zealand hGH was prepared in the USA; Qatar hGH was prepared in France. Additional possible single cases due to hGH (not included in table) in Sweden, Australia, and New Zealand

[c]One additional asymptomatic but infected red cell recipient died of an unrelated illness; another asymptomatic infected hemophilia patient who had been exposed to potentially contaminated Factor VIII also died of an unrelated illness (neither is included in the table)

Table 5.2 Clinical features of environmentally-acquired Creutzfeldt–Jakob Disease according to the source and route of infection

Source of infection	Number	Agent entry presentation	Mean incubation period (range)	Usual clinical presentation
Corneal transplant[a]	2	Optic nerve	18 months, 27 years	Dementia/cerebellar
Stereotactic EEG	2	Intracerebral	16 months, 20 months	Dementia/cerebellar
Neurosurgery	4	Intracerebral	21 months (18–28 months)	Visual/dementia/cerebellar
Dura mater graft	228	Cerebral surface	12 years (16 months–30 years)	Cerebellar (visual/dementia)
Growth hormone[b]	226	Hematogenous (?)	17 years (5–42 years)[c]	Cerebellar
Gonadotrophin	4	Hematogenous (?)	13.5 years (12–16 years)	Cerebellar
BSE-infected tissue (1°vCJD cases)	224	Oral	12–15 years	Psychiatric/sensory
RBC transfusion[e] (2° vCJD cases)	3	Hematogenous	6.5, 7.8, 8.3 years	Psychiatric/cerebellar

[a]Additional possible case in Japan

[b]Additional possible single cases in Australia, Scandinavia, and New Zealand

[c] Combined data from France, the UK, and France, based on estimated dates of Infection at the mid-point of multi-year therapy: France, 13 years; UK, 20 years; USA, 22 years

[d]Estimate based on epidemiologic data for BSE and vCJD (dates of infection for primary cases of vCJD are unknown)

[e]One additional asymptomatic but infected red cell recipient died of an unrelated illness; another asymptomatic infected hemophilia patient who had been exposed to potentially contaminated Factor VIII also died of an unrelated illness (neither is included in the table)

into a 55 year-old woman. The autopsy later revealed a diagnosis of CJD. The recipient became ill 18 months later and had a clinical course typical of CJD, also confirmed at autopsy, and subsequently by transmission of the disease to an intra-cerebrally inoculated primate in D.C. Gajdusek's laboratory at the NIH. The case is interesting for at least three reasons, apart from being the first recognized instance of iatrogenic CJD. First, the interval of 18 months between the operation and onset of disease in the recipient was short enough for the connection to have been suspected; had it been many years instead of many months, it might have gone unrecognized and never come to light. Second, it only occurred because of the "lead time" needed for scientific research to disseminate through the general medical community—in this case, the clinical features and transmissibility of CJD. Even a few years later, the diagnosis would certainly have been strongly suspected and cadaveric tissues never used for corneal (or any other) tissue transplant. And third, brain tissue from the recipient that was used in the successful transmission experiment had been stored in formalin for several months prior to inoculation.

Only two other instances of corneal transplant transmission of CJD have occurred in the 40-odd years since this case was reported, and neither can be considered definite. In one case, CJD developed 16 months after a corneal transplant, but the cause of death in the donor was not established; in the other case, both donor and recipient died of neuropathologically verified CJD, but the interval between transplant infection and clinical signs was 30 years. It seems likely that donor deferral criteria based on an ever-increasing diagnostic awareness have been largely responsible for the absence of additional cases.

A second episode of surgical contamination, reported in 1977 (Bernoulli et al. 1977), occurred in 1974 in association with depth electrodes that had been used on a 69-year-old woman with CJD, sterilized with 70% alcohol and formaldehyde vapor (standard practice at that time), and re-used in two patients with intractable epilepsy. The latter two patients developed illnesses consistent with CJD about 2½ years later, and postmortem examinations confirmed the diagnosis in each patient. Two features of this episode merit comment. First, the implicated needles were sent to Gajdusek's laboratory and implanted in the brain of a chimpanzee that subsequently died of CJD, proving the iatrogenic cause of the disease, which to this day remains the only formally proven case of iatrogenic CJD. The second point of interest is that one of the recipients was a 23-year-old woman who became pregnant 14 months after the operative procedure, and who delivered by Caesarian section a normal male infant, who was in good health when last contacted at the age of 12 years.

In two subsequent retrospective studies, neurosurgical cross-contamination of instruments was found to be probably responsible for three cases in the UK and one case in France during the 1950s (Will and Matthews 1982; El Hachimi et al. 1997). The absence of neurosurgical contamination in recent years is difficult to explain, as operations on patients with undiagnosed CJD continue to occur, and instrument sterilization protocols in many hospitals remain suboptimal. It may be due to a combination of (1) a more widespread awareness of the need to consider CJD among neurological differential diagnoses; (2) more rigorous sterilization protocols and the increasing use of disposable instruments on any suspect or known CJD

patient, especially in the UK, where a nation-wide program of optimized sterilization or one-time use of such instruments has been mandated; and (3) a failure to recognize cause and effect without long term post-operative surveillance.

5.5 The 1980s: Human Growth Hormone (hGH) and Dura Mater Grafts Human Growth Hormone

The decade began quietly enough, although well before the first intimation of trouble in the growth hormone sector, the possibility of risk was already under study in Alan Dickinson's laboratory in Edinburgh, based on his appreciation of the fact that the pituitary was closely associated with the brain and thus likely to be infectious. His instincts were correct: in 1985, four young adults dying of CJD within the previous year had all been treated in the 1960s and 1970s with human growth hormone extracted from cadaveric pituitary glands. The first case, in a 21-year-old man whose diagnosis was not established until post-mortem examination, was the subject of a letter by Dr. Raymond Hintz, a Stanford pediatric endocrinologist, to Dr. Mortimer Lipsett, Director of the NIH institute responsible for the US human growth hormone distribution program (Brown 1988):

> "…the patient was treated for 14 years with growth hormone, and I feel that the possibility that this was a factor in his getting Creutzfeldt–Jakob disease should be considered. A careful follow-up of all patients treated with pituitary growth hormone in the past 25 years should be carried out, looking for any other cases of degenerative neurological disease."

Lipsett acted immediately by notifying all prescribing pediatricians at the hormone distribution centers of a possible problem. A few days later, on a flight from Washington to a meeting in Athens, Gajdusek remarked that Lipsett had called him about a possible case of CJD in a growth hormone patient, adding that it looked like there might be an epidemic in the works (his travelling companion, who would subsequently head the NIH investigative panel, did not think it likely). Within a month, two further cases surfaced, prompting Lipsett to shut down the entire program, and the FDA to rush through the approval process for a recombinant product that was then under evaluation.

As more and more cases came to light in the USA, UK, and France, it became clear that contamination was widespread, but its severity could not be predicted—would it become a full-fledged epidemic, or would it remain limited to a comparatively small number of cases? In the event, it lay somewhere between the two extremes, with a grand total of 226 cases from 1985 through the end of 2011 (Fig. 5.2). Case numbers for the three principally affected countries were: 29 (USA), 65 (UK), and 119 (France). Considering the at-risk patient population in each country, these numbers yield frequencies of infection of 1.1% in the USA, where no case has occurred in any patient beginning treatment after 1977 when a chromatography purification step was introduced; 3.6% in the UK, where cases continue to appear in patients infected throughout the entire treatment period; and 10.2% in France, where all cases are thought to have been infected within a 2-year window between 1983 and 1985 from

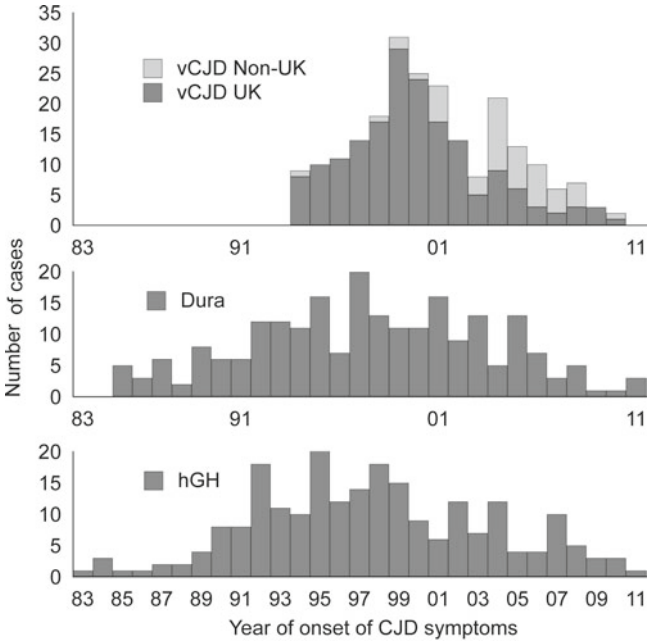

Fig. 5.2 Incidence of iatrogenic CJD due to contaminated cadaveric human growth hormone and dura mater, and of vCJD due to ingestion of BSE-contaminated tissues, 1982–2010

contamination due to both sourcing and processing deficiencies ((Abrams et al. 2011; National Creutzfeldt–Jakob Disease Research Surveillance Unit 2009), and unpublished data).

From a clinical standpoint, CJD infection from peripherally administered growth hormone produced a distinctive evolution of symptoms reminiscent of kuru, almost invariably beginning with cerebellar signs, and little or no dementia during the course of the disease (Table 5.2). The incubation period, estimated from the mid-point of what was usually a several-year course of treatment, was approximately 17 years, but again like kuru, could extend out to 30 years and beyond—the current record for the longest incubation period from any cause of iatrogenic disease is 42 years in a recently diagnosed U.S. patient. Susceptibility to infection was to some extent influenced by the polymorphism at codon 129 of the *PRNP* gene: in France and the USA, methionine homozygotes were modestly over-represented (55%) compared to the normal Caucasian population (40%); in the UK, however, valine homozygotes far outnumbered methionine homozygotes, leading to speculation that a different "strain" of CJD was being disseminated in the UK. In all three countries, heterozygotes as a group had somewhat longer incubation periods than homozygotes.

These epidemiological and clinical observations incriminating hGH as the cause of infection were bolstered by the occurrence of virtually identical disease features in four Australian women treated with human pituitary gonadotropin. Formal proof came in 1993 in a report that inoculation of archived samples of 76 US hormone lots

into over 200 monkeys and several chimpanzees had produced a transmission of disease from one lot to one of two inoculated monkeys, consistent with the occurrence of low-dose random contamination (Gibbs et al. 1993).

5.6 Dura Mater

The original publication discussing the first three cases of CJD in growth hormone recipients concluded with the following paragraph: "We are once again dramatically reminded that human tissues are a source of infectious disease, and that any therapeutic transfer of tissue from one person to another carries an unavoidable risk of transferring the infection. In this context, we must continue to worry about such products as follicle stimulating hormone. luteinizing hormone, prolactin, and human interferon, as well as skin, bone, bone marrow, dura mater, blood vessel, and nerve grafts and organ transplantation" (Brown et al. 1985). This warning was almost immediately confirmed by the onset of what would be a coincidental outbreak of CJD contamination of dura mater grafts used in neurosurgical operations (Fig. 5.2). As with the growth hormone contamination, recognition of the source of contamination could not help the many victims who were already incubating disease from treatment during the previous two decades, but the resulting substitution of synthetic or non-dural tissues for neurosurgical grafts put an end to new cases of iatrogenic disease from this source.

The first case, reported by neurosurgeons at the Yale University School of Medicine in 1987 (Koch et al. 1985), was in a patient who had received a dural graft following the resection of a cholesteatoma 19 months before the onset of CJD. A second case was reported from New Zealand in 1989, and a third case from Italy, also in 1989. As word spread, further cases came to light in several different countries, especially from Japan, which in time would be the setting for two-thirds of the 228 cases worldwide, almost all of which were the result of graft patches processed in the early 1980s by a single German company. The different national incidences were due to the frequency with which grafts were used, rather than from any particular batch contamination, as the fact that cases occurred in 18 different countries over a span of 25 years suggests that contamination was occurring on a regular basis until manufacturing ceased in 1987.

A predominance of codon 129 methionine homozygotes was heavily influenced by the large number of cases in Japan, where methionine homozygosity occurs in over 90% of the general population. Outside of Japan, heterozygotes as a group had somewhat longer incubation periods than homozygotes (similar to what was seen in growth hormone patients). The overall mean incubation period was 12 years, with a range from 1.5 to 30 years. Clinical presentations were usually cerebellar, although some patients presented with dementia, or more rarely, with visual signs. In the large Japanese case population, analysis of presenting signs according to the site of graft placement showed a significant excess of hemiparesis or hemianopsia in patients with supratentorial grafts, and of brainstem signs in patients with infratentorial grafts.

About one-third of the cases had atypical features: slow progression, non-characteristic EEG, plaque deposition (including some patients with "florid" plaques), and an atypical prion molecular "signature" in Western blots that suggested the possibility of two different strains of infecting agent. One patient also had a pulvinar sign on MRI, a feature that is usually seen only in vCJD.

5.7 The 1990s: BSE and vCJD

The following first-hand account of how BSE was discovered is described by Dr. Raymond Bradley (personal communication):

In the Report of the Chief Veterinary Officer of the Ministry of Agriculture, Fisheries and Food of 1986 there appeared an anonymous brief report of a scrapie-like disease in a single, 2¾-year-old captive female nyala in an English wildlife park (not published until 1988) (Jeffrey and Wells 1988). There was no evidence of contact with other animals affected by transmissible spongiform encephalopathy and, at the time, no suggestion that the disease had been transmitted via infected feed. A year later, a scrapie-like disease was reported in the same wildlife park, this time in a captive gemsbok, and similar cases subsequently occurred in an Arabian oryx, a greater kudu, and an eland in other zoos.

All this was surpassed in importance by the discovery in November 1986 of what is now known as bovine spongiform encephalopathy (BSE) in domestic British cattle. Several cattle with an unusual, progressive and fatal nervous disease had been investigated by staff at Veterinary Investigation Centres in southern England but without any conclusion as to the pathological definition or cause of the disease. Formalin-fixed brains from two cows in different herds were submitted to the Pathology Department of the Central Veterinary Laboratory and neuropathologically examined by Martin Jeffrey and Gerald Wells who independently concluded that they were affected by a scrapie-like spongiform encephalopathy (Wells et al. 1987).

During the course of 1987, further cases were identified and there was sufficient evidence available by the early summer to initiate a detailed epidemiological investigation conducted by John Wilesmith, Head of the Epidemiology Department. By the end of the year he concluded that the cause of the epidemic in cattle (and the similar cases in captive exotic ungulates) was due to the consumption of Meat and Bone Meal (MBM) derived from rendered animal carcasses and waste products that were included in the concentrate rations of weaned calves, especially of dairy cattle, as a protein-rich supplement (Wilesmith et al. 1988).

As is now well known, the epidemic that followed in the UK, and some years later in other European countries (Fig. 5.3), together with cases in non-European countries—mostly Japan and Canada—became headline news all over the world, seriously affected the beef industry, and led to a global surveillance for BSE. It will never be known if the outbreaks in countries other than the UK were due to infective

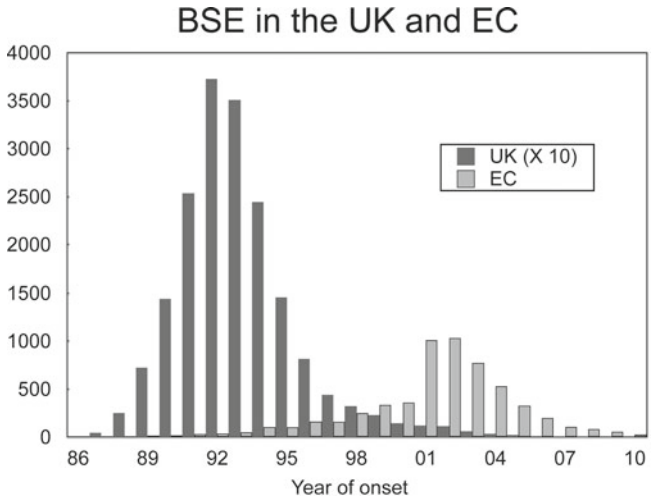

Fig. 5.3 Incidence of BSE in the UK and non-UK European Community, 1986–2010. Note that UK case totals are ten times the vertical axis numbers

tissue (dead or alive) imported from the UK, or from simultaneous endogenous mini-epidemics of BSE due to widespread similar changes in rendering practices.

The more important question was whether BSE could spread to humans, and no one had the answer. If, as thought likely, BSE had its origin in the contamination of MBM by scrapie, and scrapie did not cause CJD, how could humans be at risk? The answer lay in a few laboratory experiments that had documented the fact that a given strain of TSE in one species could be unable to transmit disease to a different species unless first passaged through an intermediate species. The analogy of sheep-to-human versus sheep-to-cattle-to-human infection was clear enough, but epidemiology carried the day, and the consensus was that human infection from BSE was highly unlikely. One speaker at a BSE meeting held in Brussels in 1992 went so far as to conclude his presentation by eating a hamburger brought at his request from the UK by a British colleague. To the chagrin of the assembled scientific experts and government authorities, the consensus was wrong: BSE turned out to be infectious for humans, causing a variant form of CJD that was first identified in 1996 in eight cases of disease in young UK adults that had occurred during the previous 2 years (Will et al. 1996). (The speaker, however, is still alive and well 20 years later.)

The author remembers having been contacted by Prof. Robert Will in late 1995 about the neuropathology of a few young adult cases of sporadic CJD in the NIH collection, and the subsequent clandestine disappearance of several members of the Edinburgh CJD Surveillance team at a meeting in Paris in March of 1996, from which, in strict confidentiality, they had been urgently recalled to the UK to make a presentation to the government's TSE advisory committee, as later described by Richard Rhodes (Rhodes 1997):

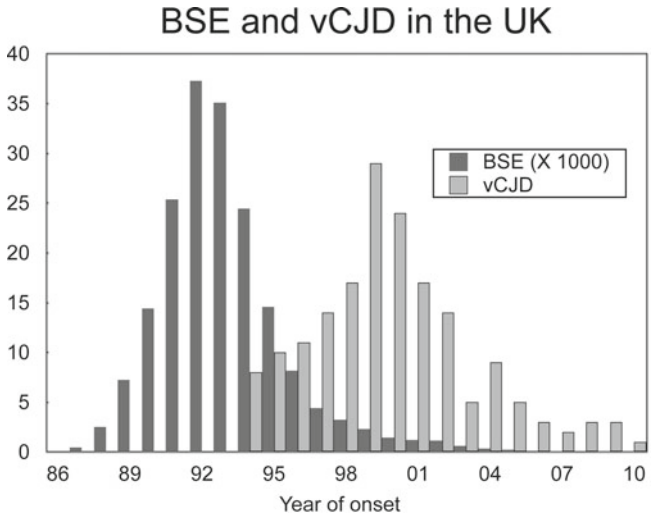

Fig. 5.4 Incidence of BSE and vCJD in the UK, 1986–2010. Note that the BSE case totals are 1,000 times the vertical axis numbers

> "Ironside opened the meeting with slides illustrating the unusual pathology. The SEAC chairman, John Pattison, remembers the moment vividly: "Before he said anything, we could see what it was. It was dramatically different". Another SEAC member, Jeffrey Almond, recalls near-panic. "The atmosphere became genuinely quite tense. Some of us were genuinely afraid of what we were hearing. We were afraid that this really maybe indicated a transmission of BSE to humans".

And with good reason—the number of cases in the UK would rapidly enlarge to attain a peak annual incidence of 29 cases in 1999, and cases also began to appear in other countries in people who had become infected during an earlier period of residence in the UK, or who became infected in their own countries as BSE spread around the world. Indigenous infections were especially prevalent in France, which had been the largest importer of MBM and cattle from the UK. The global total of vCJD through 2011 stands at 224 cases.

The incidence curves of BSE and vCJD in the UK can be used to estimate the average incubation period for vCJD (Fig. 5.4). Observations of naturally infected cattle, and oral dosing experiments using as little as 1 mg of brain (Wells et al. 2007), suggest a reasonable estimate of the incubation period of BSE to be about 5–6 years, with a considerable range upwards. Cattle can therefore be presumed to have first been infected towards the late 1970s, and maximum human exposure would have occurred in the mid-1980s, after the "silent" epidemic was well underway but before BSE had become a concern for humans. A peak incidence of vCJD applies to vCJD patients infected outside the UK, where a further delay was needed for exported BSE to become established, resulting in a non-UK vCJD peak incidence 5 years later, in 2004.

The distinctive clinical characteristic of vCJD is its presentation in the form of behavioral or sensory abnormalities, rather than the dementia/cerebellar/visual syndrome typical of sporadic CJD (Will and Ward 2004) (Table 5.2). However, as the illness progresses, most of the signs of sporadic CJD supervene, and at an advanced stage vCJD is clinically indistinguishable from sporadic disease. Two pre-mortem tests have enhanced the diagnostic presumption of vCJD: all symptomatic cases have had a methionine–methionine coding genotype at polymorphic codon 129 of the *PRNP* gene; and in up to 90% of patients the MRI shows a "pulvinar sign"—hyper-intensity of the posterior thalami. The diagnosis can only be established with certainty, however, by post-mortem examination that, as noted above, reveals the presence of "florid" plaques—globular accumulations of misfolded "prion" protein (PrP^{TSE}) surrounded by a halo of vacuoles.

5.8 The Millennium: Denouement

The era of iatrogenic CJD and BSE-induced variant CJD is rapidly passing into history, but as with most outbreaks of infectious disease, there are always at least a few cases that trail out beyond the expected dates of extinction. In 2011, single new cases of dura mater-related CJD occurred in Austria, Korea, and the Netherlands, and one new case of hGH-related CJD occurred in the UK. In 2012, two new cases of vCJD were identified in France.

With respect to vCJD, the "end" has also been complicated by the recent discovery of three secondary cases and an inapparent infection in recipients of packed red blood cells from asymptomatic vCJD donors (Llewelyn et al. 2004; Peden et al. 2004; Wroe et al. 2006; Health Protection 2006), as well as an inapparent infection in a recipient of plasma-derived Factor VIII (Peden et al. 2010). However, there are several reasons for hoping that further transmissions will not occur. The incubation periods of the three symptomatic cases were 6.5, 7.8, and 8.3 years (Fig. 5.5), (Gillies et al. 2009), and 11 of 26 other recipients of red cell transfusions from these same donors remain healthy or have died from non-vCJD illnesses after an interval of at least 10 years (Hewitt et al. 2006), (updated by Prof. RG Will, personal communication). Also, leukodepletion of red cells was instituted in the UK in 1999 and none of 13 recipients of leukodepleted red cells from these donors has developed disease after intervals of 5–10 years. And finally, the near disappearance of primary cases during the past few years signifies a parallel decrease in the risk of individuals incubating vCJD within the blood donor population.

One other possible cause of future cases needs to be mentioned: the dreaded "second wave" of cases with long incubation periods due to codon 129 genotypes other than methionine–methionine. This is certainly not an unreasonable concern in view of the tendency towards prolonged incubation periods associated with alternative genotypes in both kuru and hGH-related forms of CJD. In each outbreak, however, the alternative genotypes began to appear well before the methionine–methionine cases had been exhausted, and that has not happened with vCJD infections. We are near

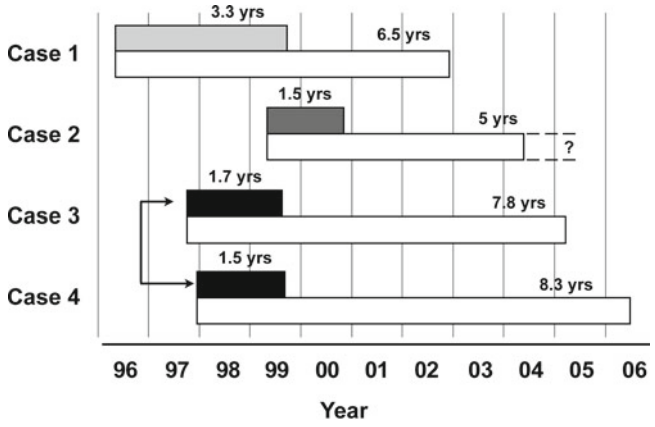

Fig. 5.5 Graph of intervals between transfusions and disease in four instances of secondary vCJD infections transmitted via packed red blood cells from donors who later died of vCJD. *Upper bars* of each pair represent donors and *lower bars* represent recipients. The second recipient died from a non-vCJD illness and was only discovered to have been infected through the use of post-mortem immunohistochemistry. The third and fourth recipients received transfusions from the same donor

the end of the outbreak and not a single symptomatic case of vCJD has occurred in a heterozygote or valine homozygote. The caveat to this observation is the finding of pre-or subclinical infection in the spleens of the heterozygous red cell and Factor VIII recipients mentioned above (Peden et al. 2004, 2010) and in the appendices removed from two homozygous valine individuals in a large UK "blinded" prevalence study reported in 2004 (Ironside et al. 2006). As these two individuals are anonymous, there is no possibility of ever knowing their ultimate fate. A more recent analysis of immunohistochemical tests performed on over 32,000 appendix samples removed between 1995 and 1999 yielded 16 positives, and an estimate of the 'carrier rate' of vCJD infection in the UK of approximately 1 per 2000. No information about the codon 129 status of the positive individuals was given (UK Advisory Committee on Dangerous Pathogens, 2012).

It has been obvious for many years that the most effective means to prevent further environmentally acquired cases of CJD would be a reliable laboratory test to detect pre- or subclinical infection. Around the turn of the century, nearly a dozen different laboratories were working to develop such a test, but all of them experienced problems in applying their methods to human plasma, and commercial interest flagged as the magnitude of vCJD regressed. Today, however, there is renewed interest as a result of a high sample throughput modification (QuIC test) of the PrP^{TSE} amplification technique that promises, finally, to produce a practical blood screening test (Orrú et al. 2011). Until that happens, we will need to continue to depend on the other two means of prevention—recognition and deferral of high-risk donors, and decontamination of instruments and therapeutic products—while maintaining a vigilant attitude towards as yet unidentified future sources of environmental infection.

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