The purpose of this technical manual is to update current knowledge on human prion diseases, in order to assist health officials in the diagnosis, surveillance and response to human transmissible spongiform encephalopathies. The goal is to improve monitoring of the form associated with bovine spongiform encephalopathy ('mad cow disease') and known as variant Creutzfeldt-Jakob disease (vCJD). This document provides required information for understanding the epidemiology, diagnosis and surveillance of human TSEs.

In issuing this document, WHO aims to help public health departments and research centres undertaking surveillance for human TSEs by collecting in one document the relevant case definitions, surveillance methods and reporting tools. In addition, substantial portions of the document describe, in detail, the diagnostic methods for the diagnosis of human TSEs. For those needing training or validation of their diagnostics, contact information for reference centres cooperating with WHO is provided.

Information about handling of potentially infectious tissues, risk communication, relevant literature and related websites is included.

Copies can be obtained from

Communicable Disease Surveillance and Response
World Health Organization
Geneva

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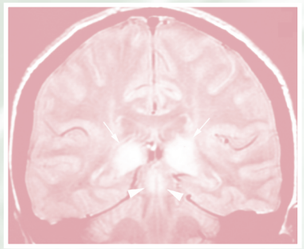
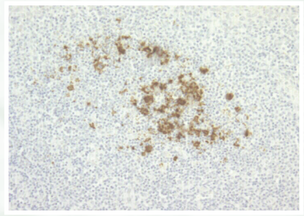
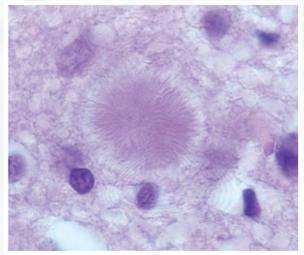
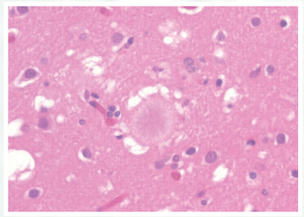
WHO MANUAL FOR SURVEILLANCE OF HUMAN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

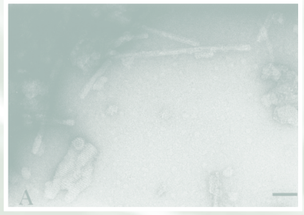
WHO











WHO manual for surveillance of human transmissible spongiform encephalopathies including variant Creutzfeldt-Jakob disease

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Contents

A brief word on nomenclature

Unfortunately much confusion surrounds the terms used to describe CJD-related disorders of humans and the comparable diseases of animals, largely reflecting disagreements over the nature of the causative agent. These conditions have commonly been referred to as: transmissible spongiform encephalopathies, prion diseases, transmissible cerebral amyloidosis and slow-virus diseases. However, no term is perfect. For example, some of the human hereditary forms lack spongiform change neuropathologically or have yet to be transmitted. This text will refer to 'transmissible spongiform encephalopathies (TSEs)' throughout.

Abbreviations

BSE	bovine spongiform encephalopathy
CJD	Creutzfeldt-Jakob disease
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computerized tomography
CWD	chronic wasting disease
EEG	electroencephalogram
fCJD	familial CJD
FFI	fatal familial insomnia
FLAIR	fluid-attenuated inversion recovery
FSE	feline spongiform encephalopathy
GSS	Gerstmann-Sträussler-Scheinker disease
MRI	magnetic resonance imaging
NFT	neurofibrillary tangles
PET	positron emission tomography
PDWI	proton-density weighted imaging
PrP	prion protein
PrP^{C}	cellular isoform of the prion protein
PrP^{Sc}	scrapie, amyloid-forming isoform of the prion protein
PSW	periodic sharp-waves
sCJD	sporadic Creutzfeldt-Jakob disease
SPECT	single-photon emission computed tomography
T1WI	T1-weighted imaging
T2WI	T2-weighted imaging
TME	transmissible mink encephalopathy
TSE	transmissible spongiform encephalopathy
vCJD	variant CJD

CHAPTER 1

Introduction and rationale for global Creutzfeldt-Jakob disease surveillance

In March 1996, 10 cases of a newly recognized form of Creutzfeldt-Jakob disease (variant CJD) were first reported in the United Kingdom. These patients exhibited a novel and distinct clinical-pathological phenotype. Given the temporal-spatial association, an etiological link with the bovine spongiform encephalopathy (BSE) agent was considered likely, although unproven.

As of November 2002, 139 vCJD cases have been reported; 129 in the UK, six in France and one in each of Canada, the Republic of Ireland, Italy and the United States of America. A consistent upward trend in the number of reports in the UK has been observed, but whether this increase will be sustained, or what the eventual number of vCJD cases will be is unknown. Countries without reported cases of either vCJD or BSE may also have been exposed to the BSE agent because of importation of cattle or cattle-derived products from BSE-affected countries.

If indeed vCJD is linked with exposure to the BSE agent, and because the incubation period may be long, the possibility of more cases occurring over the next 30 years cannot be dismissed. Clearly, the population exposed to the BSE agent is not limited to those countries reporting BSE cases. Mathematical modeling indicates that the epidemic of BSE began in the UK in mid-1970, and that while approximately 180 000 cattle developed BSE, about 1 000 000 cattle must have been infected. During this period (and before full control measures were implemented) cattle were exported to many countries in Asia, North and South America, Africa and Australasia. Cattle products sourced from the UK cattle population and meat and bonemeal (MBM) manufactured in the UK were also widely exported. Finally, foreign travellers visiting the UK during the 1980s could have been exposed to the BSE agent.

A clear demonstration of the risk from exportation of cattle and cattle products comes from the three countries (Canada, Falkland Islands and Oman), otherwise not known to have BSE, but that have reported BSE in imported cattle. It is now clear that European countries imported contaminated MBM or infected cattle. Through their own rendering industries they began recycling contaminated materials, fostering internally generated epidemics. By 1989, the first case of BSE from outside the UK was reported. As of November 2002, 21 countries have reported endemic BSE cases. In some countries the number of reports of BSE is increasing – given that control measures have been recommended, perhaps these countries have not followed these measures or have not followed them sufficiently well to control BSE. As disturbing is that some EC countries reported their first cases only after implementing active surveillance. It is clear that even in countries where there is a very high level of awareness of BSE, BSE may not have been either prevented or recognized early.

The future public health threat of vCJD in the UK, Europe and potentially the rest of the world, is of concern and currently unquantifiable. However, the possibility of a significant and geographically diverse vCJD epidemic occurring over the next few decades cannot be dismissed. WHO has responded to this concern through its activities to promote global surveillance of CJD and by holding consultations on various issues related to transmissible spongiform encephalopathy (TSE) and public health. In order to ascertain the number and distribution of any future cases of vCJD, the WHO Consultation on the Global Surveillance, Diagnosis and Therapy of Human Transmissible Spongiform Encephalopathies recommended global surveillance for all forms of human TSE. In 2001, a new case definition for vCJD was adopted by WHO. Although WHO's main concern is the identification of cases of vCJD, this is best achieved through surveillance of all forms of CJD. This is important because the clinical phenotype of vCJD may not always be distinct from that seen in other forms of CJD. Additionally, the experience gained from identifying cases of the most common CJD subtypes should enhance the ability of surveillance systems to detect vCJD.

Programmes have already been established in Europe (i.e. EuroCJD and NeuroCJD, funded by the European Commission), and some other countries (i.e. Australia, Argentina, Canada and the United States). In December 2001 a WHO programme for establishing a national surveillance for CJD in east-European countries and China was funded as a Concerted Action in the Quality of Life and Management of Living Resources by the European Commission. These programmes have helped in investigating

the possible relationships between CJD and spongiform encephalopathies in animals, as well as in further defining the epidemiology of CJD, including risk factors for the disease. Key members of each country's neurological and epidemiological communities are invited to participate in networking meetings, training and other activities, and to report numbers of cases on an annual basis.

WHO has conducted training in surveillance of human TSEs in all regions of the world (Buenos Aires, Bangkok, Beijing, Bratislava, Cairo, and Dakar). Experience from the WHO workshops held to date has indicated that difficulty may be experienced in surveillance of human TSE in developing countries. Indeed many of the countries that have participated in these meetings reported a zero incidence of CJD; experience from mature surveillance systems indicates that a 'natural' case rate is approximately 0.5–1.0 cases of human TSE per million population per annum. Case detection is hampered by extremely low autopsy rates, reflecting both cultural and religious values, safety concerns for pathology staff, and lack of facilities for diagnosis. Furthermore, neurophysiology services in some countries are scanty and the level of awareness of the diagnostic features of CJD is low.

WHO is currently promoting a number of ways to improve case detection. This includes the adoption of novel and simple diagnostic procedures (such as testing CSF), the dissemination of information, and by facilitating the training of personnel in pathological and other laboratory techniques required for CJD diagnosis. In addition to this publication, reports of numerous consultations, the pamphlet *Understanding the BSE Threat*, and fact sheets are available on the WHO web site (see Annex 5 Information Resources).

It is anticipated that WHO's activities to promote global CJD surveillance will lead to a greater understanding of CJD and its variants, including the potential causes of iatrogenic CJD and the distribution of the various hereditary forms. Importantly, it will also provide important information for enhancing the protection and planning of public health worldwide.

WHO will assist and coordinate the transfer of material and personnel, and provide training as appropriate. WHO collaborating centres and reference centres (see Annex 5) will help in providing laboratory technical support and epidemiological expertise. Collaboration with other agencies operating in the TSE arena will continue. Particularly important are the Office International des Epizooties (OIE), the Food and Agriculture Organization of the United Nations (FAO), the Commission of the European Community (CEC), and other national and international agencies with mandates in human and veterinary medicine and in public health.

CHAPTER 2

The nature of the infectious agent

2.1 Prions

The infectious nature of scrapie was recognized in 1935 following the experimental intraocular inoculation with infected spinal cord of a previously healthy ewe. Recognition of the transmissibility of the human diseases kuru (1966), CJD (1968), Gerstmann-Sträussler-Scheinker disease (GSS,1981) and fatal familial insomia (FFI,1995) followed, but the exact nature of the infectious agent remained elusive. In 1954 the concept of 'slow-virus disease' was first introduced. However, exhaustive but fruitless efforts to find the 'TSE virus', and a conspicuous lack of inflammatory response, argued against a viral etiology. Furthermore, the infectious pathogen showed a remarkable resistance to treatments that would normally inactivate viruses, such as ultraviolet and ionizing radiation. In the 1970s, a radical theory was put forward suggesting that the infectious agent could be a self-replicating protein called a "prion" (proteinaceous infectious particle). By the early 1980s, it was demonstrated that a partially protease-resistant hydrophobic glycoprotein was present in large quantities in amyloid deposits in the central nervous system (CNS) of scrapie-infected animals. It appears to constitute the major component of the transmissible scrapie agent.

Prion protein (PrP) is encoded in the host genome and is expressed both in normal and infected cells in all mammals. The entire open reading frame of all mammalian and avian PrP genes resides within a single exon. In humans, the PRNP gene is located on the short arm of chromosome 20. It codes for a protein product of 253 amino acids consisting of a repeat region in which an initial nonapeptide is followed by four octapeptide coding repeats of similar sequences at the N-terminus of the molecule. PrP molecules have been found on the outer surface of plasma membranes of nerve cells, to which they are anchored through a covalent-linked glycolipid, suggesting a role as a membrane receptor. PrP is also expressed in other tissues, indicating that it may have different functions depending on its location.

Structurally, PrP is a protein consisting of a signal peptide, followed by an N-terminal domain that contains tandem repeats of a proline/glycine rich octapeptide. This is followed by a highly conserved domain of about 140 residues that contains a disulfide bond. Finally, there is a C-terminal hydrophobic domain that is removed post-translationally when PrP is attached to the extracellular side of the cell membrane by a GPI-anchor. This normal protease-sensitive cellular form (cellular isoform of the prion protein, PrP^{C}) is transformed into the abnormal protease-resistant isoform (scrapie isoform of the prion protein, PrP^{Sc}) in the disease state. Since direct biochemical studies of PrP^{Sc} have failed to reveal a difference in post-translational modifications that could distinguish it from PrP^{C}, it is generally accepted that the ability to replicate and form amyloid results from a conformational rearrangement of the PrP molecule. This conformational transition of PrP^{C} ('normal' prion protein) into PrP^{Sc} ('infectious' prion protein) is therefore the essential factor in prion pathogenesis. The prion hypothesis states that once produced, the abnormal isoform PrP^{Sc} acts as a template for the conversion of more PrP^{C} to PrP^{Sc}. Thus, a chain reaction is set in motion with more and more PrP^{C} being transformed into the pathological PrP^{Sc} isoform.

Previous spectroscopic studies demonstrated that PrP^{C} has a high α-helical content (about 40%) whereas PrP^{Sc} contains 45% β-sheets and 30% α-helix and therefore shows remarkable amyloidogenic properties. PrP^{Sc} formation is supposed to proceed from the interaction between PrP^{C} and PrP^{Sc}, which generates a conformational change of PrP^{C} to PrP^{Sc}. Such conformational change implies a transition from a structure rich in α-helix to a structure rich in β-sheets, a recognized characteristic of the non-soluble and proteinase resistant PrP^{Sc}.

A stochastic model has been proposed to explain PrP^{Sc} formation. According to the model, spontaneous fluctuations in the structure of PrP^{C} could create a partially unfolded protein, named $PrP^{\star}$. $PrP^{\star}$ would be an intermediate in the formation of PrP^{Sc}, and it would be assumed that it would be normal for a small amount of $PrP^{\star}$ to be present in brain tissue, but with insignificant rate of PrP^{Sc} formation. $PrP^{\star}$ would have three different molecular pathways: revert to PrP^{C}, degrade, or convert to PrP^{Sc}. Each of the known forms of TSE disease (infectious, sporadic and inherited) could be explained as follows. In infectious prion diseases, exogenous PrP^{Sc} would reach the brain

and act as template to promote the conversion of $PrP^{\star}$ to PrP^{Sc}. In sporadic prion diseases, a random accumulation of $PrP^{\star}$ could lead to the accumulation of sufficient PrP^{Sc} to start the pathologic process. In inherited prion diseases, mutations of PrP gene lead to an inherited less stable form of PrP^{C} that has an increased rate of both $PrP^{\star}$ and, consequently, PrP^{Sc} formation. The location of the known PrP point mutations near or within the α-helices segments, which are important for structural stability, is consistent with this hypothesis.

It has been demonstrated that mice devoid of PrP^{C} (PrP 'knockout' mice) do not develop prion diseases when inoculated with mouse PrP^{Sc}, demonstrating that the host susceptibility to scrapie infection and prion propagation requires the expression of PrP^{C}. This finding also supports the theory that prion replication occurs when inoculated PrP^{Sc} interacts with the homologous host PrP^{C}. In any case, the accumulation of PrP^{Sc} in the brain is a hallmark of most forms of TSE. However, to date the exact mechanism by which PrP^{Sc} triggers further PrP^{Sc} production is unknown. In addition, the mechanism by which PrP^{Sc}, or the absence of normally folded PrP^{C}, causes tissue damage is still unclear.

Although the prion theory has gained increasing popularity in the last few years, some scientists still believe the transmissible agent is virus-like, and that it contains DNA. Some have argued that perhaps the infectious DNA is associated with, and protected by, a host protein (the so-called 'virino hypothesis'). The strongest argument in support of the 'viral' hypotheses is the presence of different 'strains' of agent found in hosts with identical PrP genotypes, although this has been recently challenged.

2.2 Prion strains and strain-typing

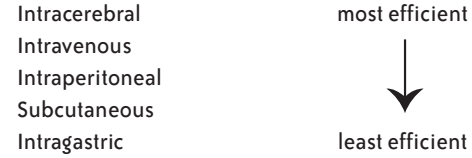
The 'strain' of a TSE is identified by a number of experimental laboratory-based methods. The most established method has been extensively used to characterize strains of sheep scrapie by identifying specific differences between strains when they are transmitted into genetically similar mice. For example, each scrapie strain leads to a consistent incubation period and pattern of neuropathology.

In humans, several PrP^{Sc} types, associated with different clinical phenotypes of CJD, have been identified. Strain-typing experiments, as described above, performed for sporadic CJD and vCJD have shown that the vCJD agent is different from the sCJD agent and similar to the BSE agent.

A second method of 'strain-typing' involves comparison of fragment size after limited proteolysis and semi-quantification of the ratio of the three PrP glycoforms (diglycosylated, monoglycosylated and unglycosylated PrP). Western blot is currently being used, in experimental settings, in an attempt to identify the 'strains' of human disease. Importantly, in extracts taken from the brain tissue of persons with vCJD, the ratio of glycosylated/non-glycosylated PrP is different from sporadic CJD and similar to experimentally transmitted BSE. The use of PrP strain typing in the differential diagnosis of sporadic CJD and vCJD has been suggested. However, since these methods are still experimental and some discrepancies between different laboratories are reported, this approach, although promising, cannot yet be recommended for clinical settings as a marker to differentiate between sCJD and vCJD.

2.3 Infection and pathogenesis

The efficiency of TSE transmission from donor to host is dependent on several factors, including route of entry. Experimental evidence indicates that ease of transmissibility decreases in the following order.



It is of note that the intragastric (i.e. oral exposure) route has the lowest efficiency, requiring in mice about 10 times more infectious material than the highly efficient intracerebral route.

The incubation period for transmission of a TSE agent within and between species is determined by a number of factors. These include the route of infection (central inoculation leading to a more rapid onset of disease than peripheral inoculation), strain, and dose. It is widely recognized that lower doses will increase the incubation period. If the agent of one species is subsequently inoculated into another, there is usually a longer incubation period than seen within the donor species. However, subsequent passage of an agent within the 'new' species is associated with a decrease in incubation period i.e. the agent becomes 'host adapted'. Whether multiple dosing over time with small, sub-infective dosages leads to transmission (i.e. a cumulative dose effect) is unclear and is currently under investigation.

A number of researchers have developed animal models to study the invasion and pathogenesis of scrapie. When orally exposed, it appears that infection occurs via the gut. Replication of the TSE agent occurs first in the spleen and lymph nodes. The agent probably reaches the brain from the spleen via the sympathetic fibres of the splanchnic nerves, which connect to the mid-thoracic spinal cord. From here

infectivity passes caudally and at a maximum rate of about 1mm/day. It is interesting that splenectomy in the early stages of the disease delays neuroinvasion, illustrating the importance of the lymphoreticular system in the initial stages of infection. Once infection has passed to the brain and spinal cord, it can pass to the peripheral tissues.

2.4 Species barrier

Species barrier is the well-recognized increase in difficulty that exists when trying to transfer infection between species as opposed to within species. With some host/agent combinations, it appears that the barrier is sufficiently high to prevent the transmission entirely (e.g. BSE has not been seen in dogs, although it has been transmitted to cats). As noted earlier, once the agent has been adapted to a species, the incubation period decreases. It has been suggested that the 'barrier' is simply a prolongation in incubation period, and that all forms of TSE are transmissible if the animal (or human) survives long enough.

Evidence suggests that the PrP structure may play an important role in the determination of the species barrier. The greater the homology between the PrP structure (particularly the central residues) of the donor and the host, the more likely it is that the host will acquire a specific strain. In human populations, there is an interesting observation that homozygosity at codon 129 increases susceptibility to both sporadic and iatrogenic forms of CJD. All vCJD cases so far described are homozygotic (methionine/methionine) at codon 129. It has been speculated that heterozygosity (methionine/valine) may induce the expression of two different PrP^{C} proteins, resulting in a slower replication of pathogenic prion proteins.

CHAPTER 3

Animal transmissible spongiform encephalopathies

Other than scrapie of sheep and goats, naturally occurring animal TSEs have historically been rare, with only relatively small numbers of cases documented in mink, elk and mule deer. There is no evidence that animal TSEs, other than BSE, have ever been transmitted to humans.

3.1 Scrapie

Scrapie is the prototypical TSE. It is an insidious degenerative disease affecting the CNS of sheep and goats. Natural scrapie has also been reported in moufflon, a primitive relative of sheep, but only in Great Britain. The term 'scrapie' describes the tendency for affected sheep to scrape themselves against trees or bushes. The disease was known as *la tremblante* (trembling disease) in France, as *Gnubberkrankheit* (itching disease) or *Traberkrankheit* (trotting disease) in Germany, *rida* in Iceland, *súrlókór* (brushing disease) in Hungary and by multiple other names in Britain. As a clinical entity, it was recognized in sheep in England as early as 1730. It has subsequently been reported in many countries and territories including Austria, Belarus, Belgium, Canada, Colombia, the Czech Republic, France, Germany, Ghana, Ireland, the Isle of Man, Israel, Japan, the Netherlands, Northern Ireland, Slovakia, Somalia, Spain, Switzerland, the United Arab Emirates and the United States of America. Although scrapie occurred secondarily to importation of sheep to Australia and New Zealand, the disease was successfully eradicated through stringent and immediate efforts to depopulate the imported sheep as well as their animal contacts.

Scrapie was first experimentally transmitted by inoculation of a ewe in the 1930s. Demonstration of transmission to mice in the early 1960s permitted the disease to be intensively studied, but in spite of this, the exact mechanism(s) of scrapie spread within herds of sheep remains obscure. It is commonly accepted that the disease is infectious and contagious but that genetic factors are also important. Infection is most commonly transmitted from ewe to lamb. Transmission may occur at parturition or afterwards when the ewe and lamb run together. There is also horizontal spread of infection between unrelated adults and this may account for some of the scrapie cases in older sheep. Placental tissue is known to be infectious and this is commonly postulated as a source of transmission both to the lamb and to unrelated animals sharing the same pasture. The exact routes of infection are unresolved, but possibilities include transplacental, oral, nasal, optic or cutaneous. Complex genetic factors involving the PrP gene, and potentially other genes, are known to affect the incubation period and thus the apparent susceptibility of sheep to scrapie. The possibility of genetically engineered animals resistant to disease has therefore been raised.

In natural scrapie, the onset of disease is often insidious. Early signs are apprehension, restlessness, hyperexcitability and aggressiveness. Some animals apparently become demented. Fine tremors of the head and neck are observed. As the disease progresses the tremors become more generalized, involve the whole body and produce a shivering effect. Fasciculations of superficial skeletal muscles may occur, and signs of cutaneous irritation, self-induced by rubbing and scratching, constitute one of the most characteristic clinical features, though do not occur in all cases, e.g. Icelandic scrapie (*rida*). As the disease evolves, the gait becomes ataxic with severely affected animals unable to stand or walk without falling. In the advanced stages of scrapie, animals become stuporous and manifest visual impairment, excessive salivation and wasting. The duration of the natural clinical course is usually less than four months.

As with other TSEs, the neuropathological triad of spongiform change, neuronal loss and astroglial proliferation occurs in scrapie. Vacuolation of the neuronal cytoplasm is a marked and pathognomonic feature, being particularly evident in the brainstem and the ventral and lateral horns of the spinal cord. Cerebral amyloidosis is seen in just over half of natural cases of scrapie. Another characteristic feature of scrapie and other TSEs is the presence of rod-shaped structures seen on electron microscopy and known as scrapie-associated fibrils (SAF) (see Figure 3.1, beetween pages 58 and 59). SAF are fibrillar forms of amyloid – the same amyloid that is contained in PrP plaques. A recent study has identified the presence of abnormal PrP in tonsillar tissue from sheep presumed to be infected with scrapie. PrP was detected before the occurrence of

clinical signs. This may be a useful finding, possibly leading to a clinical diagnostic test for the disease.

3.2 Bovine spongiform encephalopathy

Bovine spongiform encephalopathy was first reported in British cattle in November 1986 and over the subsequent decade an epidemic of about 180 000 cases occurred. Most cases were infected as calves; the modal age of disease occurrence is five years (range 29 months to 18 years) and the average incubation period is 60 months. Current evidence suggests that BSE originated from the use of cattle feed containing meat and bonemeal (MBM) contaminated by a scrapie-like agent derived from either sheep or cattle. The rendering procedure, by which animal materials were processed to produce MBM, changed in the UK during the 1970s and early 1980s. The decreased use of hydrocarbon solvents and the adoption of lower temperatures have been hypothesized as the cause for increased survival of the infective agent. These changes were adopted in response to a fall in the value of tallow (the fat-rich fraction of the process whose yield is increased by using solvent), a rise in the cost of energy and a need to replace old plants with safer systems (solvents are potentially explosive and carcinogenic). Many scientists believe that sheep scrapie, endemic in Great Britain, was the likely source of the infective agent that initiated the BSE epidemic. However, experiments indicate that BSE is associated with a single major strain of infective agent and although over 20 different scrapie strains are recognized, to date none appear to match that seen in BSE. This has led to the further hypothesis that BSE may have been an uncommon sporadic and/or hereditary disease of cattle that was dramatically amplified as a result of infected cattle material entering the modified rendering process. Whatever the origin of the agent responsible for BSE it has been epidemiologically demonstrated that the recycling of infected cattle through the rendering process in the 1980s was responsible for fuelling the large and explosive epidemic. It is of note that BSE has been experimentally transmitted via the oral route to cattle by as little as 0.1g of BSE-affected cattle brain.

The British government made BSE notifiable in June 1988 and shortly afterwards a statutory ban on the feeding of ruminant-derived protein to ruminants was introduced. In November 1989 a ban was introduced on the use of certain specified 'high risk' bovine offals (SBO) for human consumption (brain, spinal cord, tonsils, thymus, spleen and intestines from animals > 6 months old). The selection of offals to include in the SBO ban was based the infectivity of tissues of scrapie-infected sheep.

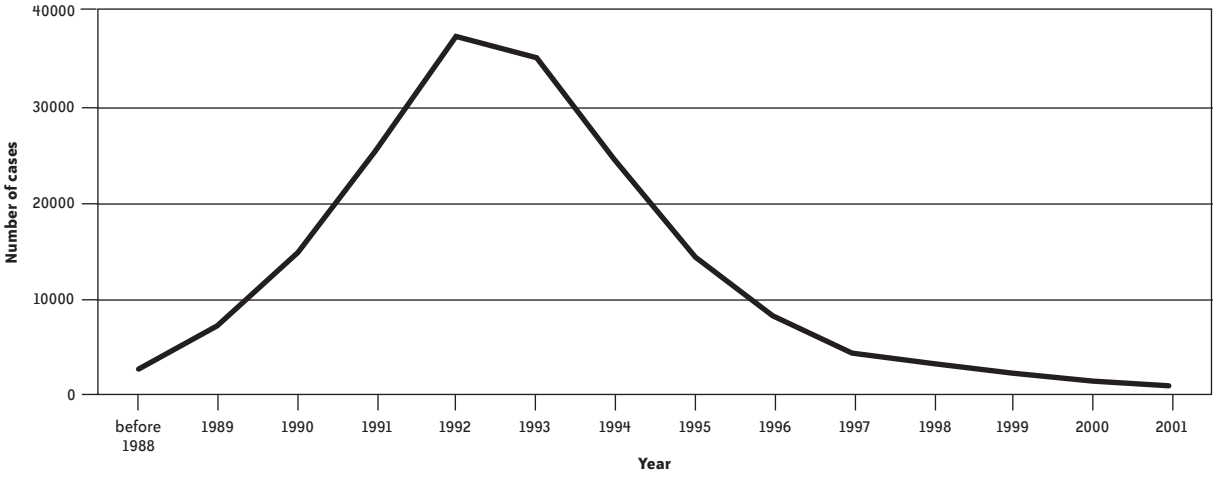
To date, BSE infectivity has been demonstrated in the following list of tissues to varying degrees: brain, eyes (retina), trigeminal ganglia, the spinal cord, the dorsal root ganglia and the distal ileum. However, muscle and milk from clinically affected cases of BSE have shown no detectable infectivity using the mouse bioassay (which has potential limitations, in particular its sensitivity due to the 'species barrier').

In September 1990 the use of SBO was prohibited for use in feed for all animals and birds in the UK. At the end of 1992 BSE reached its peak incidence in the UK but thereafter declined rapidly, almost certainly in response to the statutory measures (see Figure 3.2). As new cases continued to appear, it was clear that despite the regulations, that contaminated feed was still being consumed by ruminant animals, either intentionally or unintentionally. Cases of BSE observed in cattle that were born after the implementation of the feed ban are considered to be due combinations of the following factors: the continued use of feed rations produced before the ban, cross-contamination of cattle feed by feed containing MBM (intended only for pigs or poultry), and an incomplete compliance with the SBO ban. Further measures were instituted to address these particular issues in the UK, and following the announcement of a possible link between BSE and vCJD in March 1996, the exportation of cattle and cattle based products from the UK were largely prohibited. In addition, the UK prohibited the use of all mammalian MBM in all animal feed, and cattle >30 months old and heads from all cattle over six months old were excluded from all food or feed chains. Demonstration of the efficacy of control measures has led to the easing of restrictions in the UK, however, during the same time period, the number of cases of BSE on the European continent increased. As a result, among other measures, the EC prohibited the use of mammalian MBM in all animal feed in 2001.

Although the pattern of the epidemic remains consistent with the hypothesis that the vast majority of cases arose through infection with contaminated feed, it remains possible that other routes of transmission exist. A study to assess maternal transmission suggests that this may occur at a low rate. Maternal transmission is estimated to be responsible for only about 1% of cattle expressing disease. However, the study also tentatively suggests that genetic factors may influence susceptibility. There is no evidence of horizontal transmission of BSE between cattle.

The appearance of a number of novel TSEs, causally linked with BSE, in domestic and captive animals raises the question of whether BSE occurs, or will occur, in further animal species. Particular concern has been expressed regarding the possibility of BSE in sheep, pigs and poultry. BSE has been experimentally transmitted to sheep by feeding as

Figure 3.2 The BSE epidemic in the UK. Number of cases reported, by year of report. *N* = 183 000 reports



Source: OIE Data, 20 August 2002

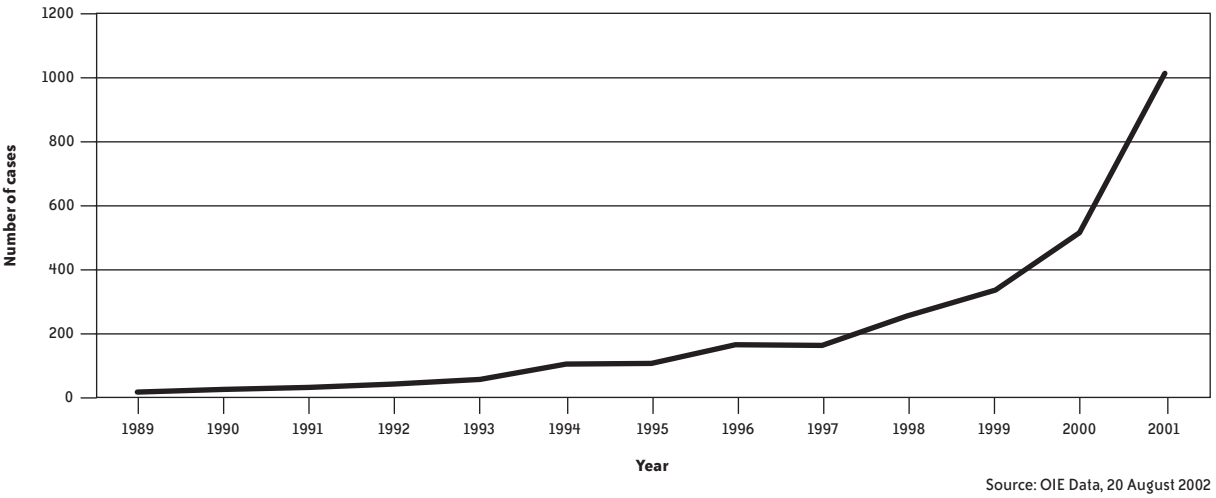
little as 0.5 g of infected bovine brain and it is known that some sheep were fed MBM until this practice was banned in 1988. In this regard, the lack of evidence of a BSE-related epidemic of sheep scrapie is reassuring. However, concern remains sufficiently high to leave the ban of ovine brain and spinal cord from sheep (over 6 months of age in the UK and over one year in France) in place as a part of a risk reduction strategy for humans in the EC.

Pigs, but not chickens, have been shown susceptible to BSE. However, while intracranial inoculation of infected bovine brain homogenate has led to disease in pigs, even very large oral doses of BSE-infected brain have failed to produce disease.

By the end of 2001, over 180 000 confirmed cases of BSE had been reported in the UK (Figure 3.2). Relatively small numbers of cases (approximately 3200) have also been reported in native-born cattle in other countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Japan, Liechtenstein, Luxembourg, Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Switzerland) (Figure 3.3). A few cases have also been reported in Canada, the Falkland Islands, and Oman, but solely in animals imported from the UK.

The duration of the clinical course of BSE is typically 1 or 2 months, but ranges from 7 days to 14 months. The most commonly observed signs are apprehension, hyperaesthesia and ataxia, but affected animals may also show a decreased milk yield and loss of condition. There is no effective treatment and the disease always progresses to death in the affected animal. A number of other cattle conditions can mimic BSE, e.g. magnesium deficiency ('staggers'). Pathological changes are similar to scrapie in many

Figure 3.3 The BSE epidemic in Europe, excluding the UK. Cases reported, by year of report. *N* = 3286



Source: OIE Data, 20 August 2002

respects with vacuolar lesions largely confined to the brainstem and accompanied by neuronal degeneration and an astrocytic reaction. Sparse cerebral amyloid plaques are seen in a small proportion of cases. In contrast to scrapie, greater diagnostic importance is attributed to the neuropil vacuolation than neuronal vacuolation.

Unfortunately, to date there is no practical and reliable diagnostic test for use in live animals. Pathological diagnosis remains fundamental although the recent introduction of rapid screening tests, now compulsory in many countries, has made possible the detection of a larger number of cases.

3.3 Chronic wasting disease of mule deer and elk

Chronic wasting disease (CWD) is a TSE of deer and elk. To date, this disease has been found only in cervids (members of the deer family) in North America. It was first recognized as a clinical syndrome in 1967. CWD is typified by behavioural changes and chronic weight loss leading to death. Species that have been affected with CWD include Rocky Mountain elk, mule deer, white-tailed deer, and black-tailed deer.

CWD has been found in free-ranging deer and in wildlife research facilities in a small number of American states. Although cases of CWD have been seen in two zoological parks, the affected animals all originated from the above-mentioned research facilities. Soon after the recognition of the disease, animal movement from these facilities was stopped. CWD also has been diagnosed in farmed elk herds in a number of states of the USA and in the Canadian province of Saskatchewan. Both the US and Canada are undertaking extensive surveillance programmes based on hunter-kills. As the investigation intensifies, the number of affected states and provinces is increasing. Whether this is due to a slow spread of the disease or whether surveillance is uncovering endemic disease is not entirely clear. However, the rate of CWD in northern Colorado and south-eastern Wyoming is much higher than in any other state, lending credibility to theories of slow spread of this disease. Both natural migration patterns of deer and movement into and out of game farms could be contributing to the spread of CWD.

Although the exact origin and mode of transmission of CWD is unknown, there is no clear evidence to suggest that CWD is caused by exposure to any other form of animal TSE. Transmission via feed is not believed to be the route of exposure, as infected animals consumed a variety of foodstuffs with no common ingredient of animal origin identified. Epidemiological studies suggest that transmission may be lateral, and possibly maternal. Between deer, transmission is highly efficacious. It is of note that painstaking attempts to eradicate CWD from captive facilities, including thorough decontamination and a 12-month period free of elk or deer, failed to prevent disease recurrence. There is ongoing research to further explore the possibility of transmission of CWD to other species. Notably, there have been no successful transmissions, by any route, of CWD to cattle. Furthermore, other ruminant species, including wild and domestic cattle, sheep and goats, have been housed in facilities in direct and indirect contact with CWD-affected deer and elk, without detected transmission.

Control measures for farmed herds have consisted of quarantine, depopulation of herds, and slaughter/testing, however, there are no control measures for wild populations of deer and elk. Hunters are advised not to eat any part of any animal that appears to be diseased, and to use gloves while dressing kills.

3.4 Transmissible mink encephalopathy

Transmissible mink encephalopathy (TME) was first described in 1967 but had occurred on mink farms in two American states (Minnesota and Wisconsin) as early as 1947. The disease occurs as outbreaks, in farmed mink only. It has been recognized in Canada, Finland, Germany, Russian Federation and the USA. The condition is rare and mortality high, with nearly all adult mink on an affected ranch succumbing to the disease during an outbreak.

Evidence points to infected feed as the cause of TME and it has been suggested that scrapie is the likely contaminant. However, experimental transmission of scrapie to mink via the oral route has not been successful to date, although TME could be caused by a different scrapie strain that those used experimentally. The possibility of a cattle origin for TME has also been raised. Products from fallen or sick cattle ('downer cows') were said to have been fed to a colony of affected mink in the USA and that these animals had been fed a diet free of any ovine material. However, surveillance of cattle in the USA has not revealed a single case of BSE, thus arguing against a cattle origin of TME infection. Furthermore, although BSE has been experimentally transmitted to mink, the incubation period, clinical signs and neuropathology show significant differences from natural TME. No convincing evidence for maternal or horizontal transmission of natural disease exists.

3.5 Feline spongiform encephalopathy

In 1990, the first case of feline spongiform encephalopathy (FSE) in a domestic cat was reported (BSE

in captive Felidae are discussed in Section 3.6). The 6-year-old animal had been referred to the Bristol Veterinary School in England with a progressive neurological condition. It failed to respond to treatment and subsequent neuropathological examination revealed a scrapie-like spongiform encephalopathy. Although no previous naturally occurring TSE had been documented in a feline, CJD had been experimentally transmitted to a cat in 1972 and several times thereafter. Since 1990, cats with FSE have been reported from most regions of the UK and single cases have been documented in indigenous cats from France, Liechtenstein and Norway. Ninety-one reports have been made as of September 2001.

Experimental evidence supports the hypothesis that these novel feline diseases are causally related to BSE. It is probable that the domestic cats were infected through the consumption of infected feed, but the precise ingredient is not known. It is noteworthy that only a single case has been reported since bovine offals were banned from use in feed or food for any species in the UK in September 1990.

3.6 Spongiform encephalopathies of captive zoo animals

In a British zoo in 1986, a nyala (an animal which belongs, like cattle, to the family Bovidae) died of a spongiform encephalopathy. Thereafter, additional cases of spongiform encephalopathy occurred in the following captive wild Bovidae in Britain: gemsbok, Arabian oryx, greater kudu, eland, and scimitar-horned oryx. Additionally, wild-captive Felidae (cheetah, tiger and lion) have developed spongiform encephalopathies. Over all 37 cases of TSEs in zoo animals have been reported (12 species, including reports among chimpanzees).

As with FSE, the temporal-spatial clustering of these novel spongiform encephalopathies would be consistent with a causative link to BSE. Both mouse transmissions and western blot strain typing confirm that the agent causing BSE and the agent causing illness in these animals is identical. It would seem likely that dietary exposure to MBM was of most importance for the Bovidae. It is noteworthy that one of the kudu was the offspring from an affected mother, thus raising the additional possibility of maternal transmission in this species. It is assumed that disease in the captive large cats arose through the consumption of uncooked infected bovine material, such as heads and necks containing CNS tissue.

3.7 Strain typing studies of the TSE agent in animals

Using a range of genetically distinct mice allows a 'strain profile' based on incubation period and neuropathological distribution of lesions (the 'lesion profile') to be produced. Such experiments have suggested that scrapie exists as over 20 separate strains, whereas BSE is due only to a single major strain of agent, which is distinct from those of scrapie. Furthermore, the transmission characteristics of vCJD, FSE, the novel spongiform encephalopathies of kudu and nyala, and experimental transmissions of the BSE agent to sheep, goats and pigs, closely resemble those of the BSE agent.

CHAPTER 4

Human transmissible spongiform encephalopathies

Creutzfeldt-Jakob disease (CJD) is the prototype of a family of rare human degenerative conditions (see Fig 4.1). They are called 'transmissible spongiform encephalopathies' (TSE) because of experimental transmissibility to animals and their characteristic neuropathology involving spongiform change. The human TSEs, of which CJD is by far the most common, appear to occur sporadically in about 85% of cases. Ten to fifteen per cent are inherited and the remaining cases are iatrogenic. Gerstmann-Sträussler-Scheinker disease (GSS) and fatal familial insomnia (FFI) are extremely rare human transmissible neurodegenerative disorders and are best considered as familial variants of CJD. The cause of sporadic CJD remains unknown despite extensive study, and, in particular, there is no evidence of an epidemiological link with scrapie, a naturally occurring TSE of sheep and goats. Iatrogenic CJD was recognized following detection of cases caused by transmission of the infectious agent during the use of contaminated human pituitary-derived growth hormone or gonadotropin, dura mater grafts, neurosurgical instruments and corneal transplantation.

CJD is thought to occur worldwide, but as systematic surveillance has only been undertaken in a minority of countries, the incidence in most of the world is unknown. A recent large study of CJD in the European Union suggested an incidence of between 0.5 and 1.5 cases per million persons per year. The higher incidence among certain populations (i.e. over 30 cases/million in Libyan-born Israelis) results from the high prevalence of familial CJD in this population. In the 1950s another human TSE, kuru, was found to be endemic in the Fore region of Papua New Guinea with a prevalence as high as 2% in some tribes. The cause of kuru was initially far from clear but detailed anthropological studies concluded that the condition was due to the transmission of the infective agent through ritualistic cannibalism. Since these practices stopped, the disease has become very rare.

Figure 4.1 Human TSEs

- Kuru
- Creutzfeldt–Jakob disease
  - Sporadic
  - Familial
  - Iatrogenic
  - Variant
- Fatal insomnia
  - Fatal familial insomnia
  - Acquired fatal insomnia
- Gerstmann-Sträussler-Scheinker disease

4.1 Kuru

Kuru is a TSE that is confined to the people living in the mountainous interior of Papua New Guinea. It was first described in 1955, although cases had probably been occurring for several decades before this. The term 'kuru' means 'shivering or trembling' in the language of the Fore, the cultural and linguistic group in which more than 80% of cases occurred. The point prevalence of the disease in this population was about 1%, although it was, at times, as high as 2%. Women and children were much more commonly affected than adult males, leading to a male/female ratio of more than 3:1 in some villages, and suggesting (incorrectly) that sex-linked genetic factors were important in disease etiology. Although the cause of kuru was initially unclear, intensive study concluded that the disease resulted from the practice of ritualistic cannibalism. During rites of mourning for dead kinsmen, conjunctival, nasal, skin, mucosal and gastrointestinal contamination with highly infectious brain tissue occurred. For cultural reasons, men were only infrequently exposed to infectious tissues during these funeral rituals, thus explaining the relative scarcity of the disease in adult males. The recognition that other tribes remained free of kuru despite cannibalistic practices similar to the Fore, led to the suggestion that kuru may have initially arisen following the ritualistic cannibalism of a sporadic or familial CJD victim in the Fore region.

Kuru has gradually been disappearing since cannibalistic rituals ceased toward the end of the 1950s, and with the passage of time progressively older age groups have become free of kuru. A few cases are reported even today, due to the very long incubation period (4.5 to over 35 years). It is noteworthy that since the cessation of cannibalism, no child born of a mother with kuru is known to have developed the disease, suggesting that direct maternal transmission rarely, if ever, occurs.

In 1959, an American veterinary pathologist, Dr William Hadlow, drew attention to the similarity between the neuropathology of kuru and scrapie. It was known at this time that scrapie was transmissible and subsequently Drs Clarence Gibbs Jr and Carleton Gajdusek demonstrated transmission of kuru to a chimpanzee in 1965.

The clinical course of kuru is remarkably uniform, with cerebellar symptoms progressing to total incapacitation and death, usually within 3 to 9 months. The disease has been divided into three clinical phases. The first, or ambulant stage, starts with unsteadiness of stance or gait and often of the hands. This is preceded in some cases by symptoms of headache and limb pains. Dysarthria starts early, and speech progressively deteriorates as the disease advances. Convergent strabismus often appears early as well, and persists. Shivering tremors are also noted during this phase. In the latter part of the first stage, the patient needs some support when walking (see Figure 4.2, beetween pages 58 and 59). The second, or sedentary stage, is reached when the sufferer can no longer walk without complete support (see Figure 4.3, beetween pages 58 and 59). Tremors and ataxia become more severe, rigidity of the limbs often develops, associated with widespread involuntary movements, particularly myoclonus with or without choreoathetosis, and a startle reaction may be seen. Emotional lability, leading to outbursts of pathological laughter, frequently occurs and although most patients show a resignation and a light-hearted attitude toward their illness, some patients become depressed. Mental slowing is apparent, but severe dementia is conspicuously absent. The third, or terminal stage, is reached when the patient is unable to sit up without support. At this time ataxia, tremor, and dysarthria become progressively more severe and incapacitating. Pyramidal, extrapyramidal and frontal release signs may be seen at this stage and in time inanition and signs of bulbar involvement develop. The patient becomes mute and unresponsive, deep decubitus ulceration and hypostatic pneumonia often occur, and the patient finally succumbs, usually, but not always, in a state of emaciation.

In keeping with the prominent cerebellar clinical features of kuru, neuropathology usually demonstrates macroscopic atrophy of the cerebellar vermis. Microscopically, changes are widespread in the CNS. The neuropathology is characterized by marked astrocytosis throughout the brain, mild spongiform change of the grey matter, diffuse neuronal degeneration that is most severe in the cerebellum and its afferent and efferent connections and minimal demyelination. Typical intracytoplasmic vacuolation is usually observed in the large neurons of the striatum. The most striking histological abnormality however is the presence of PrP-positive amyloid plaques, most conspicuous in the cerebellum, and occurring in about 80% of cases (Figure 9.18).

4.2 Sporadic Creutzfeldt-Jakob disease

4.2.1 Epidemiology of sCJD

In 1920 Hans Gerhard Creutzfeldt (1885–1964), a German neuroscientist, reported the case of a 22-year-old woman with a 6-year history of progressive cerebral dysfunction. A year later another German neuroscientist, Alfons Maria Jakob (1884–1931), described further cases and in 1922 the term 'Creutzfeldt-Jakob disease' was first introduced. Although some of these first cases would not meet modern neuropathological criteria, a retrospective analysis suggests at least half were the condition we now know as CJD.

In the past 20 years, many epidemiological surveys, including case-control studies, have been undertaken in France, Israel, Japan, the UK, the USA and more recently in the European Union. Our current understanding of CJD epidemiology is indebted to these studies, which have led to a greater insight into the clinical, pathological and aetiologic features of CJD. In addition, collaborative studies have been able to examine etiological risk factors.

The majority of CJD cases occur sporadically, and there is no evidence of geographical clustering or case-to-case transmission. The incidence is generally between 0.5 and 1.5 cases per million persons per year. The female to male ratio is representative of the general population and no distinct pattern of socio-economic incidence prevails. The mean age at onset of disease is approximately 65 years but age of onset is known to range from 14–92 years of age.

Case–control studies have yielded conflicting results. CJD has been associated with surgery to the head, trauma to the head or body, surgery requiring sutures, herpes zoster infection, tonometry, consumption of various meats, farming, and exposure to a range of animals including fish and squirrels. However, none of these associations has been consistently found and therefore they most likely reflect the difficulty of obtaining reliable associations from case-control studies. There is no clear evidence of a risk from diet (including consumption of brain), previous surgery, blood transfusion, occupation or animal exposure. However, the possibility that sporadic CJD arises through other unrecognized environmental exposures cannot be dismissed.

4.2.2 Clinical features

The classical diagnostic triad of CJD is a rapidly progressive dementia, myoclonus and a characteristic

electroencephalogram (EEG). The median and mean duration of illness is 4.5 and 8 months respectively. Only 4% of cases survive longer than 2 years.

Patients usually present (in order of decreasing frequency) with cognitive decline, ataxia or visual disturbance, either alone or in combination. Less common presenting features include behavioural disturbances or a stroke-like illness. Occasionally there is a history of non-specific symptoms in the illness prodrome such as headache, fatigue, sleeping difficulties, weight loss, malaise or anxiety. Dementia is invariably present during the course of the illness and myoclonus, although a rare presenting feature is observed at some stage in 80% of cases. Visual abnormalities are also common and include non-specific blurring, visual field defects, perceptual abnormalities and occasionally hallucinations. Seizures are observed in only 10% of patients, late in the clinical course. As the disease progresses multi-focal CNS failure occurs, with increasing global cognitive dysfunction, ataxia, dependency and urinary incontinence. The clinical picture declines as the patient becomes bed-bound, mute and unresponsive. Physical pain is an uncommon feature at any stage of the illness and, due to the rapid progression of cognitive impairment, any retained insight is usually soon lost. Terminally, the patients are usually rigid, frequently cortically blind, dysphagic and may develop Cheyne-Stokes respiration. Dysphagia predisposes toward aspiration and pneumonia, which are the commonest causes of death

Physical signs correspond with the global CNS involvement and may include a combination of cerebellar, pyramidal, and extrapyramidal signs. Primitive reflexes, paratonic (gegenhalten) rigidity, cortical blindness and akinetic mutism are also common, whereas lower motor neuron signs are rarely observed. Myoclonus is probably the most important clinical sign. It usually shows some asymmetry; is typically arrhythmic, asynchronous and stimulus sensitive. It is noted most frequently in the limbs, but also commonly affects the body and/or face. Stimulus sensitive myoclonus and/or a startle reaction can occur in response to sudden noise, visual threat, touch, noise, or muscle stretch, but usually myoclonus can also be noted at rest. Attempted movement may induce the jerks, as might a maintained posture such as holding the arms outstretched.

4.2.3 Differential diagnosis

The characteristic clinical features of CJD – rapidly progressive dementia and myoclonus – can rarely occur in patients with Alzheimer disease, the most common condition mimicking CJD. Reports exist of the characteristic EEG appearances of CJD occurring in Alzheimer disease but this is exceptional.

Figure 4.4 Differential diagnosis of sporadic CJD

Alzheimer disease
Vascular dementia (e.g. multi-infarct dementia and subcortical arteriosclerotic encephalopathy)
Diffuse Lewy body disease
Brain tumours (both primary and secondary)
Cerebellar degeneration
Frontotemporal dementia (e.g. Pick disease type and motor neuron disease type)
Progressive supranuclear palsy
Multiple system atrophy
Corticobasal degeneration
Metabolic encephalopathies
Drug-induced encephalopathies (e.g. bismuth, amitriptyline, mianserin, lithium, baclofen)
Viral encephalitis
Multiple cerebral abscesses
AIDS-dementia

Conditions that are important in the differential diagnosis are listed in Figure 4.4.

4.3 Familial (hereditary) TSEs

4.3.1 Familial CJD

Familial TSEs are inherited as an autosomal dominant trait. They account for approximately 10–15% of all known TSE cases, although some countries (Israel and Slovakia) have much higher rates of familial disease. The disorder may manifest as familial Creutzfeldt-Jakob disease (fCJD), Gerstmann-Sträussler-Scheinker disease (GSS) or fatal familial insomnia (FFI).

The clinical and neuropathological spectrum of the hereditary TSEs is extremely diverse. Familial CJD phenotypically resembles sporadic CJD, but there are variations in clinical presentation, age at disease onset, duration of illness and neuropathological findings. To a significant extent, the phenotype depends upon the causative mutation. Identification of the causative mutation (and associated polymorphisms) in the PRNP gene has become an important diagnostic test that should be performed in suspected TSE cases.

To date, 41 PRNP alleles associated with distinct phenotypes of hereditary TSEs have been reported. The prevalence of different mutations varies between countries. Figures 4.5 and 4.6 list the clinical and pathological features of the conditions associated with point mutations and octapeptide insertion mutations, respectively. Cases in which the mutation was an incidental finding to a clinical illness incompatible with a TSE, have not been included in this report. For example, deletions of one octapeptide coding repeat in the PRNP gene have been identified in

healthy control individuals, patients with other neurodegenerative diseases, and in patients with CJD. Their significance in relation to TSEs is a matter of debate.

The PRNP E200K/129M mutation is the most frequent mutation accounting for approximately 70% of the fCJD affected families worldwide. Clusters of affected families associated with this mutation were identified in isolated populations of Chile, Italy, Japan, Libyan Jewish community and Slovakia. All patients have dementia, 79% show cerebellar signs and 73% myoclonus. A periodic sharp wave discharge on an EEG is very characteristic of this form.

Approximately 50% of hereditary cases lack a clear family history of a similar disorder. Some patients with mutations were initially misidentified as "sporadic" cases, as they had no relevant family history. This might be due to low penetrance of the disease, misdiagnosis of affected family members or to a genetic abnormality occurring de novo in the affected individual.

Polymorphisms at codon M129V and E219K have been shown to affect the disease phenotype and influence the age at onset and duration of the disease. The most dramatic example is the PRNP D178N mutation. It has been linked to two phenotypically different familial disorders, fCJD and FFI. A PRNP allele with the D178N mutation and valine at polymorphic codon 129 (D178N/129V) is associated with the dementing form of fCJD, but when there is methionine at codon 129, the phenotype is FFI. Another example of the influence of codon 129 involves the 178/129V type of fCJD, which only rarely shows periodic sharp wave discharges on an EEG. Therefore, it is appropriate to identify each genotype by the causative mutation as well as by the polymorphism at these particular codons.

E200K/129M and D178M/129V have been transmitted to squirrel monkeys by inoculation with brain tissue of the patients.

Figure 4.5 Characteristics of hereditary disease associated with point mutations

PRNP allele	Condition	Epidemiology/origin	Clinical findings	Neuropathology
P102L-129M	Familial GSS (fGSS) classical form	Most common GSS mutation. Austria, France, Germany, Israel, Italy, Japan, Poland, United Kingdom and USA	The age of onset in the range of 30 to 62 years. Progressive cerebellar syndrome with late dementia, pyramidal and extrapyramidal signs. Overlap with CJD phenotype in cases with short duration. Clinical course varies from 1 to 10 years.	Numerous PrP positive uni- and multicentric amyloid deposits in the cerebrum and cerebellum, varying degrees of spongiform change, neuronal loss and astrogliosis. PrP-positive amyloid plaques and diffuse deposits.
P102L-129M-219K	fGSS	Japan	The age of onset in the range of 31 to 34 years. Similar to 'classical' GSS, but less prominent cerebellar signs.	No spongiform change or congophilic plaques. Few PrP positive plaques in the cerebral and cerebellar cortices.
P102L-129V	GSS	USA/Italian	Single case. Disease onset at age 33. Seizures, sensory abnormalities in lower extremities, gait abnormalities, dysarthria, long tract signs; no dementia. Clinical course 12 years.	No spongiform change. Neuropathological abnormalities in the corticospinal, spinocerebellar and gracile tracts. PrP-positivity in substantia gelatinosa.
P105L-129V	fGSS/ Spastic paraparetic form	Japan	The age of onset between 40 and 57 years. Spastic paraparesis progressing to quadriparesis. Late dementia without cerebellar signs, myoclonus, or periodic complexes on the EEG. Duration in the range of 6 to 12 years.	Numerous PrP-positive amyloid plaques in the cerebral cortex, severe gliosis but no spongiform changes. Cerebellum histologically preserved except for scant PrP plaques. No spongiform change. Neurofibrillary tangles (NFT) in some cases.
A117V-129V	fGSS	Alsatian family, USA/British, German, Hungarian	The age of onset between 20 and 64 years. Cognitive decline, dementia, Parkinsonism, pyramidal signs; occasional cerebellar signs; myoclonus. Duration in the range of 1 to 11 years.	Widespread PrP-positive uni- and multi-centric plaques, neuronal loss and astrogliosis; moderate spongiform change. No NFT
G131V-129M	GSS	Australia	Single case. Disease onset at 42 years. Progressive dementia, ataxia and uncontrollable aggressive behaviour. EEG: no periodic sharp-waves (PSW) Duration 9 years.	Cerebral and cerebellar atrophy. Numerous congophilic plaques in cerebellum. Intense PrP-positivity in the molecular layer of cerebellum. No spongiform degeneration. Occasional NFT in Ammon's horn and entorhinal cortex.

PRNP allele	Condition	Epidemiology/origin	Clinical findings	Neuropathology
Y145X-129V	GSS/Vascular variant	Japan	Single case. Progressive dementia starting at age 38. No myoclonus. Duration 21 years.	Diffuse cerebral atrophy with dilation of lateral ventricles. PrP-positive amyloid deposits in the cerebral and cerebellar cortices associated with NFT in the neocortex, hippocampus, and subcortical nuclei. PrP-positivity in the vessels. No spongiosis.
Q160X-129M	Familial Dementia	Austria	Single family. Onset on average at 46 years. Long duration (up to 12 years). Dementia. EEG not typical. Computerized tomography (CT), magnetic resonance imaging (MRI) severe ventricular and sulcal enlargement, cortical atrophy.	Diffuse cortical atrophy, extensive enlargement of ventricles.
D171S-129V	fCJD	Brazil	Single family. Prominent psychiatric symptoms including delusions, depression, withdrawal and violent behaviour. Mutism, incontinence and also dementia. Duration over seven years. Age at onset from mid 30s to 70s.	No description available.
D178N-129M	Fatal familial insomnia (FFI)	Italian, French, USA/ English, USA/German, German, USA, Australian/ Irish, Japanese, Austrian, British, Canadian-Chinese	Age at onset in the range of 27 to 71 years. Progressive insomnia, dysautonomia, endocrine and memory disturbances, myoclonus, ataxia, late dementia. Pyramidal and extrapyramidal signs in the case with a relatively long duration (more than 1 year). Duration between 1 to 6 months.	Atrophy of the anterior ventral and mediodorsal thalamic nuclei, olivary atrophy, varying degrees of cerebral and cerebellar cortical gliosis. Diffuse spongiform change in patients with clinical course longer than 1 year.
D178N-129V	fCJD	Finnish, French, USA/ Hungarian-Rumanian, USA/Dutch, Canadian-French, German, British and Danish families	Age at onset in the range of 26 to 56 years. Memory impairment leading to dementia, behaviour and mood changes, ataxia, speech impairments, tremor and myoclonus appear early during the course. Duration in the range of 9 to 51 months.	Considerable diversity, cerebral cortex and basal ganglia most severely involved, prominent spongiform change and gliosis, less prominent neuronal loss; cerebellum spared; no plaques.
V180I-129M	CJD	Japan, USA	All cases have no family history. Age at onset in the range of 66 to 85 years. Similar to typical sCJD but with a slower progression. Duration 1 to 2 years. EEG no PSW complexes.	Like typical sCJD; 'Kuru'-like plaques. Weak synaptic type PrP distribution.
V180I-129M& M232R-129M	CJD	Japan	Single case. Age at onset at 84 years. Duration of 1 year. Cognitive impairment, progressive dementia, akinetic mutism. No motor signs except for increased tendon reflexes and myoclonus. EEG with no typical PSW complex. MRI showed slight atrophy of cerebral white matter and basal ganglia.	Histological examination limited to the parietal lobe. Cortical spongiosis and diffuse PrP immunostaining. No plaques.

PRNP allele	Condition	Epidemiology/origin	Clinical findings	Neuropathology
T183A-129M	fCJD	Brazil (Spanish origin), Europe	Brazilian family. Average age at onset 45 years. Personality changes, aggressive behaviour, dementia, parkinsonism, myoclonus. Duration of 4 years. EEG without typical PSW. European single case, no relevant family history. Early onset at 40 years. Dementia without neurological signs of CJD. Duration of illness 4 years.	Severe spongiform change and neuronal loss in the deep cortical layers and in the putamen, but minimal gliosis in the most severely affected areas. The putamen and molecular layer of cerebellum, but no other areas of the affected brain, displayed PrP-positive immunoreactivity. Autopsy confirmed spongiform encephalopathy in a European case.
H187R-129V	fGSS	USA	Single family. Average age at onset 43. Progressive cognitive decline, limb ataxia, dysarthria, myoclonus, seizures. Duration on average 13 years. EEG showed nonspecific change	Minimal degree of astrocytosis and neuronal loss. No spongiform degeneration. Unique pattern of PrP deposits (small round or elongated "curly" granules) of laminar distribution in cortical layers and restricted 'synaptic' pattern of deposits. No amyloid plaques.
T188A-129M	CJD	Australia	Single case, no relevant family history. Onset at 82 years. During the course developed myoclonus, visual hallucinations, and cortical blindness. EEG with PSW complexes. Duration of illness 4 months	Similar to typical sCJD but minor spongiosis. Immunohistochemistry, negative for PrP.
E196K-129M	fCJD	France	Single family (three affected members, one genetically confirmed). Average age at onset 70. Proband had behavioural abnormalities, anorexia, mutism, and choreo-athetoid movements. EEG not typical. Duration of illness 12 months.	Confirmed at autopsy. No details available. PrP^{Sc}-positive.
P198S-129V	fGSS with neurofibrillary tangles	Indiana kindred	Onset in the range of 34 to 71 years. Similar to 'classical' GSS, but with more chronic course. Duration in the range of 3 to 11 years.	Similar to 'classical' GSS, more extensive PrP-positive amyloid deposits, NFT numerous in the cerebral cortex, hippocampus, and substantia innominata. Spongiform change is occasional and mild.
E200K-129M	fCJD	The most common mutation. Chile, China, England, France, Japan, Italy, Poland, Slovakia, USA, Sephardic Jews, and families of Greek and Tunisian origin	Similar to sCJD. Age at onset in the range of 35 to 71 years. Duration between 2 to 41 months.	Similar to sCJD with spongiform change, gliosis, neuronal loss, very rarely amyloid plaques.
E200K-129V	fCJD	Austria	Single family. 66-year-old female presented with a 2.5-year history of memory impairment and a 1-year history of possible ataxia. Developed progressive dementia over 3.5 months. No myoclonus. Affected family members died between age 50 and 65 years with description of dementia or depression of undetermined cause. EEG with PSW complexes.	Diffuse cerebral and cerebellar atrophy. Diffuse spongiform degeneration, astrogliosis, neuronal loss in cerebral and cerebellar cortices and basal ganglia. Diffuse synaptic type of PrP immunoreactivity in cerebral cortex, basal ganglia, brainstem, and cerebellar cortex. Unicentric plaque-like PrP deposits in the cerebellar granular cell layer and subcortical white matter.

PRNP allele	Condition	Epidemiology/origin	Clinical findings	Neuropathology
D202N-129V	GSS with NFT	England	Single case, no relevant family history. Onset at 73 years. Cognitive decline, cerebellar symptoms, dementia. No myoclonus. Duration of 6 years.	Abundant PrP-positive deposits in the cerebrum and cerebellum. NFT in the cerebral cortex. No spongiosis.
V203I-129M	CJD	France/Italian	Single case, no relevant family history. Onset at 69 years. Visual diplopia, visual hallucinations, tremor, ataxia, myoclonus. EEG with typical PSW complexes. Duration of illness 49 days	Confirmed at autopsy. No details available. PrPres positive by western blot.
R208H-129M	CJD	USA	Single case with disease onset at 62 years of age. Similar to sCJD. EEG with typical PSW complexes. Duration of 7 months.	Histology limited to the frontal cortex. Similar to sCJD. Punctate pattern of PrP immunostaining.
V210I-129M	fCJD	Italian, USA/Chinese, French, Italian and Japanese	Phenotype similar to sporadic CJD. Age at onset varies between 48 to 70 years. Asymptomatic 81 and 82 year old relatives carried mutation. EEG with typical PSW. Duration of the disease varies from 3 to 5 months.	Similar to sCJD.
E211Q-129M-124Gly(c421G)	fCJD	France	Age at onset in the range of 47 to 74 years. Similar to sCJD. EEG with typical PSW. Duration of illness in the range of 17 to 32 months.	Confirmed at autopsy. No details available.
E211Q-129M	fCJD	Italy	Age at onset in the range of 42 to 81 years. Similar to sCJD EEG with typical PSW. Duration of illness in the range of 3 to 7 months.	Autopsy was not performed.
Q212P-129M	GSS	USA	Age at onset 60 years. Similar to 'classical' GSS. No dementia. Duration of 8 years	Mild PrP-positive amyloid deposits in the cortex and cerebellum; no spongiform degeneration; corticospinal degeneration in the lateral and anterior pyramidal tracts; Lewy bodies in the neocortex and the substantia nigra.
Q217R-129V	fGSS with neurofibrillary tangles	Swedish/USA	Age at onset in the range of 62 to 66 years. Depression, cognitive decline, dementia, cerebellar and extrapyramidal signs. Duration in the range of 5 to 6 years.	Similar to GSS F198S but with most severe lesions in the cerebral cortex, thalamus, and amygdala. NFT in the cerebral cortex. PrP positive plaques in cerebral and cerebellar cortex. No spongiform change.
M232R-129M	CJD	Japan	All cases without relevant family history. Age at onset varied between 55 and 70 years. Similar to sCJD EEG with typical PSW complexes. Duration from 4 to 24 months.	Similar to sCJD. No plaques. PrP-positive immunoreactivity of punctate type.

Figure 4.6 Characteristics of hereditary disease with insertion mutations

PRNP allele	Condition	Epidemiology	Clinical findings	Neuropathology
24 bp-129Met (one extra repeat)	CJD	France	Single possible familial case. Onset at the age of 73. Similar to sCJD. EEG: diffuse periodic activity. Duration of 4 months.	Autopsy was not performed.
48 bp-129M (two extra repeats)	fCJD	North American (NA) and Dutch families	Age at onset 52 to 64 years. Similar to sCJD. EEG with PSW complexes in NA patient. Duration of 3 months to 7 years. NA patient's mother was a mutation carrier and had a slowly progressive dementia; died at the age of 86 years from pneumonia.	Autopsy of NA patient only. Typical of sCJD. Patient's mother had no neuropathological features of CJD.
72 bp (three extra repeats)	No case reported			
96 bp-129V (four extra repeats)	GSS	France	Single case with no relevant family history. Onset at the age of 82 years. Behavioural abnormalities, akinetic mutism, pyramidal signs, myoclonus. EEG with PSW complexes. Duration of 4 months.	Autopsy was not performed.
96 bp-129M (four extra repeats)	CJD	Japanese, British, Italian	Single cases. The age at onset of 62, 56, 65 years. Similar to sCJD. Duration of 5 years, and 10 and 6 months.	Focal spongiform change and PrP positive plaques in Japanese case. Consistent with typical sCJD in British and Italian patients.
120 bp-129M (five extra repeats)	fCJD	USA, USA/Ukraine, Germany	The age at onset in the 26–45 years range. Progressive dementia, extrapyramidal, pyramidal cerebellar signs and myoclonus. Duration 2–15 years.	Consistent with typical sCJD.
120 bp-129V (five extra repeats)	fCJD	USA, Germany	In the American family the age at onset in the range of 34–53 years. Progressive dementia, extrapyramidal, pyramidal cerebellar signs and myoclonus. Duration 2.5–3 months.	Autopsy was not performed.
144 bp-129M (six extra repeats)	fCJD	British and Japanese families	Age of onset 22 to 53 years. Duration 2 to 18 years. Progressive dementia +/- cerebellar, extrapyramidal and pyramidal signs, myoclonus, chorea, seizures. A long psychiatric prodrome was sometimes noted. EEG may be periodic.	Very variable from classical changes +/- plaques to cases without any specific features to suggest CJD.
168 bp-129M (seven extra repeats)	fCJD	Single Japanese case and North American family	Age at onset 23 to 35 years. Duration 7 to more than 13 years. Slowly progressive dementia, rigidity and cerebellar signs +/- myoclonus.	Varying degree of spongiform change, neuronal loss, and gliosis +/- plaques.
192 bp-129M (eight extra repeats)	fGSS	French family	The age of onset in the range of 21 to 34 years. Psychiatric features (mania), cerebellar signs, and dementia. Exact duration of illness is unknown, but it continues for many years.	Mild spongiosis; multi-centric PrP-positive plaques; microglial proliferation around plaques.

PRNP allele	Condition	Epidemiology	Clinical findings	Neuropathology
192 bp-129V (eight extra repeats)	fGSS	French and Dutch families	Age at onset 21 to 55. Duration 3 months to 13 years. Intellectual slowing, behavioural change, extrapyramidal and cerebellar signs, myoclonus.	Neuronal loss, spongiform change, gliosis and multi-centric plaques.
216 bp-129M (nine extra repeats)	fGSS	British and German	Reported cases aged 54 and 32 at onset. FH of dementia in both cases. Slowly progressive dementia in one case of more than 6 years. EEG not periodic. One case had myoclonus.	Available in one case. Marked PrP-positive amyloid plaques in cerebellum and basal ganglia. No spongiform change. A few neurofibrillary tangles.

4.3.2 Gerstmann-Sträussler-Scheinker disease

GSS is a familial disease with progressive limb and truncal ataxia, dysarthria, personality change and cognitive decline. GSS typically differs from CJD by early and prominent cerebellar ataxia, longer duration of illness, and the presence of morphologically distinct multi-centric amyloid plaques in the cerebellum. Cerebellar atrophy is documented by MRI. Myoclonic movements and periodic sharp wave discharges on an EEG are rarely observed.

The P102L/129M mutation in the PRNP gene was discovered in American and UK families and subsequently in many other families around the world. This is the most frequent GSS mutation, causing the disease in about 80% of all known GSS families. The large and well studied Indiana kindred and another family segregating GSS with the F198S/129V mutation and a Swedish family with GSS associated with the Q217R/129V mutation have a significantly different phenotype. It is mainly characterized by the presence of amyloid cores surrounded by abnormal tau-positive neurites, similar to neuritic plaques in Alzheimer disease. In addition, there are neurofibrillary tangles in the same areas of the neocortex.

4.3.3 Fatal familial insomnia

This unique hereditary syndrome is characterized by insomnia, dysautonomia and motor deficits is associated with the D178N/129M mutation. The clinical diagnosis could be established by evaluation of rapid eye movement (REM) sleep and spindle activity during non-REM sleep which are reduced and eventually disappear. Autonomic dysfunction also occurs early and includes increased lacrimation, salivation, sweating, raised body temperature, and impotence in males. Endocrine abnormalities also were noted with an increase in catecholamines and cortisol and a loss of normal circadian rhythms. Ataxia, dysarthria and dysphagia are among the early signs, while cognitive functions remain relatively spared until late in the course of the illness. Motor manifestations of advanced disease include dysarthria and limb ataxia. Cognitive functions show a lack of vigilance and attention as part of selective memory impairment.

The genetics of FFI are particularly interesting. The mutation at codon 178 is necessary for the development of disease; however, it is the presence of a polymorphism coding for methionine 'downstream' at codon 129 on the abnormal allele that appears to determine the FFI phenotype. The same 178 mutation, but coding for valine at codon 129 of the affected allele, is associated with a clinico-pathological phenotype clearly distinct from FFI. This illustrates the dramatic effect on disease phenotype that can result from a subtle change in PrP structure. Additionally, it has been noted that patients with FFI who are homozygous for the 129M allele follow a more rapid course of illness, have prominent sleep and autonomic disturbances, and signs of motor and cognitive dysfunction are mild. In contrast, patients who are heterozygous at codon 129 have a prolonged course, present with ataxia and dysarthria, tend to have prominent cognitive impairment and seizures. However, sleep disturbance and autonomic signs are less severe.

The histopathological hallmark of FFI is the loss of neurons and astrogliosis in the medio-dorsal and anterior thalamic nuclei. The extent of involvement of other thalamic nuclei varies. The inferior olives show neuronal loss and gliosis in most cases. The neocortex is mostly preserved in patients with disease duration of less than one year, but is focally affected by spongiosis and gliosis in patients with duration of disease longer than a year. FFI has been transmitted to wild type and transgenic mice following intracerebral inoculation of brain tissue from affected patients.

4.3.4 TSE associated with octapeptide coding repeat insertion mutations

With octapeptide-repeat insertion mutations, there are differences in the number of repeats as well as the order and composition of the repeat elements. The number of inserted sequences is the same in the

descendants of different lines in multi-generation families.

Clinical features are determined, to a significant extent, by the number of repeats. Patients with five and more extra repeats develop the disease earlier (35 years of age on average) than patients with one to four extra repeats (67 years of age). In addition, patients with five or more extra repeats have a longer duration of illness (8 years) when compared with patients with one to four repeats (4 months). Patients with one to four repeats have rapidly progressive dementia often associated with ataxia and visual disturbances, myoclonus and PSW on the EEG recordings. In patients with five or more octapeptide repeats, the illness is characterized by a slowly progressive mental deterioration with cerebellar and extrapyramidal signs, often without PSW on EEG examination. Patients with a low number of repeats show histopathologic changes consistent with those of sporadic TSE, including spongiform degeneration, astrogliosis and neuronal loss. In contrast, patients having seven or more octapeptide repeats have uni- or multi-centric PrP amyloid plaques located in the molecular layer of the cerebellum and the cerebral gray matter. These changes are similar to GSS.

Brain tissue from patients with five, seven and eight extra repeats has transmitted the disease to primates after intracerebral inoculation.

4.4 Iatrogenic CJD

4.4.1 Epidemiology of iatrogenic CJD

The similarity of the neuropathology of kuru and CJD led to the speculation that CJD was also transmissible. Experimental inoculation of primates with brain tissue of persons dying from CJD demonstrated that the brain of a person with CJD is infectious and suggested the risk of iatrogenic forms of CJD. As of September 2002, nearly 300 episodes of iatrogenic CJD have been identified. Figure 4.7 summarizes the known reports of iatrogenic CJD, by country reporting the cases.

The first iatrogenic CJD case was reported from the USA in 1974. The patient had received a corneal transplant at 55 years of age because of a corneal dystrophy. Eighteen months later she developed lethargy and ataxia, followed by myoclonus, spasticity and akinetic mutism. She died 8 months after the onset of her symptoms. The donor of the graft had died after a 2-month history that included ataxia, memory loss and myoclonus. Both the recipient and donor had typical neuropathological features of CJD at autopsy. Studies have subsequently demonstrated infectivity in corneas of animals inoculated with the CJD agent.

Further cases of iatrogenic CJD were reported in 1977. Two young patients from North America had undergone electrocorticography in 1974 for intractable epilepsy. During these procedures, the same two

Figure 4.7 Reports of iatrogenic CJD by country and mode of transmission

	Surgical procedures				Hormone therapy	
	Dura mater grafts	Surgical instruments	Stereotactic EEG needles	Corneal transplants	Growth hormone	Gonadotropin
Argentina	1					
Australia	5				1	4
Austria	1					
Brazil					1	
Canada	4					
Croatia	1					
France	9	1			89	
Germany	4			1		
Italy	4					
Japan	88			1		
Netherlands	2				1	
New Zealand	1				5	
Qatar					1	
Spain	6					
Switzerland	1		2			
Thailand	1					
United Kingdom	6	4			41	
United States	3			2	23	
World wide totals	**136**	**5**	**2**	**4**	**162**	**4**

From Brown P et al: Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology*, 2000 55:1075–1081; with permission. Updated 06 September 2002

silver electrodes were inserted into their cerebral cortices for several hours. The patients developed progressive neurological disease, after a delay of 16 and 20 months respectively. They subsequently died from histologically confirmed CJD. The electrode probes used in both cases had previously been implanted (for two days) into the brain of a 70-year-old woman with a 4-month history of mood disturbance, ataxia, mental deterioration and involuntary movements. She died 3 months later of histologically confirmed CJD. The electrodes had been cleaned with benzene, disinfected with 70% ethanol and sterilized in formaldehyde between each use. Twenty-eight months after their implantation in the original CJD case, the electrodes were inserted into the frontal lobes of a chimpanzee who, after a period of 18 months, developed an encephalopathy histologically confirmed as CJD.

Finally, four other instances of contaminated neurosurgical instruments transmitting CJD were retrospectively identified in the UK and France from exposures in the 1950s and 1960s. It is presumed that in these cases routine sterilization procedures were insufficient to eliminate infectivity.

In 1987, the first case of CJD linked with the use of a cadaver-derived dural homograft during a neurosurgical procedure was reported. Subsequently a further 135 similar cases have been identified. The majority of the implicated grafts were produced by a single manufacture between 1982 and 1986. At this time, the company produced the product by pooling dura while it was undergoing processing. Most if not all of the patients developing CJD following dura mater transplantation were exposed to tissues that had not been subjected to currently recommended decontamination procedures (treatment with 1 N sodium hydroxide[1] for one hour and rigorous donor selection).

Cadaver-derived human growth hormone (hGH) has been used since 1958, mainly for the medical treatment of children with growth hormone deficiency. The treatment was administered by intramuscular or subcutaneous injection. The hormone had been manufactured in batches, each batch containing up to 2000 pituitary glands. In 1985, the first case of CJD in a patient who had received hGH was reported. Subsequently, a further 161 cases have been reported, mainly in France, the UK and the USA. Cases have also been identified in Brazil and New Zealand in patients who received hGH manufactured in the USA, and in Australia in patients who had received locally produced hGH. Four cases of CJD were also recorded in Australian women treated with cadaver-derived pituitary gonadotropin. It is of note that a sample of one batch transmitted CJD to primates. In addition, experiments have shown that the infectious agent can survive methods of "inactivation" used in commercial production. The use of cadaver-derived growth hormone has now been replaced by recombinant growth hormone. Due to the long incubation period of CJD, it is possible that some further cases will appear in years to come.

The evidence supporting a causal relationship between CJD and pericardial grafts, dura mater grafts on a perforated eardrum, dura mater embolization and after liver transplant is largely absent. No reports of human TSE transmission from dental exposures have been identified. However, scrapie (not CJD) has been transmitted via abrasions with brain tissue of the dental pulp of experimental animals.

The incubation period for iatrogenic CJD can be long – up to 30 years and never less than 12 months. However, the incubation period after intracerebral exposure is shorter when compared with peripheral exposures. Additionally, the route of exposure seems to influence the clinical phenotype. Cerebellar onset is more typical in peripheral exposures (including dura mater implants onto the brain surface) and dementing onset in intracerebral exposures. See Figure 4.8

Figure 4.8 Summary of iatrogenic cases of CJD from all causes, indicating route of exposure, incubation period and clinical presentation

Mode of infection	Number of patients	Agent entry into brain	Mean incubation period, range[a]	Clinical presentation
Stereotactic EEG	2	Intracerebral	18 mo (16–20)	Dementia/cerebellar
Neurosurgery	5	Intracerebral	17 mo (12–28)	Visual/dementia/cerbellar
Corneal transplant	3	Optic nerve	18 mo[b] (16 mo–30 yr)	Dementia/cerebellar*
Dura mater graft	136	Cerebral surface	6 yr (1.5–18)	Cerebellar (visual/dementia)*
Gonadotropin	4	Haematogenous	13 yr (12–16)	Cerebellar
Growth hormone	162	Haematogenous	12 yr (5–30)	Cerebellar*

Adapted from Brown P et al: Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology*, 2000 55:1075–1081; with permission (updated 06 September 2002)

[a] Calculated from the midpoint of treatment to the onset of CJD symptoms
[b] Median
* Clinical information not available for all cases

[1] For NaOH solution, 1N = 1 mol/litre

To date, sporadic CJD is the only human TSE linked to iatrogenic transmission. In addition, transmission is a rare event and it has been confirmed in only specific and limited circumstances, as described above. Familial forms of human TSEs are extremely rare (less than 1 per 10 million population) and consequent iatrogenic exposures are extremely rare. The absence of detected transmissions of familial TSEs could reflect the rarity of exposure or a lower level of transmissibility.

4.4.2 Neuropathology of iatrogenic CJD

The main neuropathological characteristics of sporadic CJD (spongiform change, neuronal loss and astrocytosis) occur in iatrogenic disease, although the distribution of lesions varies from case to case. However, the neuropathology of hGH-related cases is noteworthy, as there is usually pronounced cerebellar atrophy associated with neuronal loss, widespread spongiform change and PrP amyloid plaque formation. In many cases, immunocytochemistry also shows widespread distribution of PrP in a diffuse pattern within the cerebellar granular layer. Furthermore, neuropathological changes in the spinal cord, particularly the presence of PrP amyloid plaques, are more frequent in hGH-related iatrogenic cases than in sporadic CJD.

4.4.3 Prevention of iatrogenic human TSEs

Recognition of the role of iatrogenic transmission of CJD led to the development, over time, of route-specific options to prevent further spread through these routes.

It is now recommended that instruments used for neurosurgical and invasive ophthalmological procedures on patients with suspected or confirmed CJD (or at risk of developing CJD, such as individuals with a family history of CJD, human dural homograft recipients and human cadaver-derived pituitary hormone recipients) should be destroyed or subjected to treatments designed to profoundly reduce the likelihood of transmission (see Chapter 6, Tissue handling and safety precautions).

Most countries of the world no longer use human-derived growth hormone, hence it can be expected that hGH-associated CJD will disappear over time.

Dura mater and cornea associated CJD can be effectively managed through better donor screening. Corneal donation from patients dying with dementia is not recommended. However, even the most stringent donor screening may not detect pre-symptomatic, but potentially infective, carriers of the TSE agent. Some countries have largely discontinued the use of commercial cadaver-derived dural homografts (Australia, New Zealand, and the United Kingdom) and are now using suitable synthetic or autologous alternatives in its place.

4.4.4 Concerns regarding the risk of CJD from blood or blood products

Increased awareness has raised concern about the risk that human TSEs are transmissible by transfusion. There have been intensive surveillance efforts directed toward investigating this possibility in the United Kingdom (focusing on vCJD) and other countries. In addition, a number of epidemiological studies (including a meta-analysis of case-control studies) have been published.

Experimental models have detected the infective agent in the blood and blood components of experimentally infected animals, transmitted infection via transfusion (rare) and transmitted infection via other routes of exposure (intracerebral inoculation). It is difficult to extrapolate from experimental data to the medical setting and in fact, there is no proven or probable instance of transmission of any human TSE by blood, blood components or plasma derivatives. Taken together, these data suggest that blood components from patients with CJD may contain low, but not transmissible levels of infectivity.

4.4.5 Concerns regarding occupational risks from TSEs

There are approximately reports of 40 cases of nosocomial exposure. It is reassuring to note that only one of these individuals had a history of occupational exposure to a human TSE – an orthopaedic surgeon had worked with human and ovine dura mater 20 years prior to his illness. The remaining reports appear to be coincidental TSE in a health care worker. Case–control studies do not suggest that individuals potentially exposed to the TSE agent in the health care setting are at an increased risk of developing CJD. It is noteworthy, and of some reassurance, that no case of CJD has been documented in any person working in a research laboratory studying human or animal TSEs.

However, the possibility that cases of CJD have rarely occurred in such circumstances cannot be confidently dismissed. Consequently, it is essential that occupational exposure be minimized wherever possible, but in particular exposure to brain and other high-risk tissues. Penetrating wounds or inoculation into mucosal tissues would logically seem to pose the highest risks.

In the UK, due to the epidemic of the known zoonotic BSE, investigations of farmers were undertaken to search for possible occupational risks from

BSE. A statistically significant excess of cases of CJD in cattle farmers has been reported in the UK since 1990. Four of these six cases were known to have had BSE-affected animals in their herds. However, analysis of the clinical and pathological features of these cases shows that none had the vCJD phenotype. This observation has been strengthened by recent molecular biological data demonstrating that the PrP glycosylation pattern characteristic of both BSE and vCJD was not present in any of these cases. Furthermore, analysis of the incidence of CJD in dairy farmers from other European countries, where BSE is rare or absent reveals a similar excess of cases. It is possible that case ascertainment in this group has been better than in other groups because of concern of a possible link between BSE and vCJD.

4.4.6 Concerns regarding vCJD

There is a reasonable speculation that the risk of iatrogenic transmission of vCJD may be higher than that of other human TSEs. Specifically, vCJD is accompanied by detectable PrP^{Sc} in peripheral tissues (including at least spleen, appendix, peripheral lymph nodes, dorsal root ganglia, and trigeminal ganglia), unlike the other human TSEs. Consequently, measures taken to avoid iatrogenic vCJD may be more stringent that those applied to sCJD (see Chapter 6 and Annex 4.3).

The weight of evidence suggests that the risk of parenteral transmission of any TSE by blood is remote and there is little evidence currently to suggest that vCJD will be different from sCJD in this respect. However, the threat of potential iatrogenic transmission of vCJD has led to extensive changes in surgical procedures within the UK and transfusion policies elsewhere. Whether these measures will be adopted worldwide will certainly depend upon emerging information about the epidemiology of BSE and vCJD globally. Some countries have chosen to implement donor deferral strategies to avoid transfusion exposure to vCJD. However, WHO does not recommend that this strategy be applied universally, noting that many countries are unable to identify sufficient donors to maintain supplies. Unless more evidence accrues to the contrary, except in areas where the loss of safe donors can safely be offset, blood services should focus their efforts on known transfusion-transmitted pathogens. They should do this by providing the appropriate donor selection and screening measures that will contribute most effectively to making blood safe in their region.

4.5 Variant CJD

4.5.1 Epidemiology

As of November 2002, 139 vCJD cases have been reported; 129 in the UK, six in France and one in each of Canada, Ireland, Italy and the USA. Compared to sporadic CJD, vCJD is characterized by a younger age of onset (mean 28 years, range 12–74, versus mean for sCJD of about 70 years) and by longer illness duration (mean 15 months, range 6–39, versus 8 months in sCJD). A consistent upward trend in the number of reports in the UK has flattened, but whether this trend will be sustained, or what the eventual number of vCJD cases will be is unclear.

4.5.2 Clinical course

Variant CJD characteristically affects persons in their mid-teens to early 40s (see Figure 4.9). The illness usually starts with non-specific psychiatric symptoms. Less often, it begins with sensory or other neurological disturbances. It progresses over months with

Figure 4.9 Age distribution at onset of vCJD and sCJD

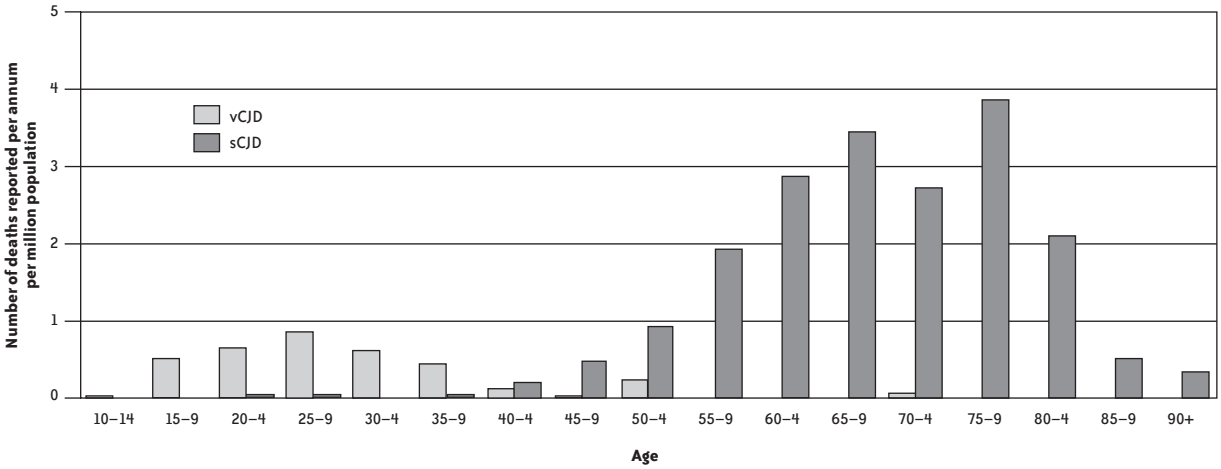


Figure 4.10 Months to clinical milestones in vCJD

Feature	Median time to development	Range 25–75th per centile
Dysphoria	0.75	0–4
Painful sensory disturbance	2.5	0–6.25
Gait or balance disturbance	4	2.5–7
Poor memory	4	1.75–7.5
Delusions	6	2–10
Disorientation	7.5	4.5–9
Involuntary movements	7.75	6–10.75
Hallucinations	8	6–10.75
Urinary incontinence	8.25	5.5–10.5

increasing ataxia, global cognitive impairment, and involuntary movements. Finally, the disease culminates in a state of incapacity and mutism. Routine blood tests are usually normal. Routine CSF examination is also typically unremarkable, except for a raised protein in about one-third of patients. The EEG characteristically shows nonspecific slow-wave abnormalities or is normal.

Although a minority of cases suffer from forgetfulness or mild unsteadiness of gait from an early stage, clear neurological signs are generally not apparent for many months after disease onset (median 6, range 4–25). During this time, the most prominent clinical features are psychiatric disturbance or sensory symptoms or both. After the onset of overt neurological dysfunction, usually ataxia, the illness rapidly progresses (see Figure 4.10) with global cognitive impairment, involuntary movements (myoclonus, chorea, dystonia, tremor), incontinence of urine, and increasing immobility leading to progressive dependency, unresponsiveness and mutism. Transient seizure-like episodes are only very rarely reported, often in patients taking potentially pro-epileptic medication. Just before death, patients are usually akinetic and mute. The mean delay from developing unsteadiness to becoming bed-bound is 6 months (range 3–13 months), and the median delay from becoming bed bound to death is 1.5 months (range 1 week–27 months) (see Figure 4.11). The most frequent cause of death is pneumonia.

4.5.3 Psychiatric features

Sixty-three percent of cases of vCJD present with a purely psychiatric onset. Fifteen percent present with a purely neurological onset. Twenty-two percent present with combined psychiatric and neurological features. Overall, by 3 months, 93% of cases have psychiatric symptoms and 69% have neurological symptoms.

Common early psychiatric features include dysphoria, withdrawal, anxiety, irritability, insomnia and loss of interest (see Figure 4.12). Forgetfulness (included in the data here as a psychiatric feature) is present at onset in only 12%, but developed in most patients before 8 months (median 4 months).

Delusions, characteristically fleeting, are a noteworthy psychiatric feature in many cases. These usually occurred a few months into the illness, but rarely were present at onset and in others did not occur until after 1 year. Examples include the belief that there were snipers in the house; that the patient had recently had a baby that died; that microscopic people were inside the patient's body; and that the patient had murdered someone. Only very rarely were the delusions sustained for more than a few hours. Nearly half of the patients hallucinate. Hallucinations tended to coincide with delusions. The hallucinations were more often visual than auditory. Examples include seeing monsters, spiders and flying firemen and hearing voices telling the patient to commit suicide.

Figure 4.11 Survival curve (in months) for sCJD and vCJD

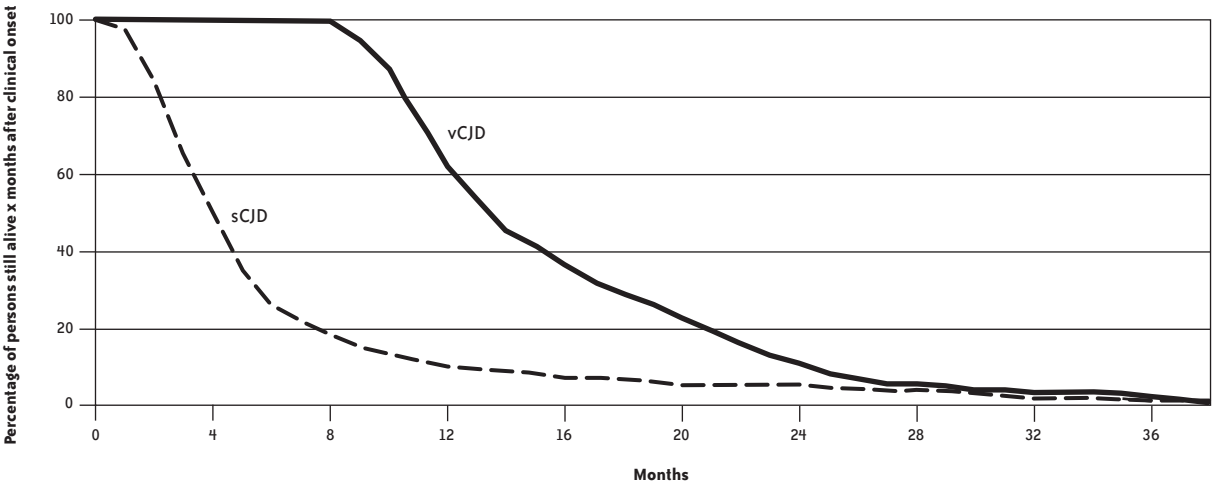


Figure 4.12 Psychiatric symptoms in vCJD

Psychiatric symptom	Percentage of cases
Dysphoria	62
Agitation or restlessness	62
Social or conversational withdrawal	61
Anxiety	58
Aggression, temper or violence	54
Insomnia	52
Tearfulness	49
Weight loss	49
Hallucinations	45
Paranoid delusions	38
Hypersomnia	36
Forgetfulness at onset	12
Suicidal ideation	9

Nearly two-thirds of patients saw a psychiatrist during their illness. Just over half of these visits were the initial referral. The mean duration of illness until psychiatric referral was 230 days. The initial psychiatric diagnosis in the majority of cases was depression, although in some the possibility of an organic basis was suspected, particularly in those with associated forgetfulness. In a small number of patients, a psychotic illness was suspected; rarely were psychiatric symptoms considered hysterical or functional only. The psychiatric treatment given to patients reflected these diagnoses and just over half over received antidepressants, often initially prescribed by their family doctor, and about one-sixth of cases received antipsychotic medication. Some patients improved transiently following treatment.

4.5.4 Sensory disturbance and other early neurological features

Fifty-seven percent of cases had at least one neurological symptom within 2 months of onset. In order of decreasing frequency they were sensory disturbance, gait disturbance or incoordination, involuntary movements (tremor, myoclonus, chorea, dystonia), dysarthria, double or blurred vision, dysphagia, taste disturbance.

All patients for whom there was sufficient clinical information available were noted to have involuntary movements. In order of decreasing frequency they were myoclonus, chorea, dystonia, and tremor. Other signs, in order of decreasing frequency, included pyramidal signs, upgaze paresis and primitive reflexes. Sensory abnormalities were noted on examination at some point during the illness in only about one-fifth of those with sensory symptoms. These included abnormalities of joint position sense, pinprick, hyperalgesia and hyperpathia. They did not appear to be persistent findings and examination was often hindered by cognitive impairment.

About two-thirds of patients had persistent sensory symptoms and in nearly one-third of these this was an initial feature. For those cases in which this was not an initial symptom, the median delay to its occurrence was 6 months (range 2–11 months). The symptoms were varied and some patients complained of more than one type of sensory disturbance. Limb pain was most common and was often nonspecific and poorly localized, usually occurring in the lower limbs. Other sensory symptoms, in order of decreasing frequency, included paraesthesia, dysaesthesia, cold feelings and numbness. Any part of the body could be affected, including the limbs, trunk, face, mouth and tongue, and the sensory disturbance could spread over time. The symptoms were lateralized in one-third of those affected. The clinical description and results of neurophysiological investigations suggest that sensory disturbance has a central rather than peripheral origin.

Formal neuropsychological examination was conducted in a minority of cases, mostly after the development of neurological symptoms and signs, and all demonstrated significant cognitive impairment, even in the rare instances when the testing was performed early in the clinical course.

4.5.5 Routine investigations

Routine haematological, biochemical and microbiological investigations, including inflammatory markers, are typically normal. Liver function tests are also usually normal, but about half of cases showed abnormalities that are minor, transient or both, most likely as a result of intercurrent infection or medication.

The EEG showed nonspecific slow-wave activity in most cases, but initial tracings were normal in a minority and some patients even had a normal EEG at a time when there was cognitive impairment, cerebellar signs and involuntary movements. No recording showed the periodic triphasic complexes typical of sporadic CJD, despite a number of patients undergoing examination in the final month of their illness (and two days before death in one case).

Routine CSF parameters (cell count, glucose, analysis for oligoclonal bands) were typically normal, with the exception of an elevation of CSF protein in nearly one-third of cases (mean, ~0.8 g/litre, range <2.3 g/litre).

4.5.6 Differential diagnosis

The most frequent conditions mimicking vCJD are sporadic CJD, Alzheimer disease and cerebral

Figure 4.13 Outcome in suspect vCJD cases with an alternative final diagnosis (*n*=51)

Degenerative
- Sporadic CJD (*n*=9)
- Alzheimer disease (*n*=6)
- Corticostriatonigral degeneration (*n*=1)
- Multi-system degeneration (*n*=1)

Infective/inflammatory
- Cerebral vasculitis (*n*=4)
- Encephalitis (*n*=1)
- Limbic encephalitis (*n*=1)
- Post-viral encephalopathy (*n*=1)
- Multiple sclerosis (*n*=1)
- Promyelocytic leukaemia (PML) (*n*=1)
- Possible encephalitis lethargica (*n*=1)

Hereditary
- Huntington disease (*n*=1)
- Probable familial spinocerebellar ataxia (*n*=1)

Metabolic
- Wilson disease (*n*=2)
- ?Metabolic disorder (*n*=1)

Other
- Cerebrovascular disease (*n*=2)
- Peripheral neuropathy (*n*=2)
- Vitamin B12 deficiency (*n*=1)
- Normal brain (*n*=1)
- No neuropathological diagnosis (*n*=1)
- Clinical recovery (*n*=4) or improvement (*n*=15)

vasculitis (see Figure 4.13). Alternative clinical diagnoses include peripheral neuropathy (perhaps reflecting the sensory symptoms described in some patients with vCJD) and causes of cognitive impairment in younger patients, including Wilson disease and vitamin B12 deficiency. The latter conditions and cerebral vasculitis are of particular importance because they are potentially treatable. Due to the great public concern regarding BSE and vCJD it is perhaps not surprising that patients with psychiatric conditions (neurosis and psychosis) have been reported who are convinced that they have developed BSE.

Figure 4.14 shows the incidence of various clinical characteristics in vCJD, sporadic CJD and cases initially thought to be vCJD, but that were subsequently found to have other diseases (non-cases). This indicates that no single feature is able to adequately distinguish these groups, although persistent sensory disturbance, chorea and upgaze paresis are much more frequently observed in vCJD than in sCJD.

It is possible that some early features may help discriminate vCJD from other conditions that present with similar nonspecific symptoms. The appearance of persistent and unexplained painful sensory disturbance within a few months of the onset of psychiatric symptoms may raise the possibility of vCJD, an assertion that would be strengthened by the presence of cognitive impairment, ataxia or involuntary movements. It is noteworthy that experience from the vCJD cases examined to date suggests that often these latter features were incorrectly attributed to side-effects of medication or were considered to be a consequence of a functional psychiatric illness. However, often the early symptoms will not point to a diagnosis of vCJD. Monitoring the evolution of the illness over the ensuing months

Figure 4.14 Clinical features of vCJD, sporadic CJD and suspect vCJD cases with alternative final diagnoses

Feature	Variant CJD[a]	Sporadic CJD[b]	Non-cases[c]
Psychiatric symptoms	98 (85)	55 (40)	90
Sensory disturbance	70 (19)	10 (5)	20
Ataxia	100 (9)	85 (40)	65
Forgetfulness	90 (12)	>95 (50)	—
Involuntary movements	100 (5)	90 (15)	—
Myoclonus	70 (0)	80 (<2)	65
Chorea	55 (0)	10 (1)	25 [d]
Dystonia	30 (4)	<15 (0)	20
Upgaze paresis	36 (0)	5 (0)	7
Dementia	100 (0)	>95	80
Akinetic mutism	51 (0)	55 (0)	30

Values are per centage of patients with the feature during the illness course and at onset in parenthesis.
[a] Details of all the clinical features were not available for some cases
[b] Values are an approximate and subjective estimation from the literature and personal experience
[c] Suspect vCJD cases with an alternative final diagnosis (*n*=27)
[d] Includes patients described as fidgety.

should be helpful, as vCJD is invariably progressive. For those patients in whom vCJD is considered, investigation for specific abnormalities on brain MRI is likely to be the most important early step toward a more definitive diagnosis. The role of MRI, CSF and tonsil biopsy is discussed in Chapter 9, Diagnostic tests for human transmissable spongiform encephalopathies, and Chapter 10, Specific protocols for the diagnosis of human transmissable spongiform encephalopathies.

4.6 Codon 129

Homozygosity of the PrP gene at codon 129 is over-represented in CJD. Seventy-nine percent of sporadic CJD cases are methionine homozygotes, compared with 37% of controls. Codon 129 also influences the nature and distribution of neuropathological lesions in sporadic CJD. In particular, PrP plaques are more common in valine homozygotes or heterozygotes (MV) than in methionine homozygotes. Evidence also suggests that the EEG from persons with valine homozygosity is much less likely to show periodic complexes than other genotypes. The duration of illness is slightly prolonged in those carrying at least one valine allele. It is noteworthy that the association with codon 129 status and susceptibility to sporadic CJD has not been seen in Japan, where the population has a different distribution of codon 129 genotype (MM = 92%, MV = 8% and VV=0%). Despite this, there appears to be no difference in the incidence of sporadic CJD in Japanese and Caucasians.

Iatrogenic cases of CJD have a high frequency of homozygosity (either methionine or valine) at codon 129. Furthermore, it has been observed that the codon 129 polymorphism influences the age of onset and clinical presentation in some cases of hereditary CJD.

Interestingly, the distribution of PrP genotypes in cases of kuru (MM = 30%, MV = 50% and VV=20%) does not differ significantly from a group of controls of the same ethnic background (MM = 30%, MV = 48% and VV=22%).

The result of the analysis of the PrP gene is available for 99 cases of vCJD. None showed a mutation and all were methionine homozygotes at codon 129.

CHAPTER 5

Care, nursing and treatment of persons with transmissible spongiform encephalopathies

Normal social and clinical contact, and non-invasive clinical investigations (e.g. X-ray imaging procedures), diagnostic tests or interventions involving non-infective tissues (e.g. blood tests, inoculations) with TSE patients do not present a risk to healthcare workers, relatives or the community.

There is no reason to defer, deny or in any way discourage the admission of a person with a TSE into any health care setting, including care at home. Based on current knowledge, isolation of patients is not necessary; they can be nursed in the open ward or at home using universal precautions. Chapter 6, Tissue handling and safety precautions, details infection control requirements that may arise as a result of invasive procedures, including CSF diagnostic testing. Otherwise, in home, hospital and laboratory settings, standard measures in handling blood or bloody tissues do not require modification to avoid sCJD.

Good nursing care to prevent the complications of immobility, such as pressure sores, is the most important treatment for a patient with CJD at this time. When in hospital settings, nursing in a single room is not required for infection control, but may be appropriate for compassionate reasons. Whether at home or in a hospital, patient waste should be handled according to recommended best practice. Contamination by any body fluid except CSF poses no greater hazard than for any other patient. No special precautions are required for eating utensils, feeding tubes, suction tubes, bed linens, or items used in skin or bed sore care in any environment.

Caregivers both in the home and health care setting should be made aware and anticipate the possibility of psychiatric symptoms, e.g. mood swings, hallucinations, or aggression. For this reason, provision of information and advice to professional and non-professional caregivers is recommended. As the disease is usually rapidly progressive, patients develop high dependency needs and require frequent assessment. It is essential to address the emotional, physical, nutritional, psychological, educational, and social needs of the patient and the associated needs of the relatives. Coordinated planning is vital in transferring care from one environment to another.

Current heightened awareness requires special sensitivity to the confidentiality of written and verbal communications. Measures to ensure the privacy of the patient and family are essential.

CJD and related disorders are invariably fatal and there is currently no available treatment for the underlying disease process. The absence of a practical test for infection during the incubation period, coupled with the rapidly progressive clinical phase of TSEs, makes these diseases difficult targets for therapy.

Therapies aimed at palliation of any distressing symptoms, such as clonazepam or sodium valproate for myoclonus, are frequently successfully administered. Sedatives may be required for agitation, but such symptoms, if present, often abate naturally as the illness progresses.

Patients are frequently given steroids, aciclovir or thiamine in the hope that they may have an occult, treatable condition such as a cerebral vasculitis, viral infection or Wernicke encephalopathy. Considering the possibility of a viral-like origin of CJD, a number of therapies have been tried unsuccessfully, including amantidine, interferon and other antiviral agents.

One of the most exploited strategies for drug discovery relies on testing compounds on cellular and animal models of prion diseases. Amphotericin B (an antifungal drug) and iododoxorubicin (an anticancer agent) have been found to delay death in hamsters or mice experimentally infected with scrapie. However, these drugs are potentially toxic and needed to be injected around the time of infection, or shortly afterward, to be effective. Amphotericin B has been tried in human CJD without benefit. A number of other therapeutic strategies have been suggested. These include drugs to block agent replication sites, polyanions, such as Dextran 500 and pentosan polysulfate, which are known to prolong the lifespan of mice infected with scrapie, and compounds that inhibit agent replication by interfering with PrP glycosylation.

Clearly, any finding which might lead to an effective treatment of CJD or vCJD is to be welcomed. However, further work is essential in order to establish whether these drugs can provide an effective treatment. Quinacrine and chlorpromazine are being offered on a compassionate protocol in some countries, however, without convincing or sustained improvements in health. A full evaluation of the benefits and risks of these proposed treatments in CJD using well-designed clinical trials is essential.

CHAPTER 6

Tissue handling and safety precautions

The well recognized resistance of the agent to conventional decontamination techniques forces infection control methods to be more stringent, and to provide guidance on situations where the risk is theoretical. A number of countries and organizations have developed guidelines to prevent transmission of the human TSEs. The reader can find further information in the guidelines prepared by various organizations on their specific websites (see Web sites of interest in Annex 5, Information resources).

6.1 General considerations

TSE agents exhibit an unusual resistance to conventional chemical and physical decontamination methods. They are not adequately inactivated by most common disinfectants, or by most tissue fixatives, and some infectivity may persist under standard hospital or healthcare facility autoclaving conditions (e.g. 121 °C for 15 minutes). They are also extremely resistant to high doses of ionizing and ultraviolet irradiation and some residual activity has been shown to survive for long periods in the environment.

The following chapter is derived from the WHO Infection Control Guideline on the prevention of iatrogenic and nosocomial exposure to TSE agents. This document followed the WHO Consultation on Caring for Patients and Hospital Infection Control in Relation to Human Transmissible Spongiform Encephalopathies, held in Geneva from 24 to 26 March 1999.

It provides guidance upon which infection control practitioners, health care practitioners, medical officers of health, and those involved in the care of persons suffering from TSE can base their care and infection control practices. It may prevent events which are either extremely rare (e.g. transmission of TSE through a surgical procedure) or hypothetical (e.g. transmission of TSE to a health care worker or family member). Throughout the chapter there is specific and assumed reference to country or region-specific guidelines for matters which lie within the legal jurisdiction of that country or region, i.e. International Air Transport Association (IATA) regulations for transportation of hazardous goods, or biosafety containment levels for laboratories. Readers should be familiar with such requirements for their own country or region.

It is recognized that some recommendations to ensure maximum safety to caregivers and the environment may under some circumstances be regarded as impractical. However, personnel involved with TSE patients or tissues are urged to endeavour to comply as far as possible. There is no reason for a patient with a TSE to be denied any procedure, as any associated risks should be reduced to negligible levels by following the recommendations in this document.

6.2 Evaluating risk in health care environments

When considering measures to prevent the transmission of TSE from patients to other individuals (patients, health care workers, or other care providers), it is important to understand the basis for stipulating different categories of risk. Risk is dependent upon three considerations:

— the probability that an individual has or will develop TSE

— the level of infectivity in tissues or fluids of these individuals

— the nature or route of the exposure to these tissues.

From these considerations it is possible to make decisions about whether any special precautions are needed. Specific TSE decontamination procedures are described below. If TSE decontamination is required, the question remains as to how stringent it should be. The specific recommendations are described in sections devoted to patient care, occupational injury, laboratory investigations and management after death.

6.2.1 Identification of persons for whom special precautions apply

Persons with confirmed or suspected TSEs are the highest risk patients. They must be managed using specific precautions, which will be described in this and subsequent sections. All precautions recommended in the body of this document apply to the care of confirmed or suspect cases of TSE, or the handling of tissues from such patients, and unless

otherwise noted, no distinction will be made between confirmed and suspect cases.

However, the concept of 'persons at risk for TSE' is useful in infection control, as it allows for the development of intermediate precautionary measures. The following persons have been regarded as 'at risk' for developing TSEs. The bracketed numbers are the number of reported occurrences of CJD transmitted through that route:

- recipients of dura mater (136 cases);
- recipients of human cadaver-derived pituitary hormones, especially human cadaver-derived growth hormone (166 cases);
- recipients of cornea transplants (four cases – one definite, one probable, two possible);
- persons who have undergone neurosurgery (7);
- members of families with heritable TSE.

The discussion and recommendations for healthy asymptomatic individuals considered to be at risk for TSE are described in Annex 4.2 and referred to in Figure 6.8.

The consultants did not extensively discuss the management of persons who have confirmed or suspected vCJD, due to the absence of specific data for review and the geographical isolation of the current cases. The discussion and their recommendations are described in Annex 4.3 and Figure 6.8.

Figure 6.1 Distribution of infectivity in the human body[a]

Infectivity category	Tissues, secretions, and excretions	
High infectivity	Brain Spinal cord Eye	
Low infectivity	CSF Kidney Liver Lung Lymph nodes/spleen Placenta	
No detectable infectivity	Adipose tissue Adrenal gland Gingival tissue Heart muscle Intestine Peripheral nerve Prostate Skeletal muscle Testis Thyroid gland Blood[b]	Tears Nasal mucus Saliva Sweat Serous exudate Milk Semen Urine Faeces

[a] Assignment of different organs and tissues to categories of *high* and *low infectivity* is chiefly based upon the frequency with which infectivity has been detectable, rather than upon quantitative assays of the level of infectivity, for which data are incomplete. Experimental data include primates inoculated with tissues from human cases of CJD, but have been supplemented in some categories by data obtained from naturally occurring animal TSEs. Actual infectivity titres in the various human tissues other than the brain are extremely limited, but data from experimentally-infected animals generally corroborate the grouping shown in the table.

[b] See discussion this section and section 5.2.

6.2.2 Tissue infectivity

From published and unpublished information, infectivity is found most often and in highest concentration in the central nervous system (CNS), specifically the brain, spinal cord and eye. This section will refer to these tissues as 'high infectivity tissues'.

Infectivity is found less often in the cerebrospinal fluid (CSF) and several organs outside the CNS (lung, liver, kidney, spleen/lymph nodes, and placenta). This document will refer to these tissues as 'low infectivity tissues'.

No infectivity has been detected in a wide variety of other tested tissues (heart, skeletal muscle, peripheral nerve, adipose tissue, gingival tissue, intestine, adrenal gland, thyroid, prostate, testis) or in bodily secretions or excretions (urine, faeces, saliva, mucus, semen, milk, tears, sweat, serous exudate). Experimental results investigating the infectivity of blood have been conflicting; however, even when infectivity has been detectable, it is present in very low amounts and there are no known transfusion transmissions of CJD. This document classifies these tissues as having no detectable infectivity ('no detectable infectivity tissues') and, for the purposes of infection control, they will be regarded as non-infectious (see Figure 6.1).

6.2.3 Route of exposure

When determining risk, infectivity of a tissue must be considered together with the route of exposure. Cutaneous exposure of intact skin or mucous membranes (except those of the eye) poses negligible risk; however, it is prudent and highly recommended to avoid such exposure when working with any high infectivity tissue. Transcutaneous exposures, including contact exposures to non-intact skin or mucous membranes,[1] splashes to the eye,[2] and inoculations via needle[3,4] or scalpel and other surgical

[1] TSE can be experimentally transmitted to healthy animals by exposing abraded gingival tissue to infected brain homogenate.

[2] By analogy with cornea transplants.

[3] A documented route of transmission in humans, from contaminated human cadaver-derived pituitary hormones (hGH and gonadotropin).

[4] Intraperitoneal, intramuscular and intravenous administration of *low infectivity* tissue extracts can cause transmission of TSE in experimental animals.

instruments[1] pose a greater potential risk. Thus, it is prudent to avoid these types of exposures when working with either low infectivity or high infectivity tissues. CNS exposures (i.e. inoculation of the eye or CNS) with any infectious material poses a very serious risk, and appropriate precautions must always be taken to avoid these kinds of exposures.

6.3 Medical interventions in home and health care settings

6.3.1 Patient care

Please see Chapter 5, Patient care and treatment. Detailed information on disposal of medical waste is provided in this chapter, Section 6.7, Waste disposal.

6.3.2 Dental procedures

Although epidemiological investigation has not revealed any evidence that dental procedures lead to increased risk of iatrogenic transmission of TSEs among humans, experimental studies have demonstrated that animals infected by intraperitoneal inoculation develop a significant level of infectivity in gingival and dental pulp tissues. Additionally, TSEs can be transmitted to healthy animals by exposing root canals and gingival abrasions to infectious brain homogenate. General infection control practices recommended by national dental associations are sufficient when treating TSE patients during procedures not involving neurovascular tissue. The participants in the WHO Consultation were unable to come to a consensus on the risk of transmission of TSEs through major dental procedures; therefore, extra precautions, such as those listed in Figure 6.2, have been provided for consideration without recommendation.

Figure 6.2 Optional precautions for major dental work

1. Use single-use items and equipment, e.g. needles and anaesthetic cartridges.
2. Reusable dental broaches and burrs that may have become contaminated with neurovascular tissue should either be destroyed after use (by incineration) or decontaminated by a method listed in Annex 1.
3. Schedule procedures involving neurovascular tissue at end of day to permit more extensive cleaning and decontamination.

[1] By analogy with transmissions following neurosurgical procedures.

6.3.3 Diagnostic procedures

During the earlier stages of disease, patients with TSE who develop intercurrent illnesses may need to undergo the same kinds of diagnostic procedures as any other hospitalized patient. These could include ophthalmoscopic examinations, various types of endoscopy, vascular or urinary catheterization, and cardiac or pulmonary function tests. In general, these procedures may be conducted without any special precautions, as most tissues with which the instruments come in contact contain no detectable infectivity (see Figure 6.1). A conservative approach would nevertheless try to schedule such patients at the end of the day to allow more strict environmental decontamination and instrument cleaning. When there is known exposure to high- or low-infectivity tissues, the instruments should be subjected to the strictest form of decontamination procedure that can be tolerated by the instrument. Instrument decontamination is discussed in more detail in Section 6.5.2, Clinical diagnostic laboratories. Decontamination methods are specifically described in Annex 4.1.

6.3.4 Surgical procedures

Before admission to a hospital or health care facility, the infection control team should be informed of the intention to perform a surgical procedure on any person with confirmed or suspected TSE. Every effort should be made to plan carefully not only the procedure, but also the practicalities surrounding the procedure, e.g. instrument handling, storage, cleaning and decontamination or disposal. Written protocols are essential. All staff directly involved in these procedures or in the subsequent reprocessing or disposal of potentially contaminated items, should be aware of the recommended precautions, and be adequately trained. The staff should be made aware of any such procedures in sufficient time to allow them to plan and to obtain suitable instruments and equipment (such as single-use items), and it may be useful to schedule the patient at the end of the day's operating list. Staff must adhere to protocols that identify specifics regarding pre-operative, peri-operative and post-operative management of the patient, disposable materials, including bandages and sponges, and reusable materials. Ancillary staff, such as laboratory and central instrument cleaning personnel, must be informed and appropriate training provided.

Basic protective measures are described in Figure 6.3. Recommendations for decontamination of equipment and environment, and for disposal of infectious waste should be followed. Supervisors should be responsible for ensuring that the appro-

Figure 6.3 Precautions for surgical procedures

Wherever appropriate and possible, the intervention should:

1. be performed in an operating theatre;
2. involve the minimum required number of health care personnel;
3. use single-use equipment as follows:
   i) liquid-repellent operating theatre gown, over a plastic apron
   ii) gloves
   iii) mask
   iv) visor or goggles
   v) linens and covers;
4. mask all non-disposable equipment;
5. maintain one-way flow of instruments;
6. treat all protective clothing, covers, liquid and solid waste by a method listed under Annex 4.1; incineration is preferred
7. mark samples with a "Biohazard" label;
8. clean all surfaces according to recommendations specified in Section 6.6.3 and Annex 4.1

priate procedures are followed and that effective management systems are in place.

Procedures which are normally carried out at the bedside (e.g. lumbar puncture, bone marrow biopsy) may be performed at the bedside, but care should be taken to ensure ease of environmental decontamination should a spillage occur.

6.3.5 Handling of surgical instruments

General measures

Methods for instrument decontamination are fully discussed in Annex 4.1. Determination of which method to use is based upon the infectivity level of the tissue and the way in which instruments will subsequently be reused. For example, where surgical instruments contact high infectivity tissues, single-use surgical instruments are strongly recommended. If single-use instruments are not available, maximum safety is attained by destruction of reusable instruments. Where destruction is not practical, reusable instruments must be handled as detailed in Figure 6.4 and must be decontaminated as described in Annex 4.1.

Although CSF is classified as a low-infectivity tissue and is less infectious than high-infectivity tissues it was felt that instruments contaminated by CSF should be handled in the same manner as those contacting high-infectivity tissues. This exception reflects the higher risk of transmission to any person on whom the instruments would be reused for the procedure of lumbar puncture.

Figure 6.4 General measures for cleaning instruments and environment

- Instruments should be kept moist until cleaned and decontaminated.
- Instruments should be cleaned as soon as possible after use to minimize adherence of tissues, blood and body fluids.
- Avoid mixing instruments used on no-detectable-infectivity tissues with those used on high- and low-infectivity tissues.
- Recycle durable items for reuse only after TSE decontamination by methods found in Section 6.6 and Annex 4.1
- Instruments to be cleaned in automated mechanical processors must be decontaminated by methods described in Section 6.6 and Annex 4.1 before processing through these machines, and the washers (or other equipment) should be run through an empty cycle before any further routine use.
- Cover work surfaces with disposable material, which can then be removed and incinerated; otherwise clean and decontaminate underlying surfaces thoroughly, using recommended decontamination procedures in Section 6.6 and Annex 4.1
- Be familiar with and observe safety guidelines when working with hazardous chemicals such as sodium hydroxide (NaOH, 'soda lye') and sodium hypochlorite (NaOCl, 'bleach') (see Annex 4.1 for definitions).
- Observe manufacturers' recommendations regarding care and maintenance of equipment.

Those instruments used for invasive procedures on TSE patients (i.e. used on high- or low-infectivity tissues) should be securely contained in a robust, leak-proof container labeled "Biohazard". They should be transferred to the sterilization department as soon as possible after use, and treated by a method listed in Annex 4.1 or incinerated. A designated person who is familiar with this guideline should be responsible for the transfer and subsequent management.

Destruction of surgical instruments

Items for disposal by incineration should be isolated in a rigid clinical waste container, labeled 'Hazardous', and transported to the incinerator as soon as practicable, in line with the current disposal of clinical waste guidance described in the *Teacher's guide: management of wastes from health-care facilities*[1]

[1] Pruess A, Townend WK. *Teacher's guide: management of wastes from health-care activities*. Geneva, World Health Organization, 1998 (document WHO/EOS/98.6).

published by WHO. To avoid unnecessary destruction of instruments, quarantine of instruments while determining the final diagnosis in persons suspected of TSEs may be used.

Quarantine

If a facility can safely quarantine instruments until a diagnosis is confirmed, quarantine can be used to avoid needless destruction of instruments when suspect cases are later found not to have a TSE. Items for quarantine should be cleaned by the best non-destructive method given in Section 6.6, Decontamination procedures, and Annex 4.1, sterilized, packed, date and 'Hazard' labeled, and stored in specially marked rigid sealed containers.[1] Monitoring and ensuring maintenance of quarantine is essential to avoid accidental reintroduction of these instruments into the circulating instrument pool. If TSE is excluded as a diagnosis, the instruments may be returned to circulation after appropriate sterilization.

6.3.6 Anaesthesia

General anaesthesia

TSEs are not transmissible by the respiratory route; however, it is prudent to treat any instruments in direct contact with mouth, pharynx, tonsils and respiratory tract by a method described in Annex 4.1. Destruction by incineration of non-reusable equipment is recommended.

Local anaesthesia

Needles should not be reused; in particular, needles contacting the CSF (e.g. for saddle blocks and other segmental anaesthetic procedures) must be discarded and destroyed.

6.3.7 Pregnancy and childbirth

TSE is not known to be transmitted from mother to child during pregnancy or childbirth; familial disease is inherited as a result of genetic mutations. In the event that a person with TSE becomes pregnant, no particular precautions need to be taken during the pregnancy, except during invasive procedures as discussed in Section 6.3.4, Surgical procedures. Childbirth should be managed using standard infection control procedures, except that precautions should be taken to reduce the risk of exposure to placenta and any associated material and fluids. Non-reusable instruments should be disposed of by incineration. Reusable instruments should be handled as for any other clinical procedure (Figures 6.3, 6.4, Annex 4.1). In home deliveries, the midwife (or any other persons in charge of delivery) should ensure that any contaminated material is removed and disposed of in accordance with correct procedures for infected clinical waste.

6.4 Occupational injury

6.4.1 Occupational exposure

Although there have been no confirmed cases of occupational transmission of TSE to humans, cases of CJD in health care workers have been reported in which a link to occupational exposure is suggested. Therefore, it is prudent to take a precautionary approach. In the context of occupational exposure, the highest potential risk is from exposure to high infectivity tissues through needle-stick injuries with inoculation; however, exposure to either high- or low-infectivity tissues through direct inoculation (e.g. needle-sticks, puncture wounds, 'sharps' injuries, or contamination of broken skin) must be avoided. Exposure by splashing of the mucous membranes (notably the conjunctiva) or unintentional ingestion may be considered a hypothetical risk and must also be avoided. Health care personnel who work with patients with confirmed or suspected TSEs, or with their high- or low-infectivity tissues, should be appropriately informed about the nature of the hazard, relevant safety procedures, and the high level of safety which will be provided by the proposed procedures described throughout this document.

6.4.2 Post-exposure management

Appropriate counselling should include the fact that no case of human TSE is known to have occurred through occupational accident or injury. A number of strategies to minimize the theoretical risk of infection following accidents have been proposed, but their usefulness is untested and unknown. For the present the following common-sense actions are recommended:

- Contamination of unbroken skin with internal body fluids or tissues: wash with detergent and abundant quantities of warm water (avoid scrubbing), rinse, and dry. Brief exposure (one minute, to 0.1 N NaOH[2] or a 1:10 dilution of bleach) can be considered for maximum safety.

[1] Although the intention of quarantine is to avoid destruction of instruments and will permit the reintroduction of instruments only if TSEs are not diagnosed, the use of a decontamination method for TSEs will confer additional safety should an instrument unintentionally come in contact with staff or patients.

[2] For NaOH solution, 0.1N = 0.1 mol/litre.

- Needle sticks or lacerations: gently encourage bleeding; wash (avoid scrubbing) with warm soapy water, rinse, dry and cover with a waterproof dressing. Further treatment (e.g. sutures) should be appropriate to the type of injury. Report the injury according to normal procedures for your hospital or health care facility/laboratory.
- Splashes into the eye or mouth: irrigate with either saline (eye) or tap water (mouth); report according to normal procedures for your hospital or health care facility/laboratory.
- Health and safety guidelines mandate reporting of injuries and records should be kept for no less than 20 years.

6.5 Laboratory investigations

6.5.1 Safety in the health care laboratory

Adherence to the following routine precautions during any diagnostic procedure or laboratory work will reduce the risk of infection. General protective measures and basic precautions as outlined in Figure 6.5 are recommended for hospital-based diagnostic laboratories as well as during decontamination procedures in those laboratories. Detailed descriptions of these general protective measures can be found in the WHO document *Safety in health-care laboratories*[1] from which Figure 6.5 is adapted. Where local or national regulations and guidelines exist, these should also be consulted. Only persons who have been advised of the potential hazards and who meet specific entry requirements (i.e. training) should be allowed to enter the laboratory working areas, or to participate in the collection of high-infectivity tissues from patients with confirmed or suspected TSEs.

6.5.2 Clinical diagnostic laboratories

The vast majority of diagnostic examinations in clinical laboratories are performed on blood (e.g. complete blood counts) and serum (e.g. chemistries), usually with automated analysing equipment. As discussed earlier, blood and its components, although found to contain very low levels of infectivity in experimental models of TSE, have never been identified to be responsible for any case of CJD in humans, despite numerous exhaustive searches. The consultation felt that this epidemiological evidence was more relevant and more persuasive than the experimental evidence, and strongly recommended that blood specimens from patients with CJD not be considered to be infectious, and that no special precautions were needed for its handling in clinical laboratories. Similarly, except for CSF, other body fluids, secretions and excretions contain no infectivity, and need no special handling (see Section 6.2 and Figure 6.1).

Figure 6.5 General protective measures

1. Eating, drinking, smoking, storing food and applying cosmetics must not be permitted in the laboratory work areas.
2. Laboratory coveralls, gowns or uniforms must be worn for work and removed before entering non-laboratory areas; consider the use of disposable gowns; non-disposable gowns must be decontaminated by appropriate methods (see Waste Disposal and Annex 4.1).
3. Safety glasses, face-shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes and particles.
4. Gloves appropriate for the work must be worn for all procedures that may involve unintentional direct contact with infectious materials. Armoured gloves should be considered in post-mortem examinations or in the collection of high-infectivity tissues.
5. All gowns, gloves, face-shields and similar re-usable or non-reusable items must be either cleaned using methods set out in Annex 4.1, or destroyed as per Section 6.7, Waste disposal.
6. Wherever possible, avoid or minimize the use of sharps (needles, knives, scissors and laboratory glassware), and use single-use disposable items.
7. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
8. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day, using methods described in Section 6.6, Decontamination procedures, and Annex 4.1.
9. All contaminated materials, specimens and cultures must be either incinerated, or decontaminated using methods described in Section 6.6, Decontamination procedures, and Annex 4.1 and Section 6.7, Waste disposal before disposal.
10. All spills or accidents that are overt or potential exposures to infectious materials must be reported immediately to the laboratory supervisor, and a written record retained.
11. The laboratory supervisor should ensure that adequate training in laboratory safety is provided and that practices and procedures are understood and followed.

[1] *Safety in health-care laboratories*. 2nd ed. Geneva, World Health Organization, 1992. This edition is under revision.

Figure 6.6 Precautions for working with high- and low-infectivity tissues from patients with known or suspected TSEs

1. Whenever possible and where available, specimens should be examined in a laboratory or centre accustomed to handling high- and low-infectivity tissues; in particular, high-infectivity tissue specimens should be examined by experienced personnel in a TSE laboratory.
2. Samples should be labeled 'Biohazard'.
3. Single-use protective clothing is preferred as follows:
   — liquid-repellent gowns over plastic apron;
   — gloves (cut-resistant gloves are preferred for brain cutting);
   — mask;
   — visor or goggles.
4. Use disposable equipment wherever possible.
5. All disposable instruments that have been in contact with high-infectivity tissues should be clearly identified and disposed of by incineration.
6. Use disposable non-permeable material to prevent contamination of the work surface. This covering and all washings, waste material and protective clothing should be destroyed and disposed of by incineration.
7. Fixatives and waste fluids must be decontaminated by a decontamination method described in Section 6.6 and Annex 4.1 or adsorbed onto materials such as sawdust and disposed of by incineration as hazardous materials.
8. Laboratories handling large numbers of samples are advised to adopt more stringent measures because of the possibility of increased residual contamination, e.g. restricted access laboratory facilities, the use of 'dedicated' microtomes and processing labware, decontamination of all wastes before transport out of the facility for incineration.

Note: This document contains recommendations designed for health care laboratories and is not intended as a guideline for scientific research laboratories. WHO has identified a number of reference laboratories[1] that may be contacted for advice on safety protocols for investigational laboratory environments.

CSF may be infectious and must be handled with care. It is recommended that analysis not be performed in automated equipment, and any materials coming in contact with the CSF must either be incinerated or decontaminated according to one of the methods listed in Section 6.6, Decontamination procedures, and Annex 4.1. There is no reason for a diagnostic test to be denied if these measures are observed.

6.5.3 Surgical pathology

Although brain biopsy tissue is (at least historically) the most likely tissue from a patient with a TSE to be examined in the surgical pathology laboratory, it may also occur that other tissues are sent to the laboratory for examination. This might happen if patients with TSE undergo surgical procedures of one sort or another for intercurrent problems during the course of their neurological illness. The tissue categories of high infectivity, low infectivity, and no detectable infectivity are listed and discussed in Section 6.2 and Figure 6.1. Precautions to be taken when handling different tissue specimens are presented in Figure 6.6. Since histopathological processing of brain tissue is most often conducted upon autopsy (WHO does not recommend brain biopsy for the diagnosis of CJD; rather it is recommended for the diagnosis of other treatable disease), detailed instructions for histopathological processing are described in Section 6.8.2, Post-mortem examination: histopathological examination.

6.5.4 Transport of specimens by air

The transportation of pathology samples by air must comply with the International Air Transport Association (IATA) Restricted Articles Regulations and any additional requirements of the individual carriers. Documentation required by the IATA includes a Shipper's Certificate for Restricted Articles, which requires that the content, nature and quantity of infectious material to be disclosed. The *WHO Guidelines for the safe transport of infectious substances and diagnostic specimens*[2] provides more information on the safe transport of material. Where properly packaged according to these guidelines, there is no danger to the carriers.

6.6 Decontamination procedures

6.6.1 General considerations

TSE agents are unusually resistant to disinfection and sterilization by most of the physical and chemical methods in common use for decontamination of infectious pathogens. Figure 6.7 lists a number of commonly used chemicals and processes that cannot be depended upon for decontamination, as they have been shown to be either ineffective or only partially

[1] *Global surveillance, diagnosis and therapy of human transmissible spongiform encephalopathies. Report of a WHO Consultation*. Geneva, World Health Organization, 1998 (document WHO/EMC/ZDI/98.9).

[2] *Guidelines for the safe transport of infectious substances and diagnostic specimens*. Geneva, World Health Organization, 1997 (document WHO/EMC/97.3).

Figure 6.7 Ineffective or suboptimal disinfectants

Chemical disinfectants	Gaseous disinfectants	Physical processes
Ineffective[1]	**Ineffective**	**Ineffective**
alcohol	ethylene oxide	boiling
ammonia	formaldehyde	dry heat (<300 °C)
β-propiolactone		ionizing, UV or microwave radiation
formalin		
hydrochloric acid		
hydrogen peroxide		
peracetic acid		
phenolics		
sodium dodecyl sulfate (SDS) (5%)		
Variably or partially effective		**Variably or partially effective**
chlorine dioxide		autoclaving at 121 °C for 15 minutes
glutaraldehyde		boiling in 3% sodium dodecyl sulfate (SDS)
guanidinium thiocyanate (4 mol/litre)		
iodophores		
sodium dichloro-isocyanurate		
sodium metaperiodate		
urea (6 mol/litre)		

effective in destroying TSE infectivity. Variability in the effectiveness appears to be highly influenced by the nature and physical state of the infected tissues. For example, infectivity is strongly stabilized by drying or fixation with alcohol, formalin or glutaraldehyde. As a consequence, contaminated materials should not be exposed to fixation reagents, and should be kept wet between the time of use and disinfection by immersion in chemical disinfectants.

6.6.2 Decontamination of instruments

Policy-makers should be guided by the infectivity level of the tissue contaminating the instrument and by the expectations of how the instrument will be re-used, as per Section 6.2, Evaluating risk in healthcare environments. In this way, the most stringent recommendations are applied to instruments contacting high infectivity tissues of a person with a known TSE, which will also subsequently be reused in the CNS or spinal column. Policy-makers are encouraged to adopt the highest decontamination methods feasible until studies are published which clarify the risk of reusing decontaminated instruments.

Annex 4.1 lists the decontamination methods available, in order of decreasing effectiveness. The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on surgical instruments is to discard and destroy them by incineration. While this strategy should be universally applied to those devices and materials that are designed to be disposable, it was also recognized that this might not be feasible for many devices and materials that were not designed for single use. For these situations, the methods recommended in Annex 4.1 appear to remove most and possibly all infectivity under the widest range of conditions.

Those surgical instruments that are going to be reused may be mechanically cleaned in advance of subjecting them to decontamination. Mechanical cleaning will reduce the bio-load and protect the instrument from damage caused by adherent tissues. If instruments are cleaned before decontamination, the cleaning materials must be treated as infectious waste, and the cleaning station must be decontaminated by one of the methods listed in Annex 4.1. The instruments are then treated by one of the decontamination methods recommended in Annex 4.1 before reintroduction into the general instrument sterilization processes. A minority opinion held that instruments should be decontaminated before mechanical cleaning, and then handled as per general instrument sterilization processes.

Annex 4.1 recommends that, where possible, two or more different methods of inactivation be combined in any sterilization procedure for these agents. Procedures that employ heat and NaOH (either consecutively or simultaneously) appear to be sterilizing under worst-case conditions (e.g. infected brain tissue partly dried on to surfaces). Moreover, hot alkaline hydrolysis reduces biological macromolecules to their constituent subunits, thereby cleaning as well as inactivating.

The Consultation recognized that complex and expensive instruments such as intracardiac monitoring devices, fibreoptic endoscopes, and microscopes cannot be decontaminated by the harsh procedures specified in Annex 4.1. Instead, to the extent possible, such instruments should be protected from surface contamination by wrapping or bagging with disposable materials. Those parts of the device that come into contact with internal tissues of patients should be subjected to the most effective decontami-

[1] Some of these chemicals may have very small effects on TSE infectivity and are not adequate for disinfection.

nating procedure that can be tolerated by the instrument. All adherent material must be removed and, if at all possible, the exposed surfaces cleaned using a decontamination method recommended in Annex 4.1. Some instruments can be partly disassembled (e.g. drills and drill bits). Removable parts that would not be damaged by autoclaving, NaOH, or bleach should be dismounted and treated with these agents. In all instances where unfamiliar decontamination methods are attempted, the manufacturer should be consulted. These cleaning procedures should be applied even if the instrument has been reused before discovery of its potential contamination.

Contaminated instruments or other contaminated materials should not be cleaned in automated washers without first having been decontaminated using a method recommended in Annex 4.1.

6.6.3 Decontamination of work surfaces

Because TSE infectivity persists for long periods on work surfaces, it is important to use disposable cover sheets whenever possible to avoid environmental contamination, even though transmission to humans has never been recognized to have occurred from environmental exposure. It is also important to mechanically clean and disinfect equipment and surfaces that are subject to potential contamination, to prevent environmental build-ups. Surfaces contaminated by TSE agents can be disinfected by flooding, for one hour, with NaOH or sodium hypochlorite, followed by water rinses (see Annex I for detailed instructions). Surfaces that cannot be treated in this manner should be thoroughly cleaned; consider use of a partially effective method as listed in Figure 6.7. Cleaning materials should be treated as potentially contaminated.

6.6.4 Decontamination of wastes and waste-contaminated materials

Decontamination of waste liquid and solid residues should be conducted with the same care and precautions recommended for any other exposure to TSE agents. The work area should be selected for easy containment of contamination and for subsequent disinfection of exposed surfaces. All waste liquids and solids must be captured and treated as infectious waste.

Liquids used for cleaning should be decontaminated in situ by addition of NaOH or hypochlorite or any of the procedures listed in Annex 4.1, and may then be disposed of as routine hospital waste. Absorbents, such as sawdust, may be used to stabilize liquids that will be transported to an incinerator; however, this should be added after decontamination.

Cleaning tools and methods should be selected to minimize dispersal of the contamination by splashing, splatters and aerosols. Great care is required in the use of brushes and scouring tools. Where possible, cleaning tools such as brushes, towelling and scouring pads, as well as tools used for disassembling contaminated apparatus, should either be disposable or selected for their ability to withstand the disinfection procedures listed in Annex 4.1.

Upon completion of the cleaning procedure, all solid wastes including disposable cleaning materials should be collected and decontaminated. Incineration is highly recommended. The cleaning station should then itself be decontaminated using one of the methods in Annex 4.1.

Automated cleaning equipment must not be used for any instrument or material that has not previously been thoroughly decontaminated following the recommendations in Section 6.5.2, Clinical diagnostic laboratories, and Annex 4.1.

6.6.5 Personal protection during decontamination procedures

Persons involved in the disinfection and decontamination of instruments or surfaces exposed to the tissues of persons with TSE should wear single-use protective clothing, gloves, mask and visor or goggles, as noted in Section 6.5.3, Surgical pathology and Figure 6.6. The recommendations found in Figure 6.6 can be adapted to different situations. All individuals involved with disinfection and decontamination procedures should be familiar with these basic protective measures and precautions. Handling of contaminated instruments during transfers and cleaning should be kept to a minimum.

6.6.6 Decontamination risk categories

The recommended levels of decontamination are shown in Figure 6.8 for different patient and tissue risk categories (including patients at risk of TSE and patients with vCJD). The table reflects the consensus of the Consultation, and should be used in conjunction with Section 6.2, Evaluating risk in health care settings (particularly Figure 6.1 which lists specific high- and low-infectivity tissues) and Annex 4.1, which describes specific decontamination options.

6.7 Waste disposal

Infectious health care waste is defined as the discarded materials that have been in contact with blood and its derivatives, or wastes from infection isolation wards. These include but are not limited to cultures,

Figure 6.8 Decontamination levels for different risk categories

Patient category	Tissue category	Decontamination options
Confirmed or suspect cases of TSE	High infectivity	Annex 4.1
	Low infectivity	Annex 4.1 (but note that CSF, and peripheral organs and tissues are regarded as less infectious than the CNS)
Persons with known prior exposure to human pituitary-derived hormones, cornea or dura mater grafts	High infectivity	Annex 4.1
	Low infectivity	Routine cleaning and disinfection procedures
Members of families with heritable forms of TSE	High infectivity	No consensus was reached. The majority felt that TSE decontamination method should be used, but a minority felt this was unwarranted.
	Low infectivity	Routine cleaning and disinfection procedures
All of the above categories	No detectable infectivity	Routine cleaning and disinfection procedures
Confirmed or suspect cases of vCJD	All tissue categories	Annex 4.1

tissues, dressings, swabs or other items soaked with blood, syringe needles, scalpels, diapers, and blood bags. The term 'TSE infectious health care waste' applies to high and low infectivity tissues from persons with confirmed or suspected TSEs, or high-infectivity tissue from persons with known prior exposure to cornea, dura matter or human growth hormone, and any disposable items that have come in contact with these tissues.

In the absence of a national standard, disposal of biological waste contaminated by a TSE is to be performed in accordance with the best practice that is most consistent with this document or equivalent standards. Practitioners should review guidelines prescribed under the laws, procedures, codes of practice or other regulatory provisions in force in the relevant state or territory. All material classified as clinical waste should be placed in secure leak-proof containers and disposed of by incineration at an authorized incineration site. Avoid external contamination of the container to ensure safe handling of clinical waste. The WHO guide, *Safe management of wastes from health care activities*,[1] provides recommendations on medical and laboratory waste disposal.

TSE infectious waste should be incinerated or treated by a method that is effective for the inactivation of TSE agents (see Annex 4.1). In regions where no incineration facilities are available, it is recommended that these wastes be chemically disinfected and then burnt in pits dedicated to final disposal. Residues should be checked for total combustion. Authorities should ensure that waste is adequately managed, as in certain big cities of the developing world it has been estimated that as much as one half of infectious waste is cleaned, repackaged and sold in the marketplace.

In hospital or health care facility environments, drainage equipment, linens or swabs contaminated by high-infectivity tissues or CSF should be collected into tough plastic bags or containers labelled 'Biohazard' and incinerated. Low-infectivity tissues and drainage from low-infectivity tissues[2] should be handled cautiously.

For tissues, secretions, or excretions with no detectable infectivity, no special requirements beyond Standard Precautions are required for the handling of body fluids or body-fluid contaminated linen, equipment or environments. Other infectious wastes from home care require no special precautions beyond those taken for any other disease. Sharp waste items (i.e. syringe needles) used during home care of TSE patients should be collected in impermeable containers and returned to the treating physician or health care establishment for disposal.

The use of enamel, heat-stable plastic or disposable trays when working with infectious specimens will help to confine contamination. If reusable, they should be treated by a method listed in Annex 4.1. Disposable items should be incinerated after use, although methods listed in Annex 4.1 may be used before disposal. Use absorbent material to soak up spills, which can then be contained and incinerated or treated by a method described in Annex 4.1. Spills of potentially TSE infectious materials in the ward should be removed using absorbent material and the surface disinfected according to Annex 4.1.

[1] A. Prüss, E. Giroult, P. Rushbrook, eds. *Safe management of wastes from health care activies*. Geneva, World Health Organization, 1999.

[2] Drainage from *low-infectivity tissue* that has not been specifically tested for infectivity, however, may retain infectivity.

Use secure leak-proof containers, e.g. double bagging, for the safe handling of clinical waste. Avoid external contamination of the waste container. Disposable gloves and an apron should be worn when removing such spills and should subsequently be disposed of by incineration, together with the recovered waste and cleaning materials, although a method described in Annex 4.1 may be used.

6.8 After death

6.8.1 Precautions for handling of the deceased patient

On the death of a patient with confirmed or suspected TSE, the removal of the body from the ward, community setting, or hospice, should be carried out using normal infection control measures. It is recommended that the deceased patient be placed in a sealed body bag before moving, in line with normal procedures for bodies where there is a known infection risk. Where the skull is open or there is CSF leakage, and where sutures do not completely control this leaking, the bag should be lined with materials to absorb any fluid, and the body should be moved in a sealed body bag. Refer to country-based guidelines and regulations for more information on care and handling of a deceased and infected patient.

6.8.2 Post-mortem examination

Post-mortem examinations remain an essential element in confirming the clinical diagnosis and the cause of death as TSE. Ideally, three people should be present during the examination: the pathologist assisted by one technician, and one further person to handle and label specimen containers. Except for training purposes, observers should be prohibited or kept to a minimum. All personnel should be made aware of the relevant history of the patient and fully informed of procedures for such post-mortem examinations.

Conducting the autopsy

To the extent possible, disposable protective clothing should be worn, including surgical cap and gown, apron, double gloves, and a face visor which completely encloses the operator's head to protect the eyes, nose and mouth. Consideration should be given to the use of hand protection, such as armoured or cut-resistant gloves.

Disposable or dedicated reusable instruments are recommended in order to minimize the risk of environmental contamination. Manual saws are recommended in order to avoid the creation of tissue particulates and aerosols and for ease of decontamination after use. Electric saws, if used, should be operated inside an aerosol-containing bag unless ventilated helmets with an appropriate filter are worn. Instruments and mortuary working surfaces should be decontaminated following the guidance in Section 6.6, Decontamination procedures, and Annex 4.1.

Restricted post-mortem examinations on TSE cases can be undertaken in any mortuary. If examination is limited to the brain, a plastic sheet with absorbent wadding and raised edges is first placed underneath the head to ensure containment of tissue debris and body fluids (e.g., CSF). The scalp is reflected in the normal way and the cranium is opened. After removal of the brain, replacement of the skullcap and suturing of the skin, the plastic sheet containing all tissue debris and drainage is bagged and sealed and sent for incineration. A full post-mortem examination is discouraged except in dedicated facilities, unless special circumstances warrant the added difficulty of infectivity containment.

Histopathological examination

Only persons who have been advised of the potential hazards and trained in the specific methods used for TSE infectious tissues should be permitted to work in laboratories where high-infectivity tissues are being processed. Facilities conducting a large number of histological examinations on high-infectivity tissues should dedicate laboratory space, processors, instruments, glassware and reagents for this purpose. Guidelines in some countries and regions require Bio-Safety Containment Level 3 for handling these tissues.

It is important to note that formalin and glutaraldehyde-fixed TSE tissues retain infectivity for long periods, if not indefinitely. As a result, they should be handled with the same precautions as fresh material and be considered infectious throughout the entire procedure of fixation, embedding, sectioning, staining, and mounting on slides, until or unless treated with formic acid. Treatment with formic acid reduces infectivity to negligible levels. Although exact procedures may vary, formic acid treatment consists of placing small pieces of fixed tissue, no more than 4 to 5 mm thick, in 50 to 100 ml of 95% formic acid for an hour, and then transferring them to fresh formalin for another two days before further processing. The entire procedure is conducted using continuous, gentle agitation.

All of the serial steps involved in bringing the blocks from formalin into paraffin and, after sectioning, bringing the mounted paraffin sections back into aqueous staining solutions, can be carried out manually, or in an automatic processor dedicated

to TSE tissues. Similarly, it would be advisable to dedicate a microtome for sectioning non-formic acid treated tissue blocks, as there is no practical way to disinfect the instrument. Formic acid treated sections can be cut on a standard microtome (if possible, using a disposable knife or dedicated blade) and processed as usual. Processing fluid should be decontaminated and debris (such as wax shavings) from section cutting should be contained and disposed of by incineration (see Annex 4.1 for decontamination methods). Formic acid treated sections tend to be brittle, but show good preservation of histological morphology.

Slides made from sections that have been treated with formic acid can be considered non-infectious. Slides made from sections that have not been treated with formic acid may also be handled without specific precautions, once the coverslip is sealed to the slide and chemically disinfected to ensure external sterility, but should be labelled as a hazardous material. These slides, if damaged, should be treated using a method described in Annex 4.1 and destroyed.

Containers used for the storage of formalin-fixed tissues should, after secure closing, be cleaned using a method in Annex 4.1, marked "Hazardous", and stored separately (e.g. in sealed plastic bags). When tissue is needed, the container can be removed from the bag, set upon a water-resistant disposable mat, and manipulation of the tissue confined to the mat. After the tissue is replaced, the area and container are cleaned according to methods described in Annex 4.1, and the container put into a new plastic bag for further storage.

Electron microscopy

Electron microscopic examination of tissue sections is not indicated for diagnostic purposes, and is not recommended except as an investigational research tool. Preparation of specimens for electron microscopy should be performed with the same precautions as for histopathology. Electron microscopy of tissue sections poses negligible risk both to the microscope and the operator due to the very small amount of tissue deposited on a grid. An electron microscope section 0.01 μm thick x 0.1 mm x 0.05 mm contains approximately 50 pg of tissue. Even the most infectious models of the disease producing 10^{10} ID_{50}/g of brain would result in less than 0.5 ID_{50} immobilized on the grid. Handling requires no special precautions except for disposal of such grids as infectious waste through incineration.

6.8.3 National and international transport of bodies

If there is a need to transport the deceased patient nationally or internationally, it will be necessary to comply with the International Civil Aviation Organization (ICAO), International Air Transport Association (IATA) Restricted Articles Regulations, and any additional requirements of the individual carriers. It should be noted that the IATA Regulations require the embalming of the body.

6.8.4 Undertakers and embalmers

General measures

Mortuary procedures may be performed on the bodies of patients who have died from CJD with a minimum of inconvenience to ensure the safety of personnel and avoid contamination of the workplace. Transportation of the unembalmed body to the mortuary should be in a sealable, impermeable plastic pouch. Ordinary contact or handling of an intact, unautopsied body does not pose a risk, and cosmetic work may be undertaken without any special precautions. If the body has undergone autopsy, care should be taken to limit contamination of the workplace by any leaking bodily fluids (especially from the cranium) when transferring the body from its transport bag to the mortuary table that has been covered with an impermeable sheet. No other precautions are required, except for embalming (see below).

Embalming

An intact (unautopsied) body can be safely managed with only minor adjustments to the usual procedures. The body should be placed on an impermeable sheet or body pouch to avoid surface contamination from perfusion drain sites, and all drainage fluids should be collected into a stainless steel container. Perfusion sites should be closed with cyanoacrylates ("super glue") and then wiped with bleach.

Embalming an autopsied or traumatized body is not encouraged, but may be safely performed when the following precautions are observed. Disposable masks, gowns, and gloves should be worn, just as is done by pathologists performing an autopsy. The body should be placed on an impermeable sheet or body pouch so that suture site leakage can be contained, and perfusion drain sites should be similarly arranged to avoid surface contamination. All drainage fluids should be collected into a stainless steel container. Perfusion and autopsy incision sites should be closed with cyanoacrylates ("super glue"). The entire body should be wiped down with bleach,

and special care taken to ensure contact of bleach with perfusion sites and closed autopsy incisions.

At the conclusion of the perfusion procedure, the container of drainage fluids should be decontaminated by adding sodium hydroxide pellets at the rate of 40 g/litre of fluid. The mixture should be stirred after a few minutes and care should be taken to avoid spillage, as the fluid will be hot. It should then be left undisturbed for at least 1 hour, after which it can be disposed of as for any other mortuary waste. Plastic sheets and other disposable items that have come into contact with bodily fluids should be incinerated. Mortuary working surfaces that have accidentally become contaminated should be flooded with sodium hydroxide or bleach, left undisturbed for at least 1 hour, then (using gloves) mopped up with absorbent disposable rags, and the surface swabbed with water sufficient to remove any residual disinfectant solution.

Non-disposable instruments and tools should be decontaminated using one of the methods recommended in Annex 4.1. At the conclusion of the decontamination procedure, the instruments are washed with water to remove residual disinfectant fluid before drying and re-use. Sodium hydroxide or bleach can be disposed of as non-infectious (but corrosive) waste fluid.

6.8.5 Funerals and cremations

Relatives of the deceased may wish to view or have some final contact with the body. Superficial contact, such as touching or kissing the face, need not be discouraged, even if an autopsy has been conducted. Interment in closed coffins does not present any significant risk of environmental contamination, and cremated remains can be considered to be sterile, as the infectious agents do not survive incineration-range temperatures (1000 °C). Transport and interment are subject to local and national guidelines. Transport overseas is governed by international regulations.

6.8.6 Exhumations

Standard procedures are conducted according to local and national guidelines. The body should be considered as having the same infectivity as at the time of burial and the precautions used for an autopsy should be followed.

6.8.7 Body donation for teaching purposes

Anatomy departments should not accept, for teaching or research purposes, any body or organs from persons confirmed, suspected, or at risk for TSE, unless they have specific training or research programmes for TSEs, including access to specialized equipment, procedures, appropriate containment facilities and training for managing TSE contaminated tissues. Departments should make inquiries of those responsible for donating the body, and of the medical staff involved in the care of the donor, to insure the rigorous adherence to this recommendation.

CHAPTER 7

Effective communication on the risks from human transmissible spongiform encephalopathies

Effective management of public health risks is a complex endeavour. It requires access to highly reliable science in order to identify potential risks and assess their likely impact upon a population. In addition, risk managers must design and implement effective strategies to manage the risks and maintain them within the agreed upon limits of acceptability. Effective communication between risk managers and the public whom they seek to protect is equally important. Public health policy based upon the best scientific assessments of risk and risk management strategies can come easily to ruin if the critical task of risk communication is not well handled.

There are several reasons for the critical role of risk communication. One is that many public safety issues are complex with significant levels of uncertainty in the scientific assessment of the risk. This means that risk managers often do not have sufficient scientific grounds to assure the public that their assessment of the risk, or their ability to manage it within accepted limits, is totally reliable. As new evidence is collected and knowledge is advanced, initial assessments may need to be revised. In such a situation, if strong assurances have been given to the public that there is no possibility of unacceptable harms, and these harms nevertheless occur, public confidence in those who made the assurances will be seriously eroded. The best efforts to restore that confidence, regardless of the science and management expertise, could be futile. One of the most important challenges of risk management is to know how to communicate openly and honestly about the uncertainties involved in the scientific assessments of risk.

The public controversy in the UK and other European countries over the risk of vCJD associated with BSE contaminated beef illustrates this dynamic dramatically. Early assurances from government officials that the observed cases of vCJD in humans were unrelated to the BSE outbreak in the cattle herds and that there were no health risks to humans from eating BSE-contaminated beef, did not take into account the scientific uncertainty about this issue. Subsequent scientific evidence of the link between BSE and vCJD undermined public confidence in public officials and the science upon which they relied. This, in turn, led the public to judge the risk unacceptable regardless of the scientific estimate of its magnitude.

However, there is an even more important reason why effective risk communication is critical to the success of risk management by public officials. Risk communication is the process by which a social consensus on safety is established. The question of whether a food, such as beef, is "safe" for public consumption, is almost never a question of whether there is, in fact, no risk. "Zero-risk" is rarely possible, or even necessarily desirable, in any human activity. To judge a food or activity as "safe" is nearly always to judge the risks associated with it as "acceptable" or not. A critical question in safety issues is that of who decides what level of risk is "acceptable" and by what standard. Clearly, the acceptability of a risk is not some objective feature of the risk that can be determined by scientific investigation or established by an algorithm. Instead, the acceptability of a risk, or its safety, is ultimately judged by its acceptance by those who are the actual or potential bearers of the risk.

Those who are responsible for public "safety" need to ensure that risks are managed, not according to what the risk experts judge as acceptable levels of risk, but according to what the public judges as acceptable. A vital aspect of risk communication is the establishment of a two-way dialogue between risk managers and the public in which the former pay careful attention to how the risks are perceived by the latter, and to those aspects of public perception that most influence their attitudes towards risk. Failure to establish this dialogue, and failure to take into account the different aspects of risks that are most significant to non-expert public perceptions of risk, can lead to risk management decisions that result in political disaster. Risk managers often assume that the role of risk communication is primarily to convince the public about the "true" nature of health risks and their acceptability. In highly controversial risk issues this strategy is rarely successful.

Public risk perceptions of technology often differ dramatically from expert assessments. Experts tend to view this as a result of scientific ignorance and irrationality. They tend to focus on the quantitative aspects of risks, such as their probability and magnitude or whether there are compensating benefits. Non-experts, on the other hand, tend to focus much

more upon their qualitative aspects, such as whether they are voluntarily assumed, their familiarity or unfamiliarity, the relative distribution of the risks and benefits, the vulnerability of the risk bearers (e.g. children and the elderly), and the trustworthiness of the risk manager.

These are some of the aspects of the risk of vCJD from BSE infected cattle that are most salient in the public mind, to which risk managers should pay close attention:

- **High levels of *uncertainties* in the science.** The TSE agents in different animals and humans are still not well understood, nor is the mode of transmission between animals and between species. In particular, the relationship between levels of BSE infectivity in cattle and levels of vCJD in humans is not yet fully understood. This uncertainty creates fear in the public mind – thus lowering the acceptability of what may well be minimal risk. It also imposes upon scientists and risk managers a responsibility to exercise great care in public pronouncements about the nature of the risk.

- **The *unfamiliarity* of the risk.** TSEs are relatively new to the public awareness. They are not well understood, and their impact upon humans is still uncertain. Consequently, public fear of the risks will likely to be much greater and tolerance for accepting risk will be much lower.

- **The *uncontrollability* of the risk.** A major factor influencing the acceptance of risk relates to their sense of whether or not they can control their exposure to the risk. Risks that are difficult to identify and for which the protective measures are unclear or unreliable will usually be viewed with much higher levels of fear and intolerance. Human TSEs, but particularly vCJD, strongly fit this profile. Even the drastic lifestyle decision for some people to exclude all beef (and other meat) from their diet is no guarantee of non-exposure via other products.

- **The *involuntary nature* of the risk.** When someone else's action or negligence leads to a risk, it will be perceived to be far less acceptable than a risk freely taken in order to enjoy the benefits of the risk taking. This is why people will gladly accept the very high risks associated with activities like smoking, while being highly averse to relatively low risks like cancer from food additives or chemical residues. The public perception is that a benefit, such as profit, is made because of the risk taking being imposed upon the public. vCJD fits this characteristic. The introduction of BSE into beef is viewed as a consequence of farming practices that benefited primarily producers, while the health risks fell upon the consumers who enjoyed no compensating benefit.

- **Low *trustworthiness* of the risk managers.** This factor is one of the most important considerations in risk acceptability. If people trust those who are responsible for controlling a particular risk they are likely to accept much higher levels of that risk acceptable. If the organizations upon which people rely for protection fail to provide the promised level of protection, even the most remote risks under their control will become matters of intense concern. Similarly, if their competence or integrity is compromised by technical or moral failures, trust is lost. Public response to the vCJD issue has been profoundly shaped by the loss of confidence in the government regulators in Europe, particularly the UK, after their strong assurances of "no risk" were called into serious question by subsequent scientific investigation. Confidence, once lost, is hard to re-establish. Consequently, public attitudes about BSE and vCJD can be expected to remain highly averse for an extended period, even if the risks are shown by further science to be substantially lower than feared.

Given these factors, what are some of the most important principles that should guide risk managers in their communication with the public about the risks of BSE and vCJD? Here are a few that are suggested by the foregoing points:

1. Remember that risk communication is a two-way dialogue between risk managers and the various "stakeholders" in the risk issue, including especially those who are most "at risk". Its primary objective is to build a consensus on the appropriate management of risk. The stakeholders need to be included early on in the process of policy development in response to newly emerging risk issues. The perceptions of stakeholders, even if they seem to be based upon inadequate scientific understanding, need to be taken seriously. Ultimately they will set the limits on a successful risk management strategy.

2. Let stakeholders know that their perceptions and opinions are being heard and are being taken seriously. Risk communication that aims only at "re-educating" the stakeholders to the experts' point of view is rarely successful, and tends to communicate a lack of appreciation for public concerns. Do not belittle or minimize public concerns. Instead, show that you take them seriously by:

   — Being candid about the real uncertainties that remain in the science. Unsubstantiated assurances can come back to haunt you.

   — Emphasizing the steps you are taking to minimize the risks. Communicating what you

are doing to minimize risks is far more effective than explaining what you think the risks are and what the public ought to think.

3. Avoid "zero-risk" messages. Claiming that there is "no risk" is a dangerous trap. It takes only one example, or more importantly, only one credible claim of an example to make a risk manager look incompetent or dishonest and destroy their public credibility. Always recognize that even the best risk assessment is fallible, and future research can always cause the revision of an opinion.
4. Finally, the profile of the risk of human TSEs, particularly vCJD, as outlined above predicts that public perceptions will be highly averse to risk, even if the magnitude of the risk may be quite low. Consequently, it is important that risk managers reflect a highly precautionary approach to the risk. This precautionary approach must be found in both the messages given to the public and in the measures taken to safeguard public health.

CHAPTER 8

Surveillance systems for human transmissible spongiform encephalopathies

8.1 Background

Historically, surveillance for human TSEs has been confined to wealthier countries. This reflected the need for advanced laboratory, neurological and neuropathological diagnostic capacity, a population sufficiently old to have developed the disease, and the clinical infrastructure required to identify and report rare diseases. In 1996, the discovery of vCJD and the subsequent epidemiologic investigations linking it to an epidemic outbreak of bovine spongiform encephalopathy in cattle led to intense international interest in determining the epidemiology of human TSEs. WHO has supported the development of surveillance systems for human TSEs in recognition of the need to determine the epidemiology of vCJD at a global level.

Internationally, only two surveillance networks are fully operational – EuroCJD (including Australia, Austria, Canada, France, Germany, Italy, the Netherlands, Slovakia, Spain and the UK,) and NeuroCJD (including Denmark, Finland, Greece, Iceland, Ireland, Israel, Norway, Portugal, Sweden and the UK). The European Commission (EC) funds both. In addition, attention is drawn to 'PrionNet', an EC-funded project to study the neuropathology of the human TSEs. A nascent surveillance project – SEEC-CJD (Surveillance for CJD in Countries of Central and Eastern Europe, and China) – in which WHO is a direct collaborator, is also funded by the EC. A small number of countries outside these surveillance systems also have surveillance systems (i.e. Argentina, USA). Otherwise, reporting of human TSEs consists of isolated case reports.

Surveillance systems for the human TSEs differ from prototypical public health surveillance systems due to two principal characteristics: human TSEs are rare, with a population prevalence that only approaches one per million, and there are no preclinical markers of disease. As a result, TSE surveillance systems must evolve from specific clinical and laboratory settings where the capacity to recognize and diagnose such a rare disease exists.

Surveillance systems for human TSEs are dependent upon reporting from physicians, particularly neurologists and neuropathologists, and those performing relevant diagnostic tests including TSE-specific CSF diagnostics, MRI scans, EEG and specialized neuropathology. TSE research groups are a valuable resource for human TSE surveillance networks. They may have already developed appropriate clinical contacts, diagnostic capacity and international collaborations, since much prion expertise develops in association with research-based efforts to understand this challenging group of diseases.

8.2 Surveillance system design

8.2.1 Core requirements for surveillance

The rarity of the human TSEs requires that case collection be centralized, typically at the national level. This is due to the need for sufficient resources to conduct surveillance and sufficient numbers of cases to permit the interpretation of the surveillance information. Without the capacity of the nationally assigned authority or authorities to collaboratively collect, investigate and analyse national referrals, public health and epidemiologic predictions are not possible.

The core requirements for surveillance for human TSEs are as follows: the use of the common case definitions for vCJD and sCJD (see Annex 1, particularly 1.2), the collection ofWHO core data elements (Minimal Monitoring Data Set – MMDS, Annex 2), and the use of internationally agreed surveillance methods at the national level (see below). The comparison of rates at an international level and the pooling of data for analysis require common surveillance methods.

Laboratory and, to a certain extent, diagnostic expertise is frequently centralized. It may be expensive and even unnecessary to provide subnational capacity to conduct certain laboratory diagnostic tests for CJD due to limitations in expertise in the conduct of the tests and scarcity of financial resources. Sometimes certain laboratories or clinical settings within national (or even regional) boundaries will have or can quickly develop expertise in a particular aspect of diagnosis. In this case a partnership of a number of different disciplines and settings can be established within a national (or regional) framework.

Some countries may prefer to send all their samples to external collaborators for analysis. A list of WHO-affiliated reference centres is provided in Annex 5.

8.2.2 Methods and strategies

There are two standard methods for conducting surveillance of human disease - passive and active.

Passive surveillance involves waiting for the referral to be received by the central site, where staff subsequently act upon the report to confirm the diagnosis and gather relevant clinical and epidemiological information. While this method is relatively inexpensive, the surveillance system depends on the interest and ability of clinicians to report cases and may result in receipt of death reports (autopsy findings) only. Active surveillance overcomes these problems by a hands-on approach in which the central coordinating site actively collaborates with clinicians to identify cases. Active surveillance systems will undertake activities to encourage reporting and to affirm that the absence of reports correctly implies the absence of cases. This can be done by sending reminder notices to clinicians and by asking them to confirm that they have not seen any cases. The choice of passive or active surveillance generally requires a compromise between competing needs: timeliness of reporting (improved through active surveillance), completeness of reporting (improved through active surveillance in settings where completeness of reporting is a problem), and resource availability (active surveillance is more expensive than passive surveillance).

In general three strategies are used to conduct surveillance of human TSEs.

The first strategy is to seek reports/referrals from the clinicians who see and diagnose the cases. This is usually done through 'advertising' strategies such as mail-outs, participation in or organization of conferences, academic rounds or other techniques. Information (see Figure 8.1) may be sent either to targeted professionals (e.g. neurologists and neuropathologists) or to the general population of medical professionals (e.g. general practitioners, psychiatrists, paediatricians, and geriatricians) plus members of the public. Reports collected by targeted professions are generally regarded as the most sensitive and specific strategy, as unusual neurological diseases, particularly among young populations, will usually be referred to these practitioners for diagnosis. Additionally, the human TSEs are inevitably progressive neurological diseases, hence people developing these diseases are most likely to be seen by either a neurologist or neuropathologist. Finally, clinicians (specifically general practitioners and psychiatrists) are unlikely to be the best source for referrals of potential CJD cases due to the high proportion of non-CJD cases among those referred. Surveillance networks with minimal capacity need to consider the amount of their resources that will be used to investigate non-cases.

Figure 8.1 Checklist for information packages

- Accurate information about the national coordinating centre, including a contact person and when they are available.
- Describe the kind of cases that the surveillance system is looking for, i.e. provide the case definition in language appropriate to the audience. Copies of the reporting form and core data reporting form may be provided immediately, or (to save resources) later when an initial screening has been conducted by the national reference centre.
- Describe the services available for diagnosis of CJD. Describe how to access these services. Ensure that protocols for informed consent and the handling of samples are prepared and can be provided rapidly upon request.
- Anticipate the need to provide post-mortem services such as autopsy.
- Provide information about the importance of the surveillance system. Indicate whether reporting human TSEs is required under the law.

The second strategy involves the development of tailored methods i.e. methods designed to identify cases from specific populations where it is anticipated that the risk of TSE would be higher than among the general population. Epidemiologically, these are called cohort studies. For example, the family members of cases of familial CJD could be identified through interviews with other family members. Another possibility is that the recipients of specific products either known to transmit TSEs (such as human growth hormone) or suspected to transmit TSEs (such as blood products) could be identified (perhaps through the review of clinic records). In either case, these persons would be included in a registry and their health outcomes periodically reviewed. Another approach is to attach CJD surveillance onto systems of surveillance created for other diseases. For example, after the first reports of vCJD in the UK, the US Centers for Disease Control and Prevention's Surveillance for Emerging Infectious Diseases added the capacity to report cases of CJD among persons under 45 years of age. This was done in response to concerns about undetected vCJD in the USA. Similarly, the Progressive Intellectual and Neurological Deterioration (PIND) surveillance systems operated by networks of paediatricians has been successfully used in the UK to search for vCJD in persons under 16 years of age.

A third commonly used strategy is the review of death certificates. It is recommended as an appropriate method in countries where death certificates are reliably completed on the entire population of

the country, and where case investigations are legally permitted if suspect vCJD cases are identified through death certificates.

Clearly, none of the systems described above will be able to identify all cases of human TSEs in a population. In the establishment of a human TSE surveillance system, most countries use a combination of all three strategies, dependent upon resources available, the need to quantify risk among populations of interest, and existing knowledge of the epidemiology of CJD.

8.2.3 National surveillance networks

Surveillance for human TSEs will require the development and maintenance of a partnership with neurologists, neuropathologists, and laboratories conducting diagnostic tests, public health departments, and international surveillance networks.

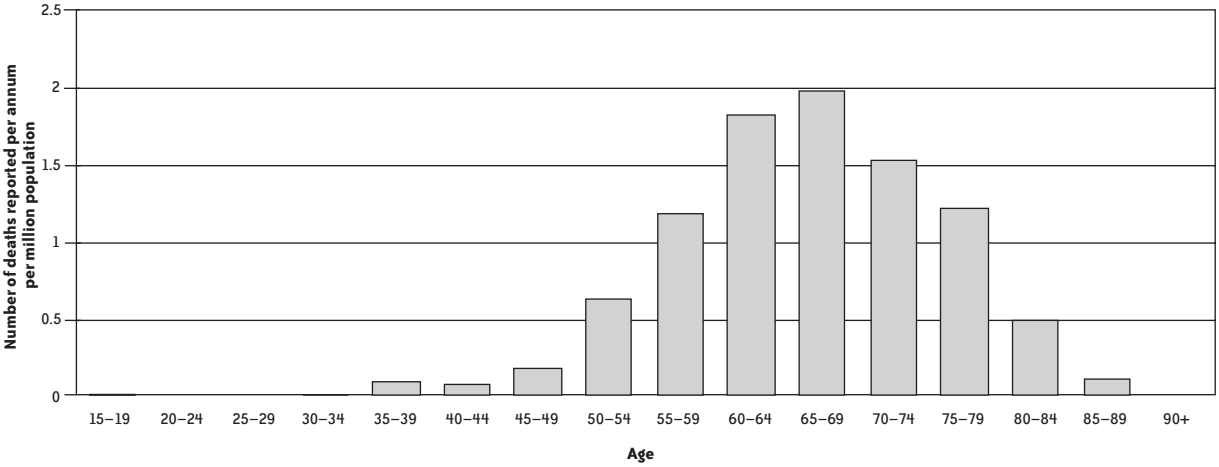
Establishment of a group of neurologists and neuropathologists who understand the purpose and value of national surveillance is the first step in obtaining case reports for the surveillance system. Advertising of the specialized diagnostic capacity of the associated services of the network will assist in this endeavour, as will mail-outs, site visits, presentations at appropriate clinical conferences and organizing specific conferences about the human TSEs. It is essential that participant neurologists and neuropathologists do not regard the system as a competitor for academic publications. This can be avoided by having a policy of acknowledging the reporting clinician, and by including all reporting clinicians (both those who report cases and those who report that they have no cases) in national activities and publications.

8.2.4 The surveillance catchment basin

Ideally, surveillance systems will include the entire national population. If, for reasons such as geographic size, finances or policy decisions, a surveillance system covers only a part of a population, i.e. a region, hospital or clinic, or even an age group or population of persons who had a specific exposure, it is important that the catchment basin of the surveillance net be described in order that epidemiologic analysis can be conducted. A catchment basin is, simply put, the population reasonably considered to be under observation by the surveillance system. Surveillance of subnational areas requires a description of the catchment basin and specific knowledge of the epidemiologic characteristics of importance to the disease under observation. It is important to anticipate where bias will be introduced if subnational surveillance is conducted. If bias is large, the accurate calculation of basic epidemiologic parameters such as annual incidence rate is impossible.

Sex and age distributions are the most important epidemiological characteristics to describe. However, the background rates of diseases easily confused with the disease under study, plus information describing the access of the population under study to relevant facilities for the diagnosis of the human TSEs is also important. Understanding whether the population under study has differential access to physicians, laboratories or specialized diagnostics is important. This is important in order to determine what fraction of the study population will be identified by the surveillance system, and consequently in order to permit extension of epidemiologic conclusions to the population of the region or country. This is particularly important in any country where geography, culture, or economics restricts access to health care and diagnostics.

Figure 8.2 Age-specific incidence of sporadic CJD



8.2.5 Predicting the case load

International experience with the surveillance of CJD indicates that the true incidence of CJD in any country with a national surveillance programme is approximately 1 case per million population per annum. However as the incidence of CJD is extremely low in persons under 45 years of age and highest in those between 65 and 69 years of age (Figure 8.2), the age distribution of the population under study will be important. With information on the total population, sex and age distribution of the population, the expected number of reports can be predicted based upon age-specific distribution from countries with high quality surveillance systems such as the UK or the USA (Figure 8.3).

8.2.6 Quality control for diagnostics

The introduction and maintenance of quality control (QC) programmes is an important component of surveillance, particularly when surveillance for a rare emerging disease such as vCJD is undertaken. The following QC programmes are recommended: neuropathology, CSF analysis, EEG, MRI, gene sequencing. Protocols for the investigation of vCJD, familial and iatrogenic cases should be developed based upon relevant case definitions (see Annex 1.1 and 1.2).

8.2.7 Services

One of the principal services, and therefore principal values, of the surveillance network is the establishment of services providing high quality and reliable diagnostics for the human TSEs. Among the services

Figure 8.3 Predicting case load

Age group	Annual death rate per million in the US (*R*[a])	Number of people in the age group in the catchment under study (*N*)	Number expected in the age group per million population (*R* x *N*/1 000 000)
0–4	0.00		
5–9	0.00		
10–14	0.00		
15–19	0.00		
20–24	0.02		
25–29[b]	0.02		
30–34	0.00		
35–39	0.03		
40–44	0.18		
45–49	0.44		
50–54	0.70		
55–59	1.74		
60–64	2.89		
65–69	3.71		
70–74	2.68		
75–79	3.60		
80–84	2.14		
85–90+	0.44		
90+	0.21		
			Total number of cases expected = sum of above
Age-adjusted death rate per million for the population		Population of country = sum of above	

[a] Taken from Holman RC et al. Creutzfeldt-Jakob disease in the United States, 1979–1994: Using national mortality data to assess the possible occurrence of variant cases. *Emerging Infectious Diseases*, 1996 2(4);333–337.

[b] Overall in the United States, only five persons died from CJD during the study period who were younger than 30 years of age.

that must be provided through the surveillance network are neuropathology, CSF testing for 14-3-3 protein and clinical capacity to assist in differential diagnosis. Where MRI is available, this service should also be offered. It has been demonstrated in many surveillance systems that the introduction of 14-3-3 CSF testing will greatly increase the referrals to the surveillance system and contribute importantly toward improving the diagnosis of CJD and hence the surveillance system. Surveillance systems are well advised to prepare information sheets describing collection requirements, storage requirements and shipping protocols.

8.2.8 Surveillance system evaluation

It is important to be able to evaluate the surveillance system and therefore, the system itself needs to be established in a way that make this evaluation possible.

The surveillance network must establish both minimum and preferred reporting requirements. The WHO Minimal Monitoring Data Set (Annex 2) is recommended as a minimum reporting requirement. A number of investigators have developed more extensive investigation protocols that may be shared upon application (PrionNet, UK National CJD Surveillance Unit).

The importance of quality assurance of surveillance data is not well appreciated in general. Upon receipt of a report of possible CJD, a person whose job is to ensure the quality of the information provided must examine all aspects of the report. Verification of facts (country of birth, city of onset, birth date etc) plus confirmation of matters relating to data analysis (correct spelling, use of appropriate fields for data entry, and the use of a data dictionary to describe what information is being requested) is required. It should be the responsibility of a specified staff member. In addition, the time between onset of symptoms and reporting to relevant national authorities must be calculated from the surveillance data. This is referred to as reporting delay and is essential to both evaluate the surveillance systems' timeliness and to provide the required information to permit statistical projections of the expected number of cases. Annual evaluation of the surveillance system should include error reporting, reviews of reporting delays, examination of information describing the reason or route of referral, and comparison of the observed number of reports with the expected numbers of reports. While electronic data systems will aid in this process, in many countries the number of reports may be sufficiently small that electronic data management is not required.

In many surveillance systems approximately twice as many referrals are received as are eventually confirmed to be human TSEs. A surveillance system should count its referrals; if the number of referrals does not exceed the number of confirmed human TSE cases by a factor of 2 or more, enhanced contact with the sources of referral is needed. In addition, since these surveillance systems are searching for vCJD, a good system will evaluate whether it is receiving an appropriate number of referrals of persons under 50 years of age in relation to the population.

Lastly, it is important that a surveillance system record information about those persons whose diseases are eventually found to be non-TSEs. This list of diseases is valuable to clinicians who will be interested in knowing which diseases are most easily mistaken for human TSEs, particularly if relevant epidemiologic, clinical and laboratory information is collected on all cases. Finally, if tissue samples (particularly blood and lymphatic tissue samples) and investigative information (e.g. MRI scans, EEGs) from these cases are collected and stored, there will be opportunities to determine whether or not new diagnostic tests can reliably distinguish between human TSEs and those diseases most easily confused with them.

Those participating in the surveillance system must review and publish information from incoming reports on a regular basis (quarterly, semi-annually or annually dependent upon the predicted number of cases) and provide feedback to the participants and other relevant audiences. The content of the surveillance report must be determined in advance and considerations of data confidentiality must be observed. No national report must allow the identification of an individual.

The surveillance data are collected principally from clinical information; some countries are able to collect data from death certificates. This supplemental reporting is valuable in identification of missed cases (thereby aiding in estimations of reporting completeness), identification of clinicians not participating in the surveillance efforts, and in determining whether the proper diagnosis is being reported on death certificates.

8.3 Additional information provided by surveillance programmes

Human TSE surveillance systems will include and develop particular expertise in this very special and unusual disease. It is important that the extent of this special knowledge is recognized by the surveillance system and that it provides nationally appropriate services where possible. The following are areas in which existing surveillance systems have been asked

for support by clinicians and national public health authorities:

- Infection control guidelines and mortuary guidelines.
- Patient and family support, i.e. treatment and patient care guidelines.
- Informed consent guidelines
  Surveillance networks must review the legal and ethical requirements of their national authorities and determine the exact requirements for informed consent. Informed consent may be required for many aspects of the data collection including research, i.e. collection and storage of samples, and their eventual use.
- Government and public advice
  Participation in advisory committees, liaison with international surveillance systems, consultant to government and public and providing response to media inquiries, i.e. leading edge information will be requested of those participating in these surveillance activities.
- Special epidemiologic studies
  Surveillance systems are the basic tool of epidemiology and it can be anticipated that epidemiologic studies including outbreak investigations may be triggered by surveillance activities. For example, Professor Robert Will of the UK CJD Surveillance Unit has developed an investigational protocol for suspect vCJD cases. It is available on request from Professor Will or WHO (for contact information see Annex 5).

Conclusion

The World Health Organization works closely with the two existing surveillance networks and is a collaborator in an EC-funded project to extend surveillance into the countries of central and eastern Europe, and China. To allow the collection and comparison of data globally, it is essential that those developing surveillance systems for human TSEs become familiar with the methods used by WHO, SEEC-CJD, EuroCJD and NeuroCJD. One goal of this manual is to collect together the relevant information to encourage the development of surveillance networks for human TSEs. Any surveillance endeavour wishing to collaborate with these ongoing surveillance networks is invited to contact the networks directly for inclusion as appropriate and possible.

WHO will continue in its role of providing training for human TSE surveillance (regional and subregional workshops have been held in Buenos Aires, Bangkok, Beijing, Bratislava, Cairo, and Dakar). In addition, WHO will provide liaison to member countries with WHO designated collaborating centres. Finally, WHO will provide a central registry site for the reports of variant CJD internationally, and will work with national and regional authorities in order to develop surveillance networks for human TSEs.

CHAPTER 9

Diagnostic tests for human transmissible spongiform encephalopathies

9.1 Routine blood tests

Routine haematological and biochemical investigations, including inflammatory markers, are usually normal in CJD and other TSE. In about one-third of CJD cases the liver function tests are mildly abnormal, often in the form of transiently elevated transaminase. The reason for this is not known.

The pathological isoform of PrP cannot be detected in blood or serum by currently available methods.

9.2 Cerebrospinal fluid

9.2.1 Sporadic CJD

The CSF of patients with CJD typically contains no inflammatory cells. A slightly elevated protein content (0.5–1.0 g/l) occurs in about one-third of cases of CJD and vCJD. The presence of oligoclonal bands confined to the CSF has only very rarely been described. The pathological isoform of PrP cannot be detected in CSF by currently available methods.

14-3-3 has been examined as a laboratory marker of CJD. 14-3-3 is a neuronal protein involved in cell signaling and is present in high concentrations within the central nervous system. It may be released into the CSF in a number of neurological and pathological conditions affecting neuronal integrity. Therefore, its presence in the CSF is not specific for CJD. However, in the appropriate clinical setting, the detection of 14-3-3 in the cerebrospinal fluid (CSF) has a high degree of sensitivity and specificity for the diagnosis of sCJD. The accuracy of this test has led the WHO and European Union's programme for the surveillance of CJD to revise their clinical criteria for the diagnosis of sCJD to include a positive 14-3-3. In patients who have a progressive dementia of less than two years duration, a positive CSF test for 14-3-3 protein is considered to have a diagnostic weight equal to that of specific EEG findings in sCJD. Helpfully, the protein seems to be stable even at room temperature for prolonged periods. The protein has been shown to be stable despite repeated freeze/thaw cycles or storage at +20 °C for 12 days. Consequently, CSF samples can be easily sent by mail to reference centers for testing.

Results from patients with familial TSE have been somewhat mixed. For example, in one group of 10 genetic cases with various point mutations, only 50% of the CSF samples were positive. However, in a series of 16 CJD cases related to the codon 200 mutation, all were positive. Most cases of iatrogenic disease test positive. A number of other conditions may also give a positive 14-3-3 CSF test result are listed in Figure 9.1.

The 14-3-3 protein is not useful as a general screening test for CJD because it may be present in the CSF of patients with other conditions. The test should be reserved for use in cases where the diagnosis of CJD is considered a reasonable possibility.

A number of other CSF protein markers for CNS injury have been evaluated for their utility in differentiating CJD from other progressive dementias. These include neuron specific enolase, NSE; S100; tau; beta-amyloid 1-42, β1-42; ubiquitin; the α-subunit of the GTP binding protein G_0, $G_{0\alpha}$; the creatine kinase BB isoenzyme, CK-BB. Some of these proteins (NSE, S100b, and tau) have been investigated in vCJD. The specificity of NSE (86%) and S100b (74%) was lower than that of 14-3-3 (94%).

At present no other brain-specific proteins found in the CSF (e.g. S100b, NSE) should be used in the routine diagnostic investigation of patients with CJD. However, CSF tau protein may be useful and current studies are under way to evaluate its diagnostic value.

At present, the above-mentioned CSF assays are available in only a few centres in Australia, Canada, some European countries and the USA. The analysis

Figure 9.1 Diseases that may have positive 14-3-3 CSF tests

- Herpes simplex and other viral encephalitides
- Recent stroke
- Subarachnoid haemorrhage
- Hypoxic brain damage
- Metabolic encephalopathy after barbiturate intoxication
- Glioblastoma
- Carcinomatous meningitis from small-cell lung cancer
- Paraneoplastic encephalopathy
- Corticobasal degeneration

of CSF 14-3-3 requires specialized technical and interpretative skills and it is strongly recommended that samples are sent to the WHO reference centres listed in Annex 5 for analysis or quality control.

9.2.2 Variant CJD

CSF pleiocytosis or low glucose concentrations are not features of vCJD and are suggestive of an alternative diagnosis.

In the appropriate clinical circumstances, 14-3-3 has a high specificity in distinguishing vCJD and sCJD from other dementing illnesses. However, the test itself is less sensitive in detecting vCJD than sCJD. For example, in the UK CJD Surveillance Unit, the sensitivity of CSF 14-3-3 detection in the diagnosis of vCJD has been reported to be 50–60% and its specificity appears to be up to 94% (Figure 9.2).

Figure 9.2 Sensitivity and specificity of 14-3-3 in vCJD in patients referred to the UK National CJD Surveillance Unit for assessment (i.e. in qualified clinical setting)

Classification according to UK surveillance criteria	Number of samples positive 14-3-3 /total samples investigated
Definite vCJD	28/53
Probable vCJD	2/15
Not CJD	3/49
Sensitivity[a]	53%
Specificity	94%

[a] A sensitivity of 50% (i.e. approximately half of the patients referred are not vCJD) is desirable in this setting.

Consequently, the finding of a positive CSF 14-3-3 in a patient with clinical features of vCJD is supportive, but not absolutely confirmatory, of the diagnosis, while a negative result cannot be used to exclude it. At present, although 14-3-3 is potentially clinically useful, it is not part of the internationally accepted diagnostic clinical criteria for vCJD. There is evidence to suggest that a positive CSF 14-3-3 is more likely to be found in the mid-stage of the disease. However this finding should not be used to determine the timing of lumbar puncture as CSF 14-3-3 has been detected at all points in the disease process.

There is no difference between the electrophoretic pattern of the CSF 14-3-3 found in vCJD and that found in sCJD (although lower concentrations tend to be found in vCJD). Consequently, CSF 14-3-3 cannot be used to distinguish between the two forms. This is particularly relevant, as sCJD is the main differential diagnosis of vCJD.

9.3 Electroencephalography

9.3.1 Sporadic CJD

The EEG was first recognized as an important aid to the diagnosis of CJD in 1954 and was included as a component of the first published diagnostic criteria in 1979. Before 14-3-3 testing, a periodic EEG recording was considered the most reliable non-invasive diagnostic test for sCJD (see Section 10.3, Specific protocols for the diagnosis of human transmissible spongiform encephalopathies: EEG). The presence of periodic sharp-wave complexes is reported to have a sensitivity of 67% and a specificity of 86% for sCJD, the remaining cases being noted to have only nonspecific slow-wave abnormalities. However, the sensitivity partly depends on the numbers of recordings taken in any given case and the stage of illness at which they are done (see below). The specificity depends on the context of testing (i.e. how carefully other possible causes of a periodic EEG pattern are excluded; see below).

The EEG may progress from showing nonspecific changes to the characteristic appearance within days. Therefore, if a non-diagnostic recording is obtained, frequent serial EEG recordings should be undertaken whenever possible. If a typical periodic EEG is obtained, it may not be necessary to repeat it, although this should be considered if there is any clinical doubt about other possible causes of the EEG pattern (such as metabolic factors). The response to diazepam is unpredictable: periodic activity can be either abolished or unaltered by its administration. A single normal EEG may be seen, particularly early in the clinical course, but a repeatedly normal EEG is not generally considered consistent with a diagnosis of sporadic CJD.

The typical EEG appearance has not been reported in kuru, vCJD or 'classical' GSS (i.e. progressive cerebellar ataxia) and has only rarely been described in growth hormone-related iatrogenic disease. Although the characteristic EEG is virtually diagnostic of sporadic CJD in the correct clinical context, similar appearances have rarely been described in other conditions, such as Alzheimer disease or

Figure 9.3 Conditions that may cause a CJD-like EEG

- Alzheimer disease
- Lewy body disease
- Binswanger disease
- AIDS dementia
- Multiple cerebral abscesses
- MELAS syndrome
- Post-anoxic encephalopathy
- Hyperammonaemia
- Hyperparathyroidism
- Hypo- and hypernatraemia hypernatraemia
- Hypoglycaemia
- Hepatic encephalopathy
- Baclofen, mianserin, metrizamide and lithium toxicity

metabolic and toxic encephalopathies (Figure 9.3). In this situation, the clinical signs and symptoms shown by the patient should help to differentiate the condition from CJD.

A selection of EEG records illustrating those felt to show minor nonspecific abnormalities (Figure 9.4, beetween pages 58 and 59), typical, demonstrating characteristic diagnostic abnormalities (see Figures 9.5, beetween pages 58 and 59), and another classified as 'suggestive' but not diagnostic (Figures 9.6, beetween pages 58 and 59)are provided.

9.3.2 Variant CJD

The characteristic periodic changes seen in sporadic CJD are not a feature of vCJD. Among 104 cases of vCJD described as of May 2001, no changes considered typical of sporadic CJD were seen. This remained true even if the EEG recording was performed late in the course of illness (i.e. more than 6 months after the onset, over 66% of duration of illness and even within 2 days of death in one case).

EEG recordings in vCJD usually show nonspecific slow-wave abnormalities but some patients have had normal tracings, even in the later phases of the disease when clinical signs and cognitive impairment were present. However, a normal EEG is in keeping with the diagnosis of vCJD especially in the early stages of illness, while a periodic record suggests sCJD. Nevertheless, it is necessary to remember that even in sCJD the EEG can be non-diagnostic for CJD.

9.3.3 Difficulties in EEG interpretation

A major obstacle of EEGs is that there are no prospectively validated criteria for their evaluation. This means that there is an important subjective appreciation component, leading to variations in the reporting of EEG tracings. Until recently, standardized criteria for a 'typical' tracing had not been widely agreed.

Section 10.3 of this document, Specific protocols for the Diagnosis of Human TSEs – EEG describes recently developed WHO criteria for a 'typical' EEG.

9.4 Neuroimaging

Characteristic magnetic resonance imaging (MRI) changes are seen in many patients with CJD and the distribution of these changes frequently allows an accurate radiological diagnosis and distinction between subtypes. The use of a consistent imaging protocol (Section10.4) and increased awareness of the characteristic changes encountered should improve the early detection of CJD.

9.4.1 Sporadic CJD – computed tomography and radionuclide imaging

Brain computed tomography (CT) is a poor discriminator of CJD from other conditions. CT performed early in the natural history of the disease is usually normal. Although neuronal loss occurs early in the disease, the accompanying volume loss is not usually evident as cerebral and cerebellar atrophy on CT until 6 months or more from symptom onset in sCJD. Focal cortical hypoperfusion has been reported on positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging in sCJD before the appearance of CT and MRI changes, but the changes are nonspecific, and the data is limited to case reports.

9.4.2 Sporadic CJD – magnetic resonance imaging

Signal abnormalities on MRI in sCJD have been recognized since 1988. Following a number of case reports, hyperintense signal changes in the putamen and caudate head (relative to the thalamus and cerebral cortex) on long repetition time (TR) (T2-weighted and proton density-weighted) MR sequences were described in 79% of a series of 29 patients with confirmed sCJD (Figure 9.7). A more recent assessment of a larger patient group (157 cases of sCJD) has reported that basal ganglia hyperintensity is 67% sensitive, and 93% specific for the diagnosis of sCJD.

The putamen and caudate nucleus high signal changes are usually symmetrical on long TR imaging, although asymmetrical involvement of the corpus

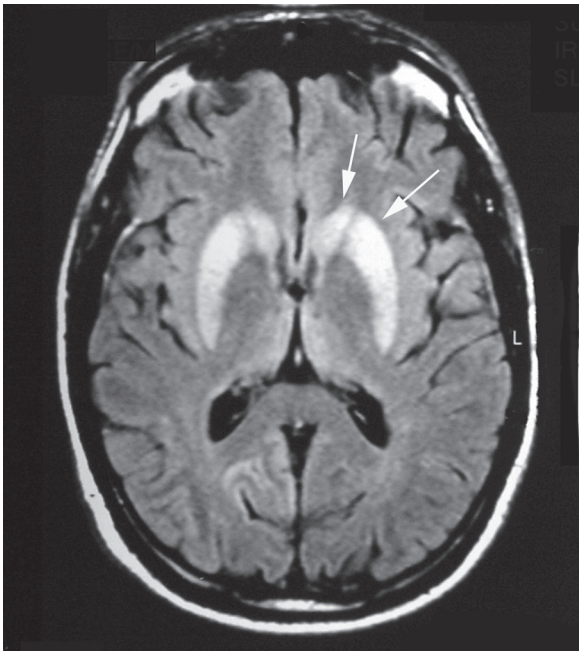


Figure 9.7 Sporadic CJD (FLAIR)

striatum occurs in 20% of cases. These MRI changes may occur early, before EEG changes are evident. Additional features include characteristic apparent accentuation of the hyperintensity of the anterior half of the putamen relative to the posterior half of the nucleus.

High signal changes are also seen in the globus pallidus (24%), thalamus (14%), and peri-aqueductal grey matter. Cortical involvement is sometimes seen and occurs early in sCJD, but may be obscured due to partial volume artefact from CSF within sulci on long TR sequences and is best demonstrated on fluid-attenuated inversion recovery (FLAIR) images and diffusion weighted imaging (DWI) (Figure 9.8). Cortical atrophy is a late feature, and the degree of atrophy correlates with the duration of disease. White matter hyperintensity is also sometimes seen in the centrum semiovale in both sCJD and vCJD (Figure 9.9).

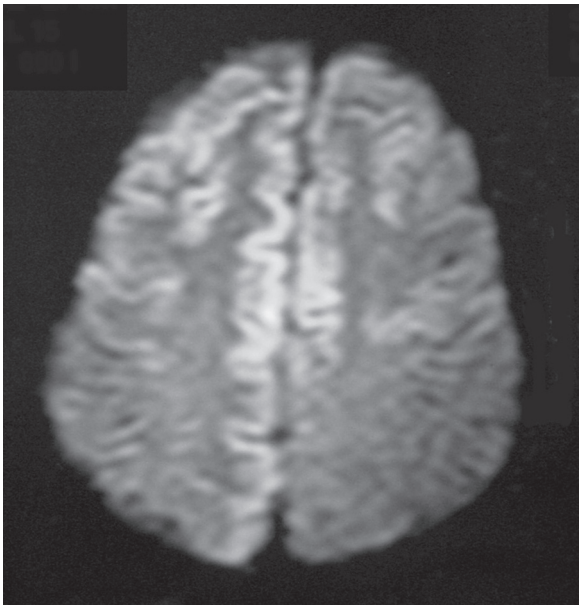


Figure 9.8 Sporadic CJD (DWI)

9.4.3 Variant CJD – computed tomography and radionuclide imaging

CT is usually normal in vCJD, and there are no specific imaging findings. Atrophy is an unusual feature of vCJD. Case reports have indicated that SPECT imaging may show focal or widespread cortical hypoperfusion (as is seen in sCJD) although these appearances are non-specific.

9.4.4 Variant CJD – magnetic resonance imaging

On MRI, a characteristic distribution of symmetrical hyperintensity of the pulvinar nucleus (posterior nucleus) of the thalamus (relative to the grey matter of the anterior putamen and normal cerebral cortex) is seen in over 90% of patients with subsequently pathologically confirmed vCJD. These changes have been named the 'pulvinar sign' (Figure 9.10), and the sign has been shown to be a highly sensitive marker of disease, being found in 79/86 (92%) of

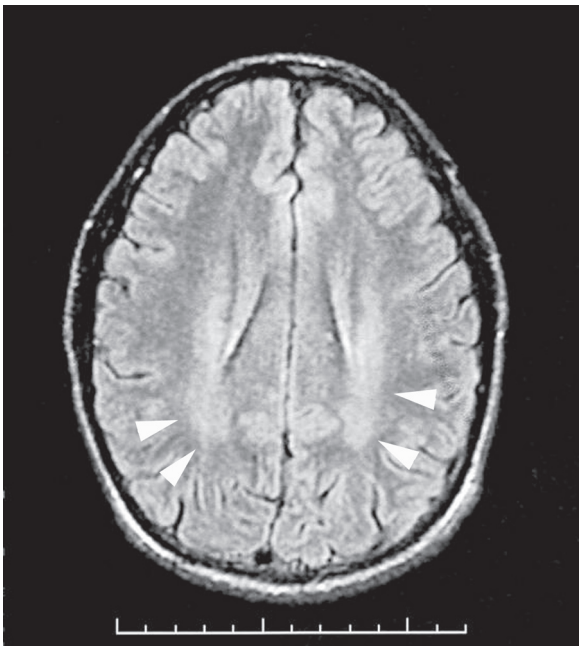


Figure 9.9 Sporadic CJD (FLAIR) White matter hyperintensity in the centrum semiovale is shown with arrows

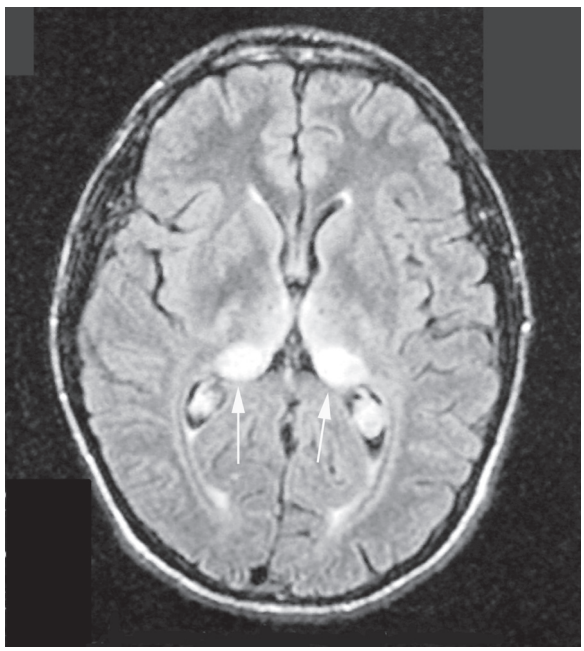


Figure 9.10 vCJD Symmetrical hyperintensity of the pulvinar nucleus of the thalamus causes the changes known as the pulvinar sign

histologically confirmed cases in a recent analysis. In the appropriate group of patients, (i.e. those with clinical features consistent with vCJD), the specificity of the pulvinar sign is over 95%. One hundred percent of 30 FLAIR sequences of pathologically confirmed vCJD were positive for the pulvinar sign in a recent study. The pulvinar sign is currently the best non-invasive in vivo diagnostic test of vCJD.

The changes of the pulvinar sign are best seen on axial images but may be seen in other planes. The pulvinar sign of vCJD has been described on T2-weighted images, proton density-weighted images, FLAIR and DWI. T1 hyperintensity occurs in 9% of patients and is thought to be due to prion protein deposition. Contrast-enhancement is not a feature. To date, the changes have been seen most consistently on FLAIR imaging.

Characteristic high-signal changes are seen in other grey matter structures in vCJD. These include the dorsomedial nuclei of the thalamus (93% of cases), giving a characteristic 'hockey-stick' appearance (Figure 9.11), peri-aqueductal grey matter (82%) (Figure 9.12), caudate head (40%) and deep white matter (36%). Cerebral atrophy is rarely seen in patients with vCJD, even with advanced disease, in contrast to the diffuse atrophy seen in advanced sCJD.

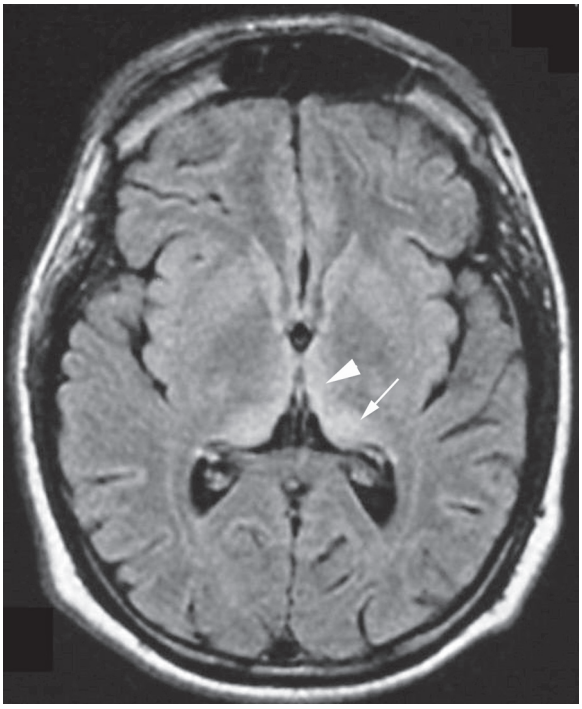


Figure 9.11 vCJD High-signal changes in the dorso-medial nuclei of the thalamus gives a characteristic 'hockey-stick' appearance

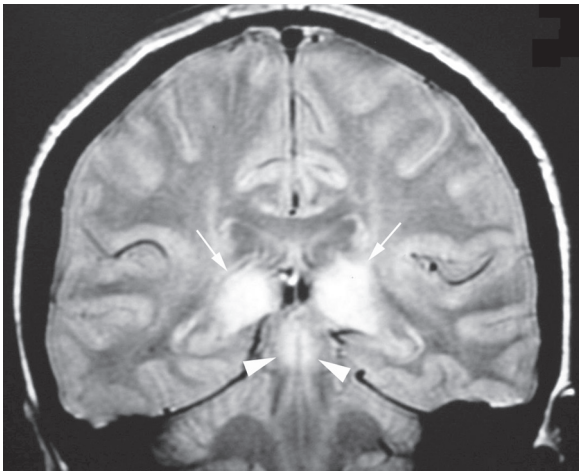


Figure 9.12 vCJD High-signal changes in the periaqueductal grey matter is shown by the arrow

9.4.5 Iatrogenic CJD – magnetic resonance imaging

Data are limited on the MRI appearances of iCJD, due to the low incidence of cases. However, preliminary analysis suggests that appearances are broadly similar to those found in sCJD, with symmetrical hyperintensity of the anterior putamen and caudate head on long TR sequences. Further work is required to analyse these findings in more detail.

9.4.6 Differential diagnosis

One of the most important clinical differential diagnoses of vCJD is sCJD, and MRI can differentiate between the two forms of the disease in most cases (Figure 9.13). Though thalamic hyperintensity (relative to cortical signal) has been described in sCJD and familial CJD, no case has been reported showing the pulvinar to be brighter than the putamen.

High signal in the thalamus has been described in

Figure 9.13 Comparison of appearances of MRI in vCJD and sCJD

MRI findings	Variant CJD	Sporadic CJD
Caudate head hyperintense	++	+++
Putamen hyperintense	+	+++
Posterior ('pulvinar') and dorsomedial thalamus hyperintense	+++	+
Periaqueductal grey matter hyperintense	++	+
Cerebral atrophy	+	+++
Cerebral cortex	+	++

a number of other conditions (Figure 9.14) but in all of these cases, the clinical and radiological features are clearly distinguishable from vCJD. Although other conditions may cause thalamic and other grey matter hyperintensity, the *highest* signal is always in the pulvinar of the thalamus in vCJD, and this is an important distinguishing feature.

Figure 9.14 Conditions with thalamic hyperintensity reported on MRI

A) Causes of thalamic high signal (involving whole thalamus or other thalamic nuclei except pulvinar)
- Carbon monoxide poisoning
- Japanese Nipositu encephalitis
- Wernicke encephalopathy
- Bithalamic glioma
- Thalamic infarction

B) Causes of pulvinar and dorsomedial nuclear group hyperintensity
- Benign intracranial hypertension (BIH)
- Cat-scratch disease
- Alpers syndrome
- Post-infectious encephalitis

Although the pulvinar sign is commonly present in vCJD, it is often overlooked during primary reporting, and has been over-reported by clinicians with limited experience of the MRI changes of vCJD. Therefore, it is recommended that MRI scans of suspected cases should be reviewed at a recognized reference centre (see Annex 5). Digital archiving of all images is recommended also to allow retrospective analysis in difficult cases.

9.5 Genetic analysis

Genetic screening of TSE cases for the mutations associated with the hereditary forms of disease can raise ethical and logistic concerns. Written consent for genetic testing is considered mandatory in many countries but may be culturally unacceptable in others. However, written consent or documented oral consent should be obtained whenever possible. Genetic counselling of patients and/or their families should be offered before any PrP gene analysis, with an emphasis on the autosomal-dominant inheritance of human TSEs (for sample consent forms see Annex 3).

WHO's surveillance programme would not recommend the genetic screening of every TSE case at present, because of the low PRNP gene mutation detection rate in sporadic cases. It is recommended, however, that genetic analysis should be offered only to patients with a family history of TSE. Analysis could be performed via one of the proposed WHO collaborating centres (see Annex 5).

All cases suspected of having vCJD should undergo PRNP gene analysis for research purposes (if consent is obtained) to exclude pathogenic mutation and to identify codon 129 polymorphism.

9.6 Pathology

9.6.1 Neuropathological characteristics of human TSEs

As with animal TSEs, no specific macroscopic abnormalities are detected outside the CNS at autopsy in CJD. Although macroscopic examination of the brain may be unremarkable, cortical or cerebellar atrophy is often found, and may vary greatly from case to case and within the various regions of the cortex in each individual. Sometimes the pattern of atrophy may correspond with the clinical syndrome, such as involvement of the occipital lobes in cases with relevant visual symptoms, or may occasionally extend to involve the basal ganglia, thalamus, hypothalamus and cerebellum. Typically, the hippocampus is preserved, even in the presence of severe brain atrophy.

The microscopic hallmarks of sporadic CJD are spongiform change, neuronal loss and astrocytosis (see Figure 9.15, beetween pages 58 and 59). Amyloid plaques, similar to those of Alzheimer disease but composed of PrP, rather than β-amyloid, are seen in about 10% of sporadic CJD cases (see Figure 9.16, beetween pages 58 and 59), but are much more common in kuru, iatrogenic CJD, and some familial forms of disease. Spongiform change consists of diffuse or focally clustered (morula-type) small round or oval vacuoles from 2 μm to 20 μm in size in the neuropil of the whole depth, or deep layers of, the cerebral cortex, cerebellar cortex (predominantly in the molecular layer), or in the subcortical grey matter. The vacuoles may become confluent to form irregular cavities. In longstanding cases severe spongiform change, neuronal loss, and astrocytosis may occur, leading to status spongiosis with collapse of the cytoarchitecture. Neuronal loss and reactive astrocytosis generally tend to be most apparent in the grey matter areas with spongiform change. However, burnt-out damage may show only prominent reactive astrogliosis without apparent spongiform change.

In some cases of CJD, cortical atrophy may be so severe that the histopathological picture is dominated by the changes of status spongiosis, with extensive neuronal loss leading to irregular microcystic cavities in the gliotic neuropil. Such changes are non-diagnostic, and the differential diagnosis would include those diseases listed in Figure 9.17.

Figure 9.17 Neuropathological differential diagnosis for CJD

Disease or condition	Neuropathological feature that aids in distinguishing listed conditions from CJD
Alzheimer disease	changes are focal or superficial
diffuse Lewy body disease	focal changes
brain oedema	irregular changes
metabolic encephalopathies	irregular changes
Pick disease	superficial changes
recent hypoxia or ischaemia	perineuronal or perivascular spongiosis
laboratory artefacts	particularly in the processes of specimen fixation, paraffin embedding, and section processing

An important adjunct to the neuropathological investigation of CJD and related disorders has come from advances in PrP staining through immunocytochemistry (see Figure 9.18, Section 9.6.5 and Annex 1.1).

The neuropathology of vCJD is significantly different from that of sCJD. In particular, a large number of amyloid plaques surrounded by a halo of spongiform change ('florid plaques', Figure 9.19) are seen, particularly in the cerebral and cerebellar cortical grey matter. The 'florid plaques' are not specific for vCJD but their widespread distribution is characteristic of the disease (Figure 9.20).

In addition to amyloid plaques, vCJD displays some other neuropathological features that make it distinguishable from other human prion diseases. For a detailed description see Annex 1.1.

The extent and intensity of pathological features increase as the disease progresses and the pathological characteristics therefore become evident in patients with a disease of long duration. Posterior thalamic astrocytosis is more extensive in vCJD than in other forms of the disease (i.e. the total number of astrocytes is higher in vCJD than in sCJD or FFI). Marked neuronal loss in vCJD, and the anatomical distribution of these changes, correlates with the abnormal hyperintensity on MRI.

Other neural tissues, including the trigeminal ganglia, dorsal root ganglia and retina, are also positive for PrP^{Sc}.

Neuropathological examination remains essential for the diagnosis of 'Definite' vCJD. (Neuropathological criteria are described in Annex 1.1.) Considering the extremely serious implications of a diagnosis of vCJD in countries not known to have the disease, neuropathology is recommended in such situations. A definite diagnosis of vCJD requires neuropathological confirmation, and furthermore, it is recommended that sample materials (frozen and fixed brain) are sent to one of the WHO reference centers listed in Annex 5. This will reduce the risk of a false diagnosis[1] due to possible technical difficulties from performing the test infrequently.

9.6.2 Brain biopsy

When used to diagnose CJD, brain biopsy typically involves the removal of a small piece of non-dominant frontal cortex under general anaesthesia. Although usually diagnostic in CJD, approximately 5% of biopsies from subsequently confirmed definite cases are non-diagnostic, reflecting the variable distribution of brain pathology in CJD. Brain biopsy can lead to serious complications, including cerebral abscess formation or haemorrhage and cannot be recommended as a procedure to confirm the clinical suspicion of CJD. Instruments used for neurosurgery on patients with CJD should be destroyed. If re-use is unavoidable, instruments must be immersed in 1N NaOH[2] or fresh undiluted hypochlorite for at least one hour, cleaned, and then autoclaved at 134 °C for 1 hour (see Annex 4.1).

9.6.3 Tonsil biopsy and lymphoreticular tissues in the diagnosis of vCJD

Although the precise role of lymphoreticular tissues in the pathogenesis of TSEs remains unclear, the preclinical involvement of various lymphoid organs has long been recognized. The diagnostic implications of this feature have been investigated in vCJD, sporadic, familial and iatrogenic CJD.

Positive results were first demonstrated in tonsil tissue taken at necropsy from a neuropathologically confirmed vCJD patient. A subsequent analysis of various lymphoid tissues, including palatine tonsil, obtained at necropsy did not test positive for PrP^{Sc} in a small number of patients with sCJD or GSS disease. When using post-mortem tonsillar tissue, all vCJD cases showed the presence of PrP^{Sc}. In contrast, none of the tonsillar tissue samples from sCJD or iatrogenic CJD were positive.

The reason tonsillar tissue contains PrP^{Sc} positivity in vCJD but not in other forms of CJD is unknown, but presumably reflects either a property of the agent strain in humans or the peripheral route of infection.

[1] False-negative results related to problems in PrP^{Sc} detection are possible, as well as false-positive results related to PrP^{C} detection.

[2] For NaOH solution, 1N = 1 mol/litre.

The above results suggested that ante-mortem tonsil biopsy could be used as a diagnostic test in patients suspected to have vCJD, and a positive result has now been incorporated into the criteria for a probable case of vCJD. However, the use of this procedure in patients suspected to have vCJD is controversial and concern has been expressed regarding its potential morbidity (e.g. bleeding, infection and the risk of general anaesthesia). The most common differential diagnoses in cases of suspect vCJD that have died are sporadic CJD and cerebral vasculitis. Tonsil biopsy cannot provide specific information on these or any other of the differential diagnostic possibilities for vCJD and there are insufficient current data to exclude the possibility of a false-negative or false-positive tonsil biopsy. Expertise is required in the accurate assessment of lymphoreticular tissues to diagnose vCJD and it is suggested that such samples should be sent to one the WHO reference centres listed in Annex 5 for review. Based on the published data, tonsil biopsy cannot be recommended as a routine diagnostic procedure in the investigation of vCJD. It is not recommended as a screening procedure, not least because most suspect cases will not have vCJD. For some suspect cases without bilateral pulvinar high signal on MRI, tonsil biopsy has a role in the classification of Probable but not Definite vCJD.

Other lymphoreticular tissues of vCJD patients also contain PrP^{Sc}, with deposition in the germinal centers and apparently involving follicular dendritic cells and macrophages. Germinal centre involvement occurs in all lymphoid tissues, but is most evident in the tonsil and spleen. All adequate samples of tonsil, spleen and lymph node taken from confirmed vCJD cases have tested positive for PrP^{Sc} whereas negative results have been obtained in those sporadic CJD and iatrogenic cases studied.

Consequently, immunocytochemistry of lymphoreticular tissue samples can be used to diagnose Probable vCJD. Various lymphoreticular tissues can be utilized, but tonsils are by far the best candidate due to the large number of germinal centres (Figure 9.21). If tonsils are not available, the next best candidate is spleen tissue; if spleen is unavailable, then peripheral lymph nodes can be sampled. If peripheral lymph nodes are used, multiple sampling is necessary in order to increase the chances of detecting PrP. In this situation, an extremely careful examination is required, since the germinal centres of lymph nodes are small. Some additional frozen material should always be saved for western blot examination. A western blot negative for PrP will be useful to confirm a negative immunohistochemistry finding, and can rule out a false positive due to incomplete digestion of PrP^{C}. In countries where post-mortem examination is not accepted or cannot be performed, post-mortem needle biopsy of the spleen, which is not disfiguring, is suggested.

It is to be emphasized that a lymphoid tissue positive for PrP together with the characteristic clinical symptoms and signs can only support a diagnosis of Probable vCJD. The finding of negative lymphatic tissues cannot exclude the diagnosis of vCJD, but if multiple large samples are all negative, the likelihood of vCJD is reduced. As stated earlier, lymphoreticular samples should be sent for confirmation to the WHO reference centres.

9.6.4 Suggested approaches to post-mortem examination

In countries where post-mortem examination is not accepted or cannot be performed, post-mortem biopsy can be used as an alternative. In this case multiple sampling (four to five large pieces) from different brain regions is required in order to perform an adequate histopathological evaluation. Nevertheless, in cases where only brain biopsy is available, a negative result does not exclude the diagnosis of vCJD.

It is emphasized that the only rationale for a brain biopsy in life remains the diagnosis of another treatable disease; brain biopsy in a living person is not recommended for the diagnosis of vCJD or sporadic CJD.

A hierarchy for histopathological diagnosis of vCJD is provided in Figure 9.22.

Figure 9.22 Hierarchy for tissue diagnosis of vCJD

1. Full autopsy with fixed and frozen tissue (for western blot examination) retained from CNS, lymphoid and other tissues
2. Limited autopsy with fixed and frozen tissue retained from CNS tissues
3. Limited autopsy with fixed CNS tissue only
4. Brain biopsy with fixed and frozen tissue retained
5. Brain biopsy with fixed tissue only
6. Lymphoreticular tissue 'biopsy' post-mortem with fixed and frozen tissue retained
7. Lymphoreticular tissue 'biopsy' post-mortem with fixed tissue only

9.6.5 Prion protein (PrP) immunocytochemistry

Immunocytochemistry for PrP is a recent, very useful addition to the repertoire of tools for CJD neuropathological diagnosis. However, it is a delicate procedure that WHO recommends should be used only by experienced reference laboratories for the

present time. More details on pretreatment and antibodies are available in the literature (e.g. Bell et al., 1997, Kovacs et al., 2002).

The basic procedure is as follows. PrP monoclonal and polyclonal antibodies are applied to paraffin sections of various tissue specimens from different regions of the brain of a suspected case. As PrP^{C} is normally present, pretreatment is required to ensure that only the pathological PrP^{Sc} is detected (e.g. with pretreatment by hydrated autoclaving). Although the pattern of PrP positivity may vary, certain generalities can be made. Three distinct patterns of PrP deposition are seen, as follows:

— diffuse synaptic pattern, usually comprising abundant tiny immunolabelled dots throughout the neuropil, with the occasional bigger and coarse deposits;

— patchy/perivacuolar type deposits, where immunolabelling is predominantly deposited around vacuoles, in a patchy distribution;

— plaque deposits, usually as small to medium sized compact "kuru-type" amyloid plaques with a fringed outline. These plaques are usually also reactive to periodic acid-Schiff, Congo red, and alcian blue stains; the Congo red staining disappears after formic acid treatment. The most frequent sites of plaque formation include the granular layer and, to a lesser extent, the central white matter and molecular layers of the cerebellum. Well defined plaques may also be seen in the basal ganglia, thalamus, brainstem and cerebral cortex. Plaque-like PrP deposits, in contrast to kuru-type plaques, are detectable only with immunocytochemistry but not with routine haematoxylin and eosin staining

More subtle staining patterns are often seen. In particular, some neurons or neurites may be outlined by granular PrP deposition. The types of PrP depositions and the amount of immunoreactive PrP may vary between cases, but tend to be relatively constant between different regions of a given brain. PrP deposition may be focal; hence the taking of multiple specimens from different regions of the brain is advisable. Presence of Alzheimer-type brain amyloidosis can modify PrP deposition. The brain stem is involved less frequently.

In the majority of cases, the diagnosis of 'Definite' CJD can be made by the one or more of the above-mentioned neuropathological exams. Rare cases may require additional studies, such as immunoblotting or electron microscopic examination of scrapie-associated fibrils (SAF), molecular biological studies or experimental transmission in animal models.

9.7 Western blot

Recently, several human PrP^{Sc} types associated with different clinical phenotypes of CJD have been identified. The fragment size after limited proteolysis, and the ratio of the three PrP glycoforms (diglycosylated, monoglycosylate and unglycosylated PrP) visible after electrophoresis on polyacrilamide gel, is maintained after passage in transgenic mice expressing human PrP. According to these data, possible molecular classifications of CJD have been published. However, some discrepancies between different laboratories have been reported.

In extracts taken from the brain tissue of persons with vCJD, the ratio of glycosylated to non-glycosylated PrP differs from sporadic CJD and is similar to experimentally transmitted BSE i.e. with the diglycosylated band being most prominent. Although the use of PrP strain typing by western blot in the differential diagnosis of sporadic CJD and vCJD is promising, it is still too preliminary to be used as a routine marker in clinical settings (Figure 9.23).

9.8 Future diagnostic tests

Prion protein is normally expressed in white blood cells and platelets and the possibility exists that some blood cells may express the abnormal PrP isoform in affected individuals. This raises the possibility of a blood test for CJD, but would require an assay for PrP with a much higher degree of sensitivity than is currently available. However, progress is being made in this direction. Improved concentration and amplification methods coupled with the use of antibodies specific to the abnormal PrP isoform only bring hope for the future of more accurate, more sensitive and simpler diagnostic techniques. A recently developed highly sensitive immunoblot method has made possible the detection of PrP in some vCJD tissues. A further refinement of this or similar methods will hopefully make possible the detection of PrP in the tissue and blood of patients manifesting or incubating the disease. Nevertheless, it is unlikely that there will be a blood test for vCJD in the near future.

CHAPTER 10

Specific protocols for the diagnosis of human transmissible spongiform encephalopathies

10.1 Blood

10.1.1 Collection, separation and storage of blood and blood components from TSE-infected humans for routine blood examinations

Blood specimens from patients with CJD are not considered infectious. Clinical laboratories should use standard precautions as established for other infectious diseases for all blood samples. In the clinical laboratory there are no precautions particular to the handling of blood from persons with human TSEs.

10.1.2 Collection, separation and storage of blood and blood components from TSE-infected humans for storage or investigation

The following protocol is taken from the Report of the Working Group for International Reference Material for Diagnosis and Study of TSEs (held in Geneva, Switzerland, 1–2 March 2001). This working group is developing reference reagents and reference panels to facilitate the development and comparison of various diagnostic approaches and assay systems for the diagnosis of TSEs. All reports of the working group, which first met in 1999, can be found on the WHO web site (Annex 5).

The working group has developed the following protocol. It can be used to collect small amounts of blood from humans with TSEs. This may be useful for future investigations of blood-based diagnostic tests. The protocol may not be suitable for studies localizing infectivity in various components of blood.

1. Collect whole blood anticoagulated with sodium citrate (final concentration should be 0.5% citrate)
2. Remove a 10% aliquot for storage at -80 °C
3. Centrifuge the remainder at 2280*g* for 4 minutes at room temperature
4. Use a transfer pipette to remove the plasma layer, leaving the last 5 mm on the buffy coat. Store the plasma layer as platelet-rich plasma (more strictly designated as "2280*g*" plasma) at -80 °C
5. Use a separate transfer pipette to collect buffy coat including 5 mm of the underlying red blood cell layer. Store at -80 °C
6. Collect the remaining red blood cells with a third transfer pipette, taking care not to touch the tube walls. Store at -80 °C

10.2 14-3-3 protein in CSF

Sample collection: Collect no less than 1 ml of CSF and send for usual diagnostic procedures.

Freeze a sample for 14-3-3 analysis (no less than 1 ml) as quickly as possible (within 20 minutes is preferred). It is important that samples are not contaminated by excess red cells. Store at -70 °C or colder until shipment on dry ice.

Western blot detection of 14-3-3: After SDS-PAGE electrophoresis of 20 µl of CSF, transfer the proteins on a nitrocellulose membrane by western blot. Immunostain using commercially available antibody raised against the η-subunit of the 14-3-3 protein, at a 1:500 dilution. A positive and a negative sample should always be included as controls. In case of weakly positive result, repeat the procedure twice and eventually, repeat a spinal tap at a reasonable time interval.

If sending a specimen to a reference centre for analysis, please include a completed CSF request form (see Annex 3) and any significant patient information. Generally, the sample must arrive at the centre during regular working days, since appropriate storage cannot be guarantied during the weekend. Please contact the responsible person for more information before sending samples.

10.3 Electroencephalography

In 1998, WHO held a consultation to standardize case definitions for human TSEs. The consultation specifically addressed problems relating to diagnostic EEG. The criteria devised by Steinhoff and Knight (unpublished) were proposed and adopted. It is expected that the results will be further evaluated.

Preliminary notes

- The finding of a characteristic periodic EEG pattern is very helpful in the diagnosis of sporadic CJD.
- Some cases of sporadic CJD never show this

pattern. A 'negative` result cannot exclude the diagnosis.
- A periodic EEG like that seen in CJD may rarely be found in a number of other conditions and these must be considered in the clinical context. A list of these conditions is given in Figure 9.3.
- The EEG changes in CJD undergo evolution. A periodic pattern may not be seen in the early phases of disease. The EEG may progress from showing non-specific abnormalities to the characteristic appearance within days. Therefore, frequent serial EEG recordings should be undertaken whenever possible.
- If a typical periodic EEG is obtained, it may not be necessary to repeat it, although this should be considered if there is any clinical doubt about other possible causes of the EEG pattern (such as metabolic factors).
- A repeatedly normal EEG is not consistent with a diagnosis of sporadic CJD.

Specific features

- Strictly periodic activity
  - Variability of intercomplex intervals is <500 ms
  - Continuous periodic activity for at least one 10-second period.
- Bi- or triphasic morphology of periodic complexes.
- Duration of majority of complexes ranges from 100 to 600 ms.
- Periodic complexes can be generalized or lateralized, but not regional or asynchronous.

Technical notes

- Bipolar montages including the vertex should be used.
- Referential montages including vertex and CZ reference electrodes should be used.
- The ECG should be co-registered.
- External alerting stimuli should be used.
- The whole record should be viewed whenever possible and a 5-minute continuous sequence as a minimum.

Note: The EEG criteria are based on considerable experience with the EEG in CJD but have not been formally evaluated prospectively in large numbers of suspect CJD cases. Such evaluation is being undertaken and the results of this may necessitate some revision.

10.4 Magnetic resonance imaging

10.4.1 T1-weighted imaging (T1WI)

T1-weighted imaging is often unremarkable. Very heavy deposition of PrP^{SC} protein in the basal ganglia can result in T1 high signal (in <10%). Enhancement with gadolinium is not a feature of CJD.

10.4.2 T2-weighted imaging (T2WI)

Basal ganglia changes are frequently seen on T2WI, and this is a consistent finding across the different CJD subtypes. The conspicuity of the changes is greater on other sequences (vide infra).

10.4.3 Proton-density weighted imaging (PDWI)

Changes on PDWI may be more conspicuous than on T2WI. The appearances of high signal on PDWI alone (without corroborative T2WI signal change) should be interpreted with caution, as some MRI systems may produce intrinsic high signal in the basal ganglia in young, normal patients.

10.4.4 FLAIR imaging

FLAIR imaging has proved the most sensitive of the long TR sequences to thalamic high signal in vCJD. It is probable that this is also the case in sCJD. If available, an axial FLAIR sequence aligned to the AC-PC line (hence allowing direct comparison of the basal ganglia and thalamic signal on the same slice) is the optimal imaging sequence for the detection of hyperintensity in the deep grey matter nuclei.

10.4.5 Diffusion-weighted imaging (DWI)

Recent reports have shown that DWI may show restriction of diffusion in the basal ganglia and cerebral cortex in sCJD, and thalamus in vCJD, early in the disease. These changes may become much less conspicuous later in the disease course. Most modern scanners are capable of standard DWI imaging, and the sequence is short (<60 seconds); it should be included in the imaging protocol where available.

10.4.6 Magnetic resonance spectroscopy (MRS)

The role of spectroscopy in CJD is not clarified. Non-specific reductions in *N*-acetyl-aspartate (NAA) have been seen in the cortex and subcortical white matter in sCJD. This probably reflects neuronal loss. At present MRS should be regarded as an investigational technique.

10.4.7 Optimal imaging parameters and sequences in suspected CJD

A wide variety of MRI protocols are used when scanning patients with suspected CJD at different institutions (particularly image plane and sequence

Figure 10.1 Recommended MRI parameters and sequences in suspected CJD

Magnet strength	Magnet field strength 1.5 T or greater
Patient preparation	The majority of patients can be scanned without general anaesthetic. If very marked movement artefact, a general anaesthetic may be required.
Minimum sequences	T2 axial images (slice thickness 3–5mm) FLAIR axial (3–5mm) +/- FLAIR sagittal (3–5mm) **IF FLAIR unavailable**: Proton density axial images (slice thickness 3–5mm)
Preferred additional sequences[a]	Diffusion-weighted imaging (DWI) if available T1-weighted volume acquisition whole brain
Image plane[b]	Axial images parallel to anterior commissure-posterior commissure line preferred to allow comparison of signal intensity of grey matter nuclei (especially thalamus and putamen) on same slice
Sequence and imaging variables	Imaging sequences vary widely depending on scanner age and manufacturer. Sequence parameters should be selected to obtain good contrast between grey matter in the basal ganglia and cortex, and the adjacent white matter. The highest resolution obtainable in acceptable time should be selected. Ideal parameters include: matrix 190/256 or greater, FOV 18 x 30, no interslice gap, slice thickness 5 mm or less.
Archive	Digital data or original film may be sent to a reference centre (see Annex 5) for review if desired. Original digital data may be requested for further quantitative analysis.

[a] To date the proton density axial image has proved the most useful widely available sequence for diagnosing vCJD. Sensitivity of sequences to CJD varies approximately: DWI > FLAIR > PD > T2 > T1.

[b] Subtle deep grey matter nucleus hyperintensity may be hard to perceive on coronal imaging in particular.

selection). Appropriate parameters and sequences are outlined in Figure 10.1. It is important to scan in the axial plane (parallel to the AC-PC line) and to obtain FLAIR images (or proton-density images where FLAIR is not available) in addition to T2-weighted images. Additional imaging with diffusion-weighted sequences is also often helpful. Archiving of all digital images is recommended to allow retrospective analysis in difficult cases.

10.5 Genetic analysis

Sample collection

Collect 5 ml of blood per tube (total of 20 ml) in two tubes containing heparin and two tubes containing EDTA. Storage and shipment can be done at room temperature.

Genetic material can also be obtained from other tissues, e.g. brain obtained at autopsy.

Please include a completed consent form (see Annex 3) and any significant patient information. Please contact the responsible person for more information before sending samples.

10.6 Neuropathology

10.6.1 Procedure for brain autopsy

The definite diagnosis of CJD, including vCJD, requires neuropathological confirmation. Autopsy should be strongly encouraged in any suspect case of CJD. Where autopsy is not possible or permitted, post-mortem biopsy of the brain should be sought. The use of cerebral biopsy in living patients is discouraged except to make an alternative diagnosis of a treatable disease.

When handling tissues and other materials from suspected CJD cases, specific safety precautions (see Chapter 6, Tissue handling and safety precautions) are mandatory to avoid accidental transmission and to eliminate any infectivity.

Extensive sampling, from different areas of the brain, is mandatory on autopsy, including as a minimum:

- frontal lobe
- temporal lobe
- occipital lobes
- basal ganglia
- cerebellum

Especially important is the comparison between the involvement of the cerebrum and the cerebellum. Generally, neuropathological confirmation is important because of the ongoing recognition of the potentially broad spectrum of clinical and pathological manifestations of human TSEs. Factors that may play a role include PrP gene mutations, genotype and as-yet unidentified factors or cofactors, including potential prion strains.

PrP immunocytochemistry testing is especially helpful, in the absence of typical or characteristic changes appreciable on routine histopathological examination.

Full procedure

The autopsy should be performed as soon as possible after death. However, the tissue can be successfully examined up to 48–72 hours post-mortem, especially if the body is refrigerated. The brain, the hemispheric dura and the pituitary gland should be split in half, sagitally. The left cerebral hemisphere with the left dura and the left pituitary gland, left cerebellar hemisphere, left vermis and left brain stem should

be put in formalin, fixed for two weeks, sliced and sampled. Tissue samples should be treated with formic acid (98%) for one hour and then placed in formalin for 24 hours before dehydration and paraffin embedding to reduce infectivity. Alternatively, the left half of the brain can be sent to the centre performing the diagnostic procedure at any time following immersion in formalin. Before shipping, formalin should be absorbed with paper towels so that there is no free formalin but the tissue is exposed to formalin vapors.

The right cerebral hemisphere should be separated from the right cerebellar hemisphere and brain stem with a horizontal cut at the level of the upper midbrain. It should than be sliced coronally in ~1.5-cm slices. The right cerebellum and brain stem should be sliced horizontally in slices of ~1.0 cm. The right half of the dura and the right half of the pituitary gland should be frozen uncut. The brain slices should be frozen in a -70 °C freezer (or, lacking that, in a -20 °C freezer) individually, inside plastic bags (to avoid drying) while lying flat on a tray. They can then be put together in a plastic bag when they are frozen. Alternatively, the right half of the brain can be sent to the diagnostic centre uncut surrounded by dry ice.

Short procedure

If the above procedure cannot be followed, 1–5 grams of brain tissue, including the cerebral cortex, should be removed and frozen and an adjacent brain tissue sample should be fixed as above.

Please include a completed autopsy request form (see Annex 3) and any significant patient information. Generally, the sample must arrive at the centre during regular working days, since appropriate storage cannot be guarantied during the weekend. Please contact the responsible person for more information before sending samples.

10.6.2 Procedure for brain biopsy

Freeze 0.5 g of tissue (for western blot of PrP as little as 10 mg is enough) in a -70 °C freezer (or in a -20 °C freezer). Ship in dry ice to the centre performing the procedure.

Fix the remaining tissue in 10% formalin for at least 24 hours. Transfer formic acid for 1 hour and then again in formalin for at least 24 hours. The tissue can then be embedded using routine procedures.

Please include a completed biopsy request form (see Annex 3) and any significant patient information. Generally, the sample must arrive at the centre during regular working days, since appropriate storage cannot be guarantied during the weekend. Please contact the responsible person for more information before sending samples.

10.6.3 Neuropathology staining

The methods and procedures for PrP immunocytochemistry, western blot and immunodetection of PrP are well described by Bell et al., 1997 and Kovacs et al., 2002.

CHAPTER 11

National and international shipping

When sending TSE material by air freight, the company handling it must hold a dangerous goods licence. Most routine couriers do not have this. It is the responsibility of the sender to comply with these regulations and failure to do so can result in a large fine or even imprisonment. The tissue must be packed in UN-certified packaging according to the guidelines and the necessary documentation must be completed.

Regulations regarding shipment of infectious substances require the shipper to make advance arrangement with the consignee and the operator to ensure that the shipment can be transported and delivered without unnecessary delay. The following statement must be included in the Additional Handling Information area of the Shipper's Declaration: "Prior arrangements as required by the IATA Dangerous Good Regulations have been made".

11.1 Packing

CJD tissue, blood and CSF samples must be wrapped, marked and labelled in accordance with the Dangerous Goods Packing Instruction Code 602. Cardice, used when sending frozen tissue, must be used according to the regulations in Code 904 III.

The following packing instructions for frozen and fixed tissue and blood fulfill these requirements.

11.1.1 Frozen tissue

The maximum amount of frozen CJD tissue allowed for transportation by air freight is 50 grams (g). The minimum amount of cardice suggested is 10 kilograms (kg) since this ensures that the tissue will remain frozen for at least 72 hours (security requirements may lead to holding times approaching 72 hours). Two hundred kilograms is the maximum amount allowed in the plane's cargo hold. The frozen tissue is packed as follows:

1. Place tissue in watertight primary receptacle(s) (i.e. vial). The primary receptacle must be leak-proof (watertight). Screw caps must be reinforced with adhesive tape. Only glass, metal or plastic containers are allowed.
2. Place in watertight secondary container. The secondary receptacle must be leak-proof (watertight) and it must contain sufficient absorbing material to absorb the entire content of the primary receptacle. The primary and secondary receptacles must withstand, without leakage temperatures in the range of -40 °C to +55 °C and pressure of air transportation. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.
3. Place in outer fibreboard box containing all of the necessary UN certificates and markings.
4. Place in thermal overpack filled with dry ice (minimum of 5 kg, better if 10 kg). This overpack must have the class 6.2 and dry ice warning labels (Dry ice. UN1845, Class 9, net-weight 10 kg) and UN number 2814 attached to the outside.
5. Place any correspondence from the pathologist inside the box before sealing.

11.1.2 Fixed tissue

Tissue must be packed in three layers so that it remains leak-proof. For the first layer, the tissue is placed in a polyethylene bag, which is then sealed, preferably heat-sealed. This is then placed in a small plastic bucket that has been labelled with the patient's name and number. The small bucket is then surrounded with absorbent material (i.e. cotton wool or tissue) and placed inside a metal bucket. The Biohazard warning sign and label "only to be opened under laboratory conditions" is taped to the lid of the metal bucket. This is then placed into a bio-transporter cargo fibreboard box. This box must conform to cargo aircraft regulations (provided by the courier) and have the class 6.2 and UN certificate warning label printed on the outside.

11.1.3 Blood and CSF

For infectious diseases, the maximum amount of blood allowed for transportation by air freight is 50 ml. The sample is packed in three layers, as described for the frozen tissue samples.

11.2 Dry ice

If dry ice is used, the outside packaging must be able to release carbon dioxide gas. Dry ice must not be placed inside the primary or secondary receptacle because of the risk of explosions.

The outside package must carry the "MISCELLANEOUS" hazard label for dry ice in addition to the Infectious Substance label.

11.3 Marking and labelling

Each package and overpack containing infectious substances must be clearly and durably marked on the outside with the following:

1. Shipping name and technical name of the contents, "**Infectious Substances affecting humans** – Creutzfeldt-Jakob Disease – (solid or liquid)", and UN number (UN 2814)
2. Full name and address of the shipper and the consignee
3. Name and telephone number of a person responsible for the shipment
4. Net weight of dry ice contained in the package
5. Diamond-shaped hazard labels (Infectious Substance label and Dangerous Goods label).

11.4 Documentation

The following documentation is required:

11.4.1 Shipper's declaration form for dangerous goods

This form gives the address and the international telephone number of both the shipper and consignee and states the nature and quantity of the dangerous goods being sent. CJD is entered as "**Infectious Substances affecting humans** – Creutzfeldt-Jakob Disease –(solid or liquid)". Its classification is 6.2 and its United Nations (UN) number is UN 2814. This form also states the amount of tissue (net quantity in each package) and number of packages being sent and how it is packed (i.e. cardice or fibreboard box). The packaging instruction for Infectious Substances is 602. An emergency contact person is also named and a telephone number is provided. Three typed copies of this form are required, each one having the original signature of the sender. The shipper retains one copy; the carrier retains two.

11.4.2 Emergency Response Sheet

This sheet (to be attached to the outside of the box being sent) provides information necessary in case of an accident.

1. The dangerous goods are **Infectious Substances affecting humans** (solid) Creutzfeldt-Jakob Disease. Class 6.2 UN 2814.
2. There is no immediate hazard to health unless the goods are ingested or injected into the body.
3. There is no risk of fire or explosion.
4. In the event of an accident, disposable gloves are to be worn for handling the material. Surfaces that have been in contact with dangerous goods must be wiped with 2 mol/litre sodium hydroxide. The sodium hydroxide is left on the surface for one hour before being washed off.
5. In the event of fire the infectious agent is destroyed.
6. Spillage in the absence of fire. Wipe areas with 2 mol/litre sodium hydroxide and leave for one hour, then wash off.
7. First-aid measures. Skin that has had contact with dangerous goods may be wiped with 2 mol/litre sodium hydroxide for a couple of minutes then washed well.

11.4.3 Customs declaration sheet

This should be typed on headed notepaper. It should state the number and weight (or volume) of samples and states that these samples are human tissue (or blood/CSF) specimens of Creutzfeldt-Jakob disease e.g. "**Infectious Substances affecting humans** (solid); For medical research only". A nominal value is generally assumed per box by national customs authorities. It must also state that the samples have been sent from the address on the headed notepaper. Normally five customs declaration sheets are required.

Note: All documentation must be attached to the outside of the box.

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ANNEX 1

Surveillance case definitions for the classification of human transmissible spongiform encephalopathies

Annex 1.1 Clinical and neuropathological classification of cases

1. **CJD** sporadic, iatrogenic or familial (same disease in first-degree relative or disease-associated PrP gene mutation)
   - — spongiform encephalopathy in cerebral and/or cerebellar cortex, and/or sub-cortical grey matter and/or
   - — encephalopathy with PrP immunoreactivity (plaque and/or diffuse synaptic and/or patchy/peri-vacuolar types).
2. **Gerstmann-Sträussler-Scheinker disease (GSS)** in a family with dominantly inherited progressive ataxia and/or dementia and one of a variety of PrP gene mutations:
   - — encephalomyelopathy with multi-centric PrP plaques
3. **Familial fatal insomnia (FFI)** (in a member of a family with a PrP^{178} gene mutation in frame with methionine at codon 129):
   - — thalamic degeneration, variable spongiform changes in cerebrum.
4. **Kuru**
   - — spongiform encephalopathy in a member of the Fore populations in Papua New Guinea.

The above criteria are modified from Budka H et al. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathology,* 1995, 5(3):459–466.

5. **vCJD**

Major features:

- — abundant kuru-type fibrillary PrP plaques, often surrounded by a halo of spongiform change (the "florid" plaque).
- — multiple small PrP plaques occurring in clusters within the cerebral and cerebellar cortex and not related to spongiform changes.
- — amorphous PrP deposits around neurons and blood vessels in the cerebral and cerebellar cortex.

Immunocytochemistry for PrP is an invaluable aid to diagnosis, although the large fibrillary plaques are easily visualized by haematoxylin/eosin stain. Plaques can also be identified by periodic acid/Schiff or Gallyas silver stains, but the amorphous PrP deposits are best visualized by immunocytochemistry.

Additional neuropathologic characteristics:

- — spongiform change most prominent in the basal ganglia, with dense perineuronal and periaxonal PrP deposition
- — severe thalamic astrocytosis and neuronal loss, particularly involving the dorsomedial and posterior nuclei (including the pulvinar)
- — massive accumulation of PrP, often with focal distribution, in the cerebellar cortex including the molecular and granular layer with occasional plaques in the white matter.
- — punctate neuronal staining for PrP in the pontine nuclei.

Annex 1.2 Classification into Possible, Probable and Definite cases

1. Sporadic CJD

Possible

- — Progressive dementia and
- — EEG atypical or not known and
- — Duration <2 years and
- — At least 2 of the following clinical features:
  - myoclonus
  - visual or cerebellar disturbance
  - pyramidal / extrapyramidal dysfunction
  - akinetic mutism.

Probable (in the absence of an alternative diagnosis from routine investigation)

- — Progressive dementia and
- — At least 2 of the following 4 clinical features:
  - myoclonus

- visual or cerebellar disturbance
- pyramidal/extrapyramidal dysfunction
- akinetic mutism and

— A typical EEG, whatever the clinical duration of the disease, and/or
— A positive 14-3-3 assay for CSF and a clinical duration to death <2years

Definite CJD
— Neuropathological confirmation; and/or
— Confirmation of protease-resistant prion protein (immunocytochemistry or western blot) and/or
— Presence of scrapie-associated fibrils.

2. Iatrogenically transmitted CJD
Probable

a. Progressive cerebellar syndrome in human pituitary hormone recipients.

b. Probable CJD with recognized iatrogenic risk

Definite
Definite CJD with a recognized iatrogenic risk.

3. Genetic human TSEs
Probable

a. Probable TSE plus definite or probable TSE in a first-degree relative.

b. Progressive neuropsychiatric disorder plus disease-specific mutation.

Definite
Definite TSE with a recognized pathogenic PrP mutation plus definite or probable TSE in a first-degree relative.

Note: For purposes of surveillance, this includes GSS and FFI.

4. vCJD

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
E No evidence of a familial form of TSE

Definite: I A **and** neuropathological confirmation of vCJD[1]
Probable: I **and** 4/5 of II **and** III A **and** III B **OR** I **and** IV A
Possible: I and 4/5 of II and III A

II A Early psychiatric symptoms[2]
B Persistent painful sensory symptoms[3]
C Ataxia
D Myoclonus or chorea or dystonia
E Dementia

III A EEG does not show the typical appearance of sporadic CJD[4] (or no EEG performed)
B MRI brain scan shows bilateral symmetrical pulvinar high signal[5]

IV A Positive tonsil biopsy[6]

[1] Spongiform change and extensive PrP deposition with florid plaques, throughout the cerebrum and cerebellum.
[2] Depression, anxiety, apathy, withdrawal, delusions.
[3] This includes both frank pain and/or dysaesthesia.
[4] Generalized triphasic periodic complexes at approximately one per second.
[5] Relative to the signal intensity of other deep grey matter nuclei and cortical grey matter.
[6] Tonsil biopsy is not recommended routinely, or in cases with EEG appearances typical of sporadic CJD, but may be useful in suspect cases in which the clinical features are compatible with vCJD and where MRI does not show bilateral pulvinar high signal.

ANNEX 2

Minimal monitoring data

Annex 2.1 Minimal monitoring data set

Administrative information

Date of report to National Reference Centre MM/YYYY

Report completed at ☐ time of death ☐ time of referral

Is case closed ☐ Yes ☐ No

Is follow-up data available ☐ Yes ☐ No

Date of report to WHO MM/YYYY

Country reporting case[1]

Identification number (10000)

Full contact information for the person submitting report; indicate if neurologist, psychiatrist, neuropathologist, physician, GP, or other

Case-related information

Month/Year of birth (numerical MM,YYYY)

Gender M/F/U

Country of birth (ISO code)[1]

Residence at onset (country, ISO code)[1]

NB. At this time it is agreed that cases of vCJD will be attributed to country of residence at onset of symptoms, with consideration given for countries in which the case was a long-time resident.

Onset (numerical MM/YYYY at time of onset of symptoms)

Clinical presentation at onset, ie initial symptoms that brought patient into clinical care (please see definitions in Annex 2.2). Choose one

☐ Rapidly progressive dementia
☐ Slowly progressive dementia
☐ Cortical blindness (Heidenhain)
☐ Psychiatric onset
☐ Cerebellar onset

[1] ISO code located at http://www.iso.ch/iso/en/prods-services/iso3166ma/02iso-3166-code-lists/index.html
Countries associated with EuroCJD, NeuroCJD and SEEC-CJD have separate numerical coding.

☐ Extrapyramidal onset
☐ Stroke-like onset
☐ Other, specify (15 character field)
☐ Missing

Sporadic CJD	**Yes**	**No**	**vCJD**	**Yes**	**No**
Myoclonus			Early psychiatric disorder		
Visual or cerebellar problems			Persistent painful sensory symptoms		
Pyramidal or extrapyramidal features			Myoclonus or chorea or dystonia		
Akinetic mutism			Ataxia		
			Dementia		

Date of death (numerical, MM/YYYY)

Did a member of the national reference centre see the patient while the patient was alive? ☐ Yes ☐ No

Classification at death
(see Annex 1.2, Classification into Possible, Probable and Definite cases, and Annex 1.1, Clinical and neuropathological classification of cases)
Select all that apply

☐ Sporadic
☐ Iatrogenic
☐ Familial
☐ Variant
☐ GSS
☐ FFI

Classification according to diagnostic criteria
(See Annex 1.2 Classification into Possible, Probable and Definite cases)

☐ Definite
☐ Probable
☐ Possible

Disease-specific PRNP mutation

☐ No ☐ Yes ☐ Pending ☐ Not done

If yes, describe the mutation letter / 3 numbers / letter

Codon 129

☐ Methionine/Methionine ☐ Methionine/Valine ☐ Valine/Valine
☐ Pending ☐ Not done

EEG sufficiently typical to support the diagnosis of CJD?
See Annex 1 for EEG Interpretation

☐ Yes ☐ No ☐ Not done ☐ Not known ☐ Missing

Was the EEG reviewed by the national reference centre?

☐ Yes ☐ No

14-3-3 in CSF

☐ Performed ☐ Not performed ☐ Missing
CSF is ☐ Positive ☐ Negative ☐ Equivocal

MRI

☐ Performed ☐ Not performed ☐ Missing

MRI shows	**Yes**	**No**
High signal in caudate and putamen		
Bilateral high signal in posterior thalamus, greater than other areas		

Has the MRI been reviewed by the national reference centre? ☐ Yes ☐ No

Pathology

Neuropathology

☐ Performed ☐ Not performed ☐ Not known

☐ Brain biopsy or ☐ Post-mortem?

Has the neuropathology been reviewed by the national reference centre? ☐ Yes ☐ No

Cases submitted via the PrionNet project by contacting Professor Budka? ☐ Yes ☐ No
(Contact information in Annex 5, Information resources)

Tonsil biopsy

☐ Performed ☐ Not performed ☐ Not known
☐ Positive ☐ Negative

Has the tonsil biopsy been reviewed by the national reference centre?

☐ Yes ☐ No ☐ Not known

Tissue collection

Brain tissue ☐ Yes ☐ No
Blood ☐ Yes ☐ No
Serum ☐ Yes ☐ No
CSF ☐ Yes ☐ No
Other tissues ☐ Yes ☐ No

Identify which tissues

Annex 2.2 Field definitions for clinical presentation

The following is an attempt to classify the different modes of presentation. Since it may not be possible to accurately classify a particular case, if the case does not clearly fit one of the specified categories the code 7 "other" value should be used.

CODE 0: RAPIDLY PROGRESSIVE DEMENTIA

The majority of cases will probably be in this category. The precise presenting symptom will vary from case to case, but the picture is an encephalopathic illness with dementia (and other neurological features). It progresses rapidly over weeks to a few months, with no individual cognitive or physical deficit being present alone for more than two weeks.

CODE 1: SLOWLY PROGRESSIVE DEMENTIA

These cases present with a slowly progressive dementia, developing over months to years, without any other significant neurological features for the first six months.

CODE 2: CORTICAL BLINDNESS (HEIDENHAIN)

These cases present with impairment of visual acuity and/or field, progressing to cortical blindness, without other significant clinical deficit for the first two weeks of illness. The visual symptoms might include visual loss, visual inattention, visual illusions and visual hallucinations. It is essential that the symptoms progress to cortical blindness. Cases with other onsets that progress to include cortical blindness are NOT included in this category.

CODE 3: PSYCHIATRIC ONSET

These cases present with psychiatric symptoms such as depression, anxiety, paranoia, and delusions, without the presence of other features for a period of at least four weeks. Nonspecific malaise or apathy is not a valid psychiatric feature unless accompanied by some of the above symptoms. Similarly, visual or auditory hallucinations in isolation are not considered to fulfil the requirements of 'psychiatric onset', unless they accompany the above features.

It may be difficult to distinguish between the early features of dementia and a more specifically psychiatric onset. Behavioural change straightforwardly due to a developing dementia is not included in this category. The essential characteristics of this presentation are that patients present with a disturbance that suggests a psychiatric disturbance rather than an obvious dementia, and that specific neurological features are absent.

CODE 4: CEREBELLAR ONSET

The presentation is with a progressive cerebellar syndrome without other significant features, for at least two weeks.

CODE 5: EXTRAPYRAMIDAL ONSET

The presentation is with an extrapyramidal syndrome involving Parkinsonian features with or without chorea, athetosis or dystonia, but without other significant features for at least two weeks.

CODE 6: STROKE-LIKE ONSET

The presentation is abrupt enough for a diagnosis of stroke to be entertained in the initial stages.

CODE 7: OTHER

None of the above-described presentations is applicable.

CODE 8: MISSING

There is no clear clinical information available or the information does not allow a definite classification according to the above criteria.

ANNEX 3

Sample request forms

Annex 3.1 CSF test request form

Please provide the following information

Drawing laboratory

Laboratory/Hospital: _____

Street address: _____

City/State/Postal Code: _____

Telephone: _____ Fax: _____

CSF specimen drawn date: _____

How was specimen stored before shipping? (check one):

☐ Frozen -70 °C (recommended) ☐ Frozen -20 °C ☐ Refrigerator 4 °C

Sending laboratory

☐ Same as above, or

Laboratory/Hospital: _____

Street address: _____

City/State/Postal Code: _____

Telephone: _____ Fax: _____

CSF specimen drawn date: _____

How was specimen stored before shipping? (check one):

☐ Frozen -70 °C (recommended) ☐ Frozen -20 °C ☐ Refrigerator 4 °C

Referring physician

Name: _____

Hospital/Institution: _____

Street address: _____

City/State/Postal Code: _____

Telephone: _____ Fax: _____

E-mail: _____

Patient information

Name: ID#

Date of birth: Sex Onset of symptoms (dd/mm/yyyy) / /

Clinical symptoms and investigations	**Yes**	**No**	**Not done or known**
Dementia			
Myoclonus			
Ataxia			
EEG typical for CJD			
MRI typical for vCJD			

Other (Please provide further information, if significant)

Possible differential diagnosis			
Cerebral infarction			
Herpes encephalitis			
Other viral encephalitis			
Paraneoplastic syndrome			
Familial history of prion or other neurodegenerative disease			

Other (Please specify)

To whom should the test result should be sent?

☐ Drawing/sending laboratory

☐ Referring physician

Annex 3.2 Brain autopsy request form

Please provide the following information

Referring physician

Name:

Hospital/Institution:

Street address:

City/State/Postal Code:

Telephone: Fax:

E-mail:

Patient information

Name: ID#

Date of birth: Sex Onset of symptoms (dd/mm/yyyy) / /

Clinical symptoms and investigations	**Yes**	**No**	**Not done or known**
Dementia			
Myoclonus			
Ataxia			
EEG typical for CJD			
MRI typical for vCJD			

Other (Please provide further information, if significant)

Possible differential diagnosis			
Cerebral infarction			
Herpes encephalitis			
Other viral encephalitis			
Paraneoplastic syndrome			
Familial history of prion or other neurodegenerative disease			

Other (Please specify)

Tentative diagnosis: _____

_____

Specimen information:

☐ Frozen tissue
☐ Fixed tissue
☐ Slides / Blocks

Treated with formic acid:

☐ Yes ☐ No

For blocks/slides, please indicate specific brain regions: _____

_____

_____

Annex 3.3 Brain biopsy request form

Please provide the following information

Referring physician

Name:

Hospital/Institution:

Street address:

City/State/Postal Code:

Telephone: Fax:

E-mail:

Patient information

Name: ID#

Date of birth: Sex Onset of symptoms (dd/mm/yyyy) / /

Clinical symptoms and investigations	**Yes**	**No**	**Not done or known**
Dementia			
Myoclonus			
Ataxia			
EEG typical for CJD			
MRI typical for vCJD			

Other (Please provide further information, if significant)

Possible differential diagnosis			
Cerebral infarction			
Herpes encephalitis			
Other viral encephalitis			
Paraneoplastic syndrome			
Familial history of prion or other neurodegenerative disease			

Other (Please specify)

Tentative diagnosis: _____

_____

Specimen information:

☐ Frozen tissue
☐ Fixed tissue
☐ Slides / Blocks

Treated with formic acid:

☐ Yes ☐ No

For blocks/slides, please indicate specific brain regions: _____

_____

_____

Annex 3.4 Genetic information and consent form

Information to be provided to the patient or relatives of the patient at the time consent is being obtained for blood collection for genetic studies.

The cause of TSE in the great majority of patients is unknown.

Only a small number of cases (10–15%) have the hereditary form of the disease due to a faulty gene. The disease is not sex-linked and both men and women could have the same chance of inheriting the abnormal gene.

In nearly all the hereditary cases, the family is already aware of the disease in other family members. About half of the family members can be affected by CJD. The disease may have occurred in the preceding generation and may occur in subsequent generations. Additionally, some family members may carry the faulty gene but never develop the disease in their lifetime. In rare instances a suspected TSE case may have a family history of dementia that is of different nature (Alzheimer disease). In this case, the first-degree blood relatives do not have an increased risk of developing TSE. The chance of finding a gene mutation in a TSE patient who does not have any other affected family members is very small, probably less than 1 in 50.

Purpose

We are offering you an analysis of your PRNP gene to clarify whether your disease is caused by mutations in that gene. Your decision is voluntary. You may choose not to have the analysis of your gene performed. You may receive no benefit from the analysis. If you approve, we would like to store a small sample of your blood and your DNA for future research purposes. In this way, we hope to advance knowledge of TSEs, which may in the future lead to a better understanding and treatment of the disease.

Procedure

If you decide to allow the analysis of your gene, a medical person will take 20 ml (a little more than a tablespoon) of your blood from a vein in your arm. A portion of your blood (10 ml) will be sent to a reference laboratory for the DNA extraction and PRNP gene analysis. A small volume (10 ml) of your blood will be frozen for future research.

Future uses of sample

If you do not wish samples of your blood or DNA to be used for future research, you have to indicate this below:

☐ I approve use of the sample of my blood and DNA for future research

☐ I do not approve use of the sample of my blood and DNA for future research

Confidentiality

Benefits and risks

The only risks to you are those of blood collection, which include slight discomfort, the possibility of bruising, fainting and possible infection at the site of collection.

Alternative procedure

There is no alternative procedure for obtaining a sufficient amount of blood for this research.

Compensation and cost

You will not be paid for your blood sample and there will be no charge to you for the shipping or testing of your blood.

Subject's rights

Your decision whether or not to take part in this study is voluntary. It will not change your future relationships with …… or affect your medical care or treatment.

Contact person

If you have any questions, please ask your physician now. If you have additional questions you may call ……

Consent authorization

My signature indicates that I have read the above explanation of this research study about TSE diseases. I have been given the opportunity to ask questions and my questions have been answered. The potential risks and benefits have been explained to me. Based on this information, I agree to have my blood drawn for the analysis of my gene and to have a portion of my blood and DNA stored for future studies of TSEs.

Printed or typed name _____

Signature _____ Date _____

Addendum

Since some neurodegenerative diseases cause dementia, the subjects themselves may not be able to provide informed consent. In that event, the legal guardian, next of kin or individual with health care power of attorney for the subject can provide the consent and any other necessary information.

Printed name of legal guardian _____

Signature _____ Date _____

Printed name of person administering consent _____

Signature _____ Date _____

Use of translator

Fill in this section if the subject is not fluent in English and a translator was used to obtain consent:

Print name of translator: _____ Date: _____

Signature of translator: _____ Date: _____

An oral translation of this document was administered to the subject in ..... (state language) by an individual proficient in English and ..... (state language).

ANNEX 4

Tissue handling and safety precautions

Annex 4.1 Decontamination methods for transmissible spongiform encephalopathies

The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments and other material is to discard and destroy them by incineration. In some health care situations, as described in Chapter 6, one of the following less effective methods may be preferred. Wherever possible, instruments and other materials subject to reuse should be kept moist between the time of exposure to infectious materials and subsequent decontamination and cleaning. If it can be done safely, removal of adherent particles through mechanical cleaning will enhance the decontamination process.

The following recommendations are based on the best available evidence at the time of the Consultation and are listed in order of more or less severe treatments. These recommendations may require revision if new data become available.

Incineration

1. Use for all disposable instruments, materials, and wastes.
2. Preferred method for all instruments exposed to high-infectivity tissues.

Autoclave/chemical methods for heat-resistant instruments

1. Immerse in sodium hydroxide (NaOH)[1] and heat in a gravity displacement autoclave at 121 °C for 30 minutes; clean, rinse in water and subject to routine sterilization.
2. Immerse in NaOH or sodium hypochlorite[2] for 1 hour; transfer instruments to water; heat in a gravity displacement autoclave at 121 °C for 1 hour; clean and subject to routine sterilization.
3. Immerse in NaOH or sodium hypochlorite for 1 hour; remove and rinse in water, then transfer to an open pan and heat in a gravity displacement (121 °C) or porous load (134 °C) autoclave for 1 hour; clean and subject to routine sterilization.
4. Immerse in NaOH and boil for 10 minutes at atmospheric pressure; clean, rinse in water and subject to routine sterilization.
5. Immerse in sodium hypochlorite (preferred) or NaOH (alternative) at ambient temperature for 1 hour; clean; rinse in water and subject to routine sterilization.
6. Autoclave at 134 °C for 18 minutes.[3]

Chemical methods for surfaces and heat sensitive instruments

1. Flood with 2N NaOH[4] or undiluted sodium hypochlorite; let stand for 1 hour; mop up and rinse with water.
2. Where surfaces cannot tolerate NaOH or hypochlorite, thorough cleaning will remove most infectivity by dilution and some additional benefit may be derived from the use of one or other of the partially effective methods listed in Figure 6.7.

Autoclave/chemical methods for dry goods

1. Small dry goods that can withstand either NaOH or sodium hypochlorite should first be immersed in one or the other solution (as described above) and then heated in a porous load autoclave at ≥121 °C for 1 hour.
2. Bulky dry goods or dry goods of any size that cannot withstand exposure to NaOH or sodium hypochlorite should be heated in a porous load autoclave at 134 °C for 1 hour.

Notes about autoclaving and chemicals

Gravity displacement autoclaves. Air is displaced by steam through a port in the bottom of the chamber. Gravity displacement autoclaves are designed for

[1] Unless otherwise noted, the recommended concentration is 1N NaOH. Since the publication of this recommendation in 1999 (WHO/CDC/CSR/APH/2000.3), correspondents have described spills, corrosion and, in one case, an explosion in the autoclave when this technique was attempted.

[2] Unless otherwise noted, the recommended concentration is 20 000 ppm available chlorine.

[3] In worse-case scenarios (brain tissue bake-dried on to surfaces) infectivity will be largely but not completely removed.

[4] For NaOH solution, 2N = 2 mol/litre.

general decontamination and sterilization of solutions and instruments.

Porous load autoclaves. Air is exhausted by vacuum and replaced by steam. Porous load autoclaves are optimized for sterilization of clean instruments, gowns, drapes, towelling, and other dry materials required for surgery. They are not suitable for liquid sterilization.

Sodium hydroxide (NaOH, or soda lye). Be familiar with and observe safety guidelines for working with NaOH. 1N NaOH is a solution of 40 g NaOH in one litre of water. 1N NaOH[1] readily reacts with CO_2 in air to form carbonates that neutralize NaOH and diminish its disinfective properties. 10 N NaOH solutions do not absorb CO_2, therefore, 1N NaOH working solutions should be prepared fresh for each use either from solid NaOH pellets, or by dilution of 10 N NaOH stock solutions.

Sodium hypochlorite (NaOCl solution, or bleach). Be familiar with and observe safety guidelines for working with sodium hypochlorite. Household or industrial-strength bleach is sold at different concentrations in different countries, so that a standard dilution cannot be specified. Efficacy depends upon the concentration of available chlorine and should be 20 000 ppm available chlorine. One common commercial formulation is 5.25% bleach, which contains 25 000 ppm chlorine. Therefore, undiluted commercial bleach can be safely used. If solid precursors of hypochloric acid are available, then stock solution and working solutions can be prepared fresh for each use.

Cautions regarding hazardous materials

In all cases, hazardous materials guidelines must be consulted.

Personnel

NaOH is caustic but relatively slow-acting at room temperature, and can be removed from skin or clothing by thorough rinsing with water. Hot NaOH is aggressively caustic and should not be handled until cool. The hazard posed by hot NaOH explains the need to limit boiling to ten minutes, the shortest time known to be effective.

Hypochlorite solutions continuously evolve chlorine and so must be kept tightly sealed and away from light. The amount of chlorine released during inactivation may be sufficient to create a potential respiratory hazard unless the process is carried out in a well-ventilated or isolated location.

[1] For NaOH solution, 1N = 1 mol/litre.

Material

In principle, NaOH does not corrode stainless steel. However, in practice some formulations of stainless steel can be damaged (including some used for surgical instruments). It is advisable to test a sample or consult with the manufacturer before dedicating a large number of instruments to decontamination procedures. NaOH is known to be corrosive to glass and aluminum. Hypochlorite does not corrode glass or aluminum. It has also been shown to be an effective sterilizing agent. It is, however, corrosive to both stainless steel and autoclaves and (unlike NaOH) cannot be used as an instrument bath in the autoclave. If hypochlorite is used to clean or soak an instrument, it must be completely rinsed from the surfaces before autoclaving. Other decontamination methods may need testing, or consultation with the manufacturer, to verify their effect on the instrument.

Annex 4.2 Management of healthy 'at risk' individuals

Tissue recipients

The risk from *recipients of dura mater, cornea transplants* and *human pituitary hormones*, and from *persons who have undergone neurosurgical procedures*, is no longer considered sufficient to warrant classifying this population as a risk for transmitting TSEs, except under conditions where there could be exposure to their high-infectivity tissues. Appropriate control measures have immensely reduced or eliminated exposure to contaminated dura mater and pituitary hormones, and only three cases of TSE transmission through cornea transplantation and six (all before 1980) of transmission via neurosurgical instruments have been reported. In addition, recipients of dura mater are largely unaware of the fact, making identification of many of the dura mater recipients unlikely.

Countries not applying appropriate control measures cannot assume similarly low levels of current risk among tissue recipients.

Familial transmissible spongiform encephalopathies

At the time of the WHO Consultation on Infection Control Guidelines for TSEs, no consensus was reached on whether asymptomatic persons at risk for *familial TSE* should be classified as 'at risk' when determining appropriate infection control levels. It was argued that the identification of familial risk among asymptomatic people would confer a lifetime

requirement for high-level infection control for a transmission risk that remains only hypothetical. Discrimination against such persons and legal implications regarding their access to insurance, employment and health care were described by several participants in the Consultation. It was proposed that such discrimination would inevitably lead to a harm that exceeded any evidence of risk posed by them to others.

Others argued that if a familial risk were identified, more stringent levels of infection control could be adopted, even in the absence of firm evidence of risk, particularly during procedures involving *high-infectivity tissues*. All consultants agreed that persons 'at risk' for familial TSE should not be denied access to treatment or surgical procedures, particularly given the range of decontamination options available. Scientific resolution of these issues was impossible because of a lack of precise information about tissue infectivity during the preclinical phase of human disease. The consultants emphasized the need to study any available tissues (including blood) from mutation-positive, but still asymptomatic, members of TSE families.

Annex 4.3 Management of individuals with confirmed or suspected vCJD

Since PrP is detected in a range of lymphoreticular tissues in vCJD, patients with vCJD might pose a greater risk of transmitting iatrogenic infections than sporadic CJD. However, this hypothetical risk has to be balanced against the real danger of stigmatizing patients and causing distress and anxiety to the patient's relatives by the introduction of rigorous and possibly unnecessary infection control procedures in general patient care.

On current evidence, the infection control procedures in nursing care settings for sporadic CJD may be applied to cases of vCJD, without the need for additional precautions. A more conservative approach may be taken for interventions involving surgical procedures, or when handling tissues and body fluids in the laboratory. As stated in Figure 6.8, measures that have been recommended for high-infectivity tissues in patients with other forms of TSE, could be applied to all tissues of persons with vCJD. It is noted that considerable safety is afforded through the measures described above and in Annex 4.1, and that no person should be denied a diagnostic test given the efficacy of the recommended measures.

ANNEX 5

Information resources

WHO associated laboratories and diagnostic reference centres

The following centres have kindly agreed to perform tests on suspected human TSEs cases if required. Please contact the named person for more information before sending samples.

Clinical evaluation or any diagnostic test

Professor John Collinge, MRC Prion Unit, The National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, England. Tel: +44 0207 837 4888, Fax: +44 0207 837 8047. E-mail: j.collinge@prion.ucl.ac.uk

Dr Richard Knight, National Creutzfeldt-Jakob Disease Surveillance Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, Scotland. Tel: +44 131 537 3108/332 2117. E-mail: r.knight@ed.ac.uk

Professor Colin Masters, Department of Pathology, University of Melbourne, Parkville, Victoria 3010, Australia. Tel: +613 8344 5867. Fax: +613 8344 4004. E-mail: c. masters@pathology.unimelb. edu.au

Professor Robert G. Will, National Creutzfeldt-Jakob Disease Surveillance Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, Scotland. Tel: +44 131 537 2128. Fax: +44 0131 343 1404. E-mail: r.g.will@ed.ac.uk

MRI evaluation

Dr Donald Collie, Consultant Neuroradiologist, UK National CJD Surveillance Unit, Bryan Matthews Building, Western General Hospital, Crewe Road South Edinburgh EH4 2XU, Scotland. Tel: +44 131 537 2475 (direct), +44 131 537 3093 (secretary). Fax: +44 131 537 3083. E-mail: dac@skull.dcn.ed.ac.uk

Pathology and western blot

Professor Herbert Budka, Institute of Neurology, University of Vienna, AKH 4J, POB 48, A-1097 Viena, Austria. Tel: +43 140400 5500. Fax: +43 140400-5511. E-mail: h.budka@akh-wien.ac.at

Professor Pierluigi Gambetti, Institute of Pathology, Case Western Reserve University School of Medicine, 10900 Euclid Ave., Cleveland, OH 44106, USA. E-mail: drn3@po.cwru.edu

Professor James Ironside, National CJD Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland. Tel: +44 131 537 1980. Fax: +44 131 537 3056. E-mail: j.w.ironside@ed.ac.uk

Professor Nicolas Kopp, Neuropathologie, Hôpital Neurologique, 59 Boul Pinel, 69003 Lyon, France. Tel: +33 472357630. Fax: +33 472357067. E-mail: nicolas.kopp@chu-lyon.fr

Professor Ana Lia Taratuto, Head, Department of Neuropathology, Institute for Neurological Research FLENI, Montanese 2325, 1428, Buenos Aires, Argentina. Tel: +54 (11) 4 788 3444 Fax: +54 (11) 4 784 7620. E-mail: ataratuto@fleni.org.ar

14-3-3 in CSF

Dr Alison Green, The National CJD Surveillance Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Scotland. Tel: +44 131 537 3075. Fax: +44 131 537 3056. E-mail: Alison.Green@ed.ac.uk

Professor Inga Zerr, Prionforschungsgruppe, Neurologische Klinik und Poliklinik, POB 37 42, Robert-Koch-Strs. 40, 370 75 Gottingen, Germany. Tel: +49 551396636. Fax: +49 551397020. E-mail: IngaZerr@aol.com

WHO

This document, the *WHO Manual for Surveillance of Human Transmissible Spongiform Encephalopathies including variant Creutzfeldt-Jakob disease*, will be posted on the WHO web site www.who.int/emc/diseases/bse/index.html. Copies may be ordered through the WHO Information Resource Centre (cdsdoc@who.int).

Other recent WHO publications on BSE and vCJD can be accessed electronically at http://www.who.int/health-topics/tse.htm, including:

- Report of a WHO consultation on public health issues related to human and animal transmissible spongiform encephalopathies. WHO/CDS/VPH/95.145, 1995
- Report of a WHO consultation on public health issues related to human and animal transmissible spongiform encephalopathies. WHO/EMC/DIS/96.147, 1996
- Report of a WHO Consultation on clinical and neuropathological characteristics of the new variant of CJD and other human and animal TSEs. WHO/EMC/ZOO/96.1, 1996
- Report of a WHO consultation on medicinal and other products in relation to human and animal transmissible spongiform encephalopathies. WHO/EMC/ZOO/97.3, 1997
- Report of a WHO consultation on the global surveillance, diagnosis and therapy of human transmissible spongiform encephalopathies. WHO/EMC/ZDI/98.9, 1998
- Report of a WHO Consultation on public health and animal TSEs: Epidemiology, risk and research requirements. WHO/CDS/CSR/APH/2000.2, 1999
- WHO Infection Control Guidelines for TSEs: Report of a WHO Consultation. WHO/CDS/CSR/APH/200.3, 1999
- vCJD Update on the hazard of transmission by blood and blood products. *Weekly Epidemiological Record*. Vol. 49 p. 390, 14 December 2001
- Report of a WHO Consultation: The revision of the surveillance case definition for variant CJD. WHO/CDS/CSR/EPH/2001.5, 2001
- Proceedings of the Joint WHO/FAO/OIE Technical Consultation on BSE: Public health, animal health and trade. 2001
- Unerstanding the BSE threat. WHO/CDS/CSR/EPH/2002.6.

Reports and minutes from the WHO Working Group on International Reference Materials for the Diagnosis and Study of TSEs is available at http://www.who.int/biologicals/B1

The home page for the Department of Communicable Disease Surveillance and Response is at http://www.who.int/emc/index.html, and inquiries can be posted from this site or sent directly to vCJDBSE@who.int

The home page for the Department of Food Safety is at http://www.who.int/fsf/

Web sites of interest

WHO Regional Office for Europe http://www.who.it/HT/bse.htm

EuroCJD and NeuroCJD surveillance networks http://www.eurocjd.ed.ac.uk

United Kingdom National CJD Surveillance Unit http://www.cjd. ed.ac.uk/

French National CJD Surveillance System http://www.invs.sante.fr/

United States Centers for Disease Control and Prevention http://www. cdc. gov/ncidod/diseases/cjd/cjd_inf_ctrl_qa. htm

United Kingdom Spongiform Encephalopathy Advisory Committee http://www. doh. gov. uk/seac. htm

Australia Department of Health and Aging. Infection Control Guidelines: for the prevention of transmission of infectious diseases in the health care setting http://www. health. gov. au/pubhlth/strateg/communic/review/

Advisory Committee on Dangerous Pathogens, Spongiform Encephalopathy Advisory Committee Guidelines on the Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection http://www.archive.official-documents.co.uk/document/doh/spongifm/report. htm

Health Canada Creutzfeldt-Jakob disease website: http://www.hc-sc.gc.ca/pphb-dgspsp/hcai-iamss/cjd-mcj/index.html

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Dr Andrew Cunningham
Dr Thomas Esmonde
Dr Michael Finkenstaedt
Dr Jim Fraser
Dr Alison Green
Dr Lev Goldfarb
Dr William Hadlow
The family of Mr Peter Hall
Dr James Ironside
Dr Richard Knight
Miss Jan MacKenzie
Dr Margaret Ann MacLeod
Dr Colin Masters
Radiology Society of North America
Dr Bernard Steinhof
Dr Gillian Stewart
Professor Bob Will
Professor Beth Williams
Dr Inga Zerr
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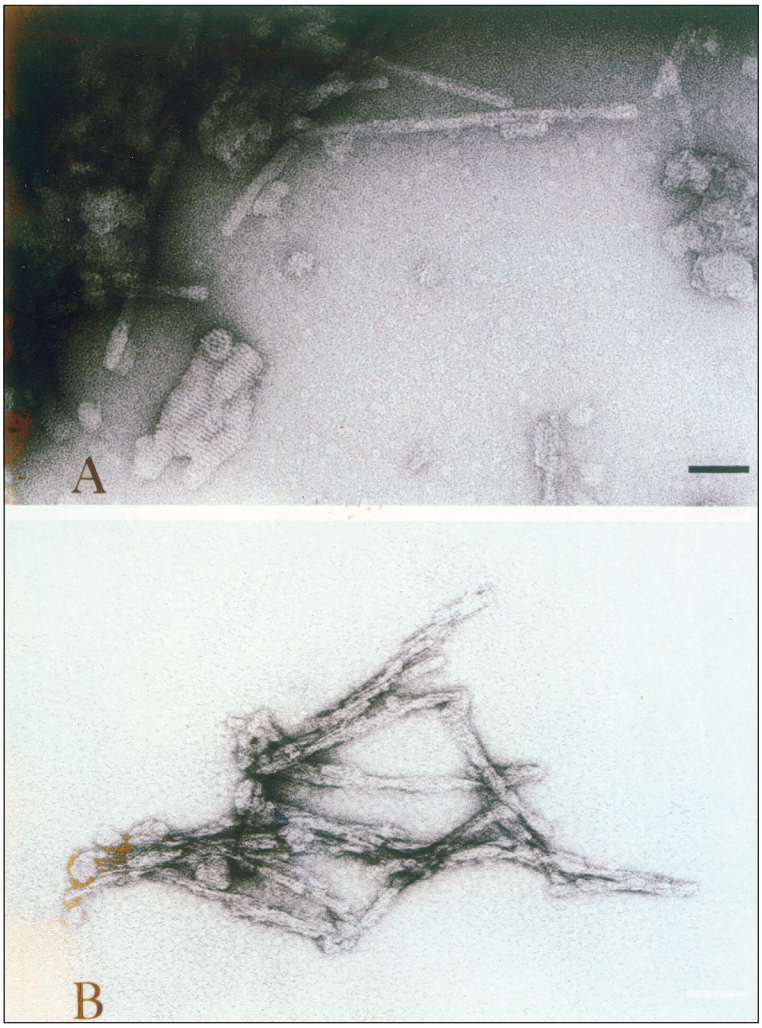

Figure 3.1 Scrapie-associated fibrils. (Courtesy of Dr. C. J. Gibbs)

Figure 4.2 Kuru

Figure 4.3 Kuru

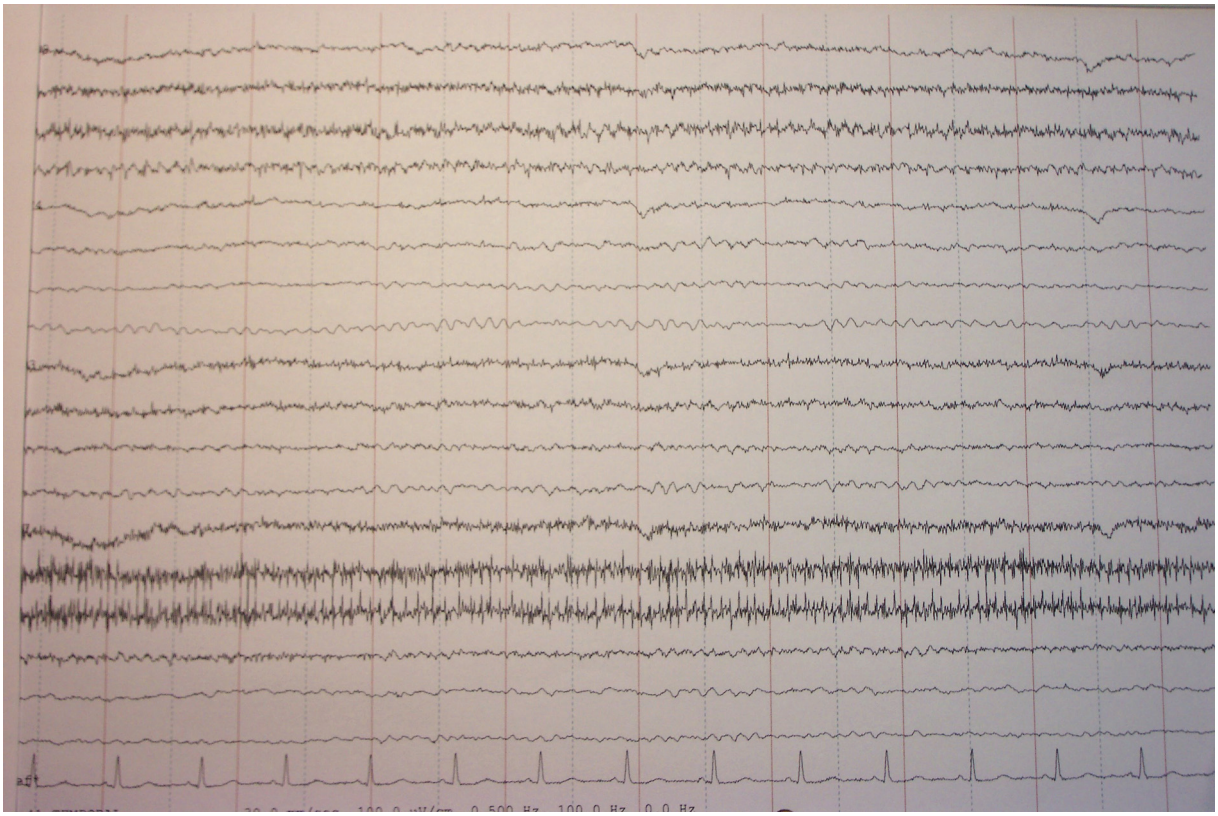

Figure 9.4 This EEG recording shows only some minor non-specfic irregularities. It was taken during the early stages of illness in a case of CJD.

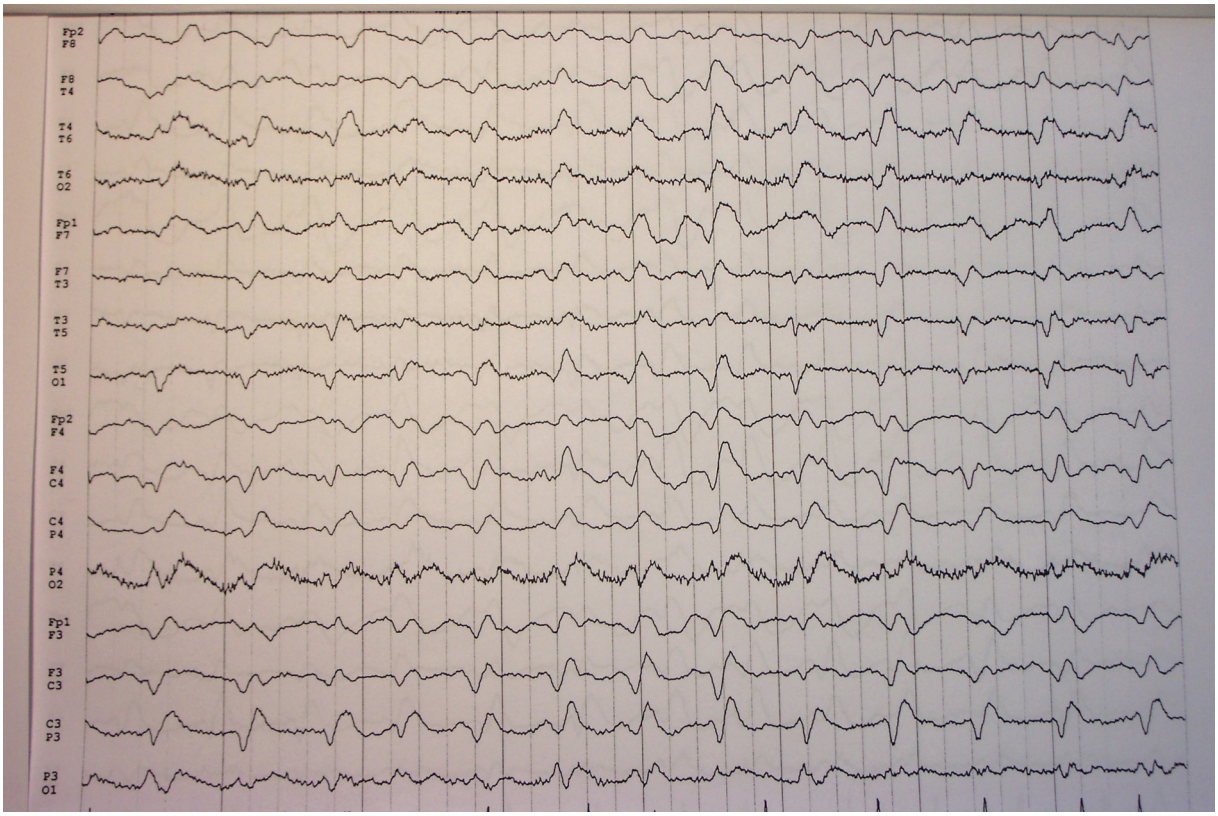

Figure 9.5 This EEG recording shows the typical generalized periodic discharges in a case of sporadic CJD. This finding allows a case otherwise classified as 'Possible sCJD' to be classified as 'Probable sCJD'.

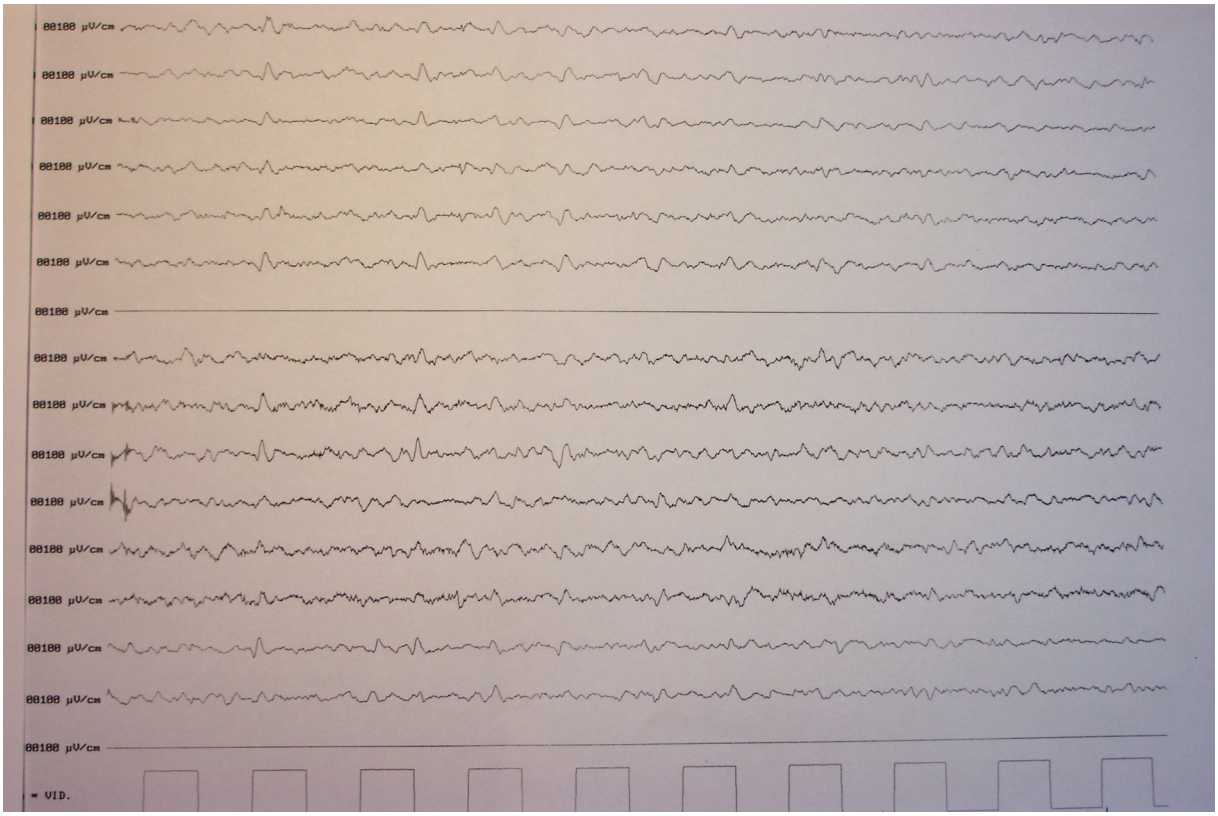

Figure 9.6 This EEG recording shows changes which are at least 'suggestive' of sporadic CJD. However, an EEG of this sort should not be used to reclassify a 'Possible sCJD' case as 'Probable sCJD'. If possible, further EEGs should be undertaken to see if the changes progress to the typical findings as seen in Figure 9.5.

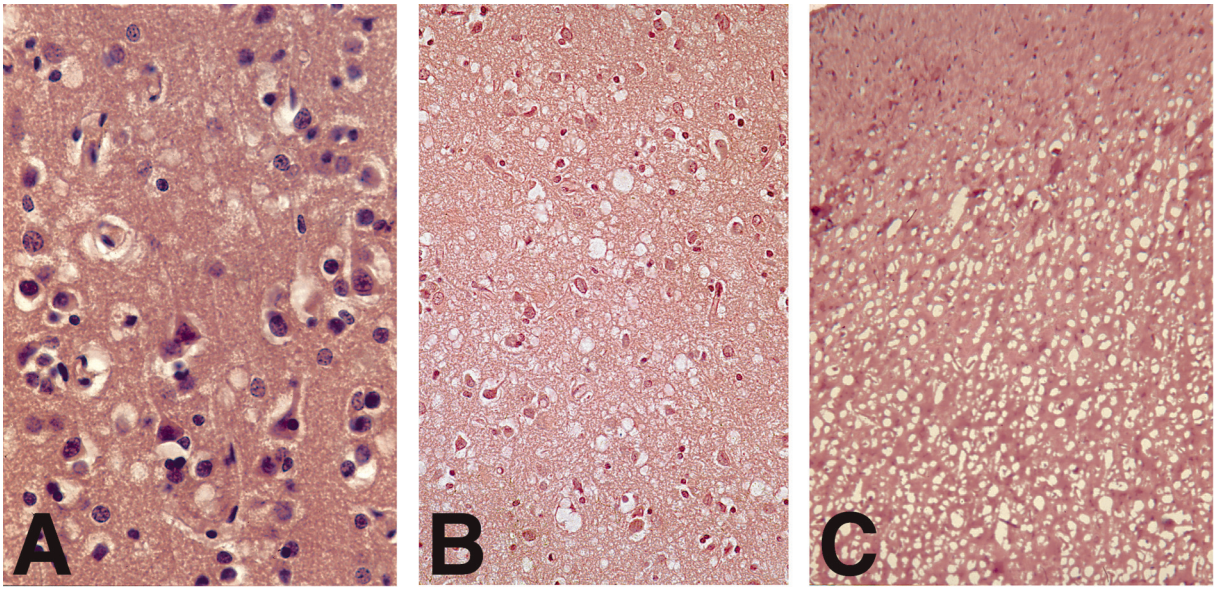

Figure 9.15 Examples of spongiform change in the cerebral cortex. Mild (A) moderate (B) and very severe (C,D) Haemotoxylin and eosin (H&E) stain.

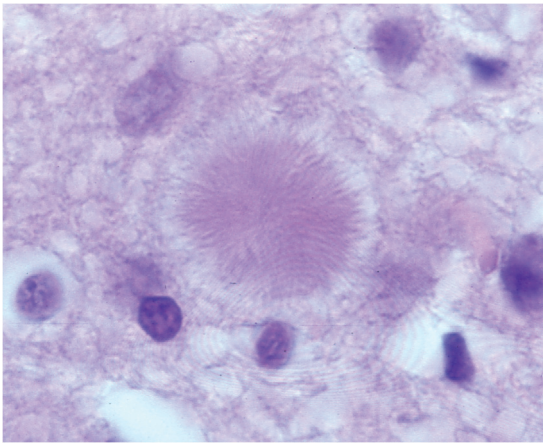

Figure 9.16 Kuru type plaque. Haemotoxylin and eosin (H&E) stain.

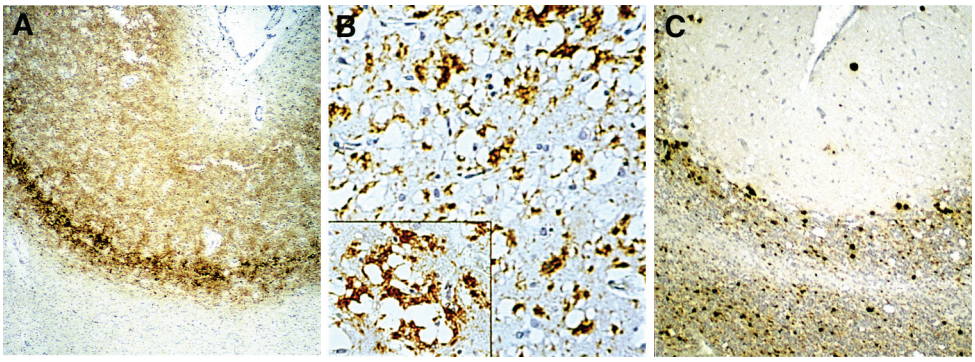

Figure 9.18 Main patterns of prion protein (PrP) deposition in CJD: synaptic (A), patchy/perivacuolar (B), and plaque-like (C) in the cerebral (A,B) and cerebellar (C) cortex. Immunocytochemistry for PrP.

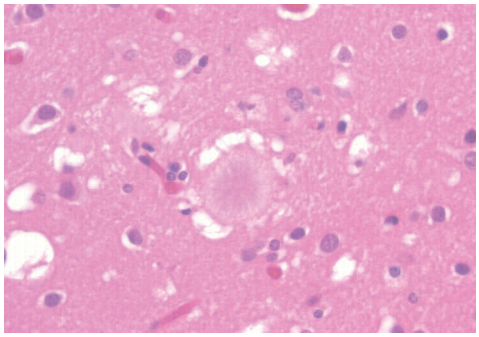

Figure 9.19 The brain in vCJD contains florid plaques (centre), which are large fibrillary aggregates of abnormal PrP surrounded by a halo of spongiform change. These lesions are widespread in the cerebral cortex and cerebellum. Haematoxylin and eosin stain (H&E), original magnification x 200

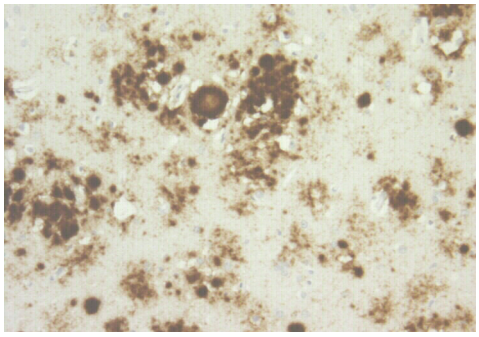

Figure 9.20 Immunocytochemistry for PrP in the cerebral cortex in vCJD shows strong staining of the large plaques, but also reveals numerous smaller plaques and amorphous PrP deposits that are not visible on routinely stained sections. KG9 anti-PrP antibody, x150

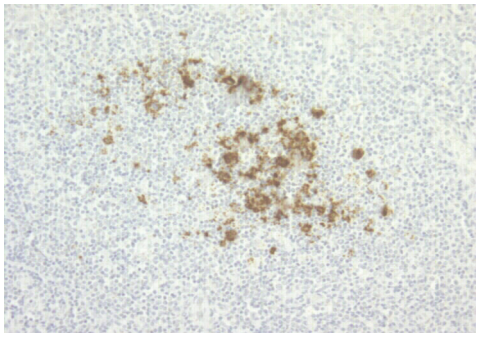

Figure 9.21 PrP immunocytochemistry in the tonsil in variant CJD shows strong staining of follicular dendritic cells within a germinal centre. This finding appears to be specific for vCJD and infectivity has been detected in the tonsil and other lymphoid tissues on bioassay in mice. KG9 anti-PrP antibody, x 200

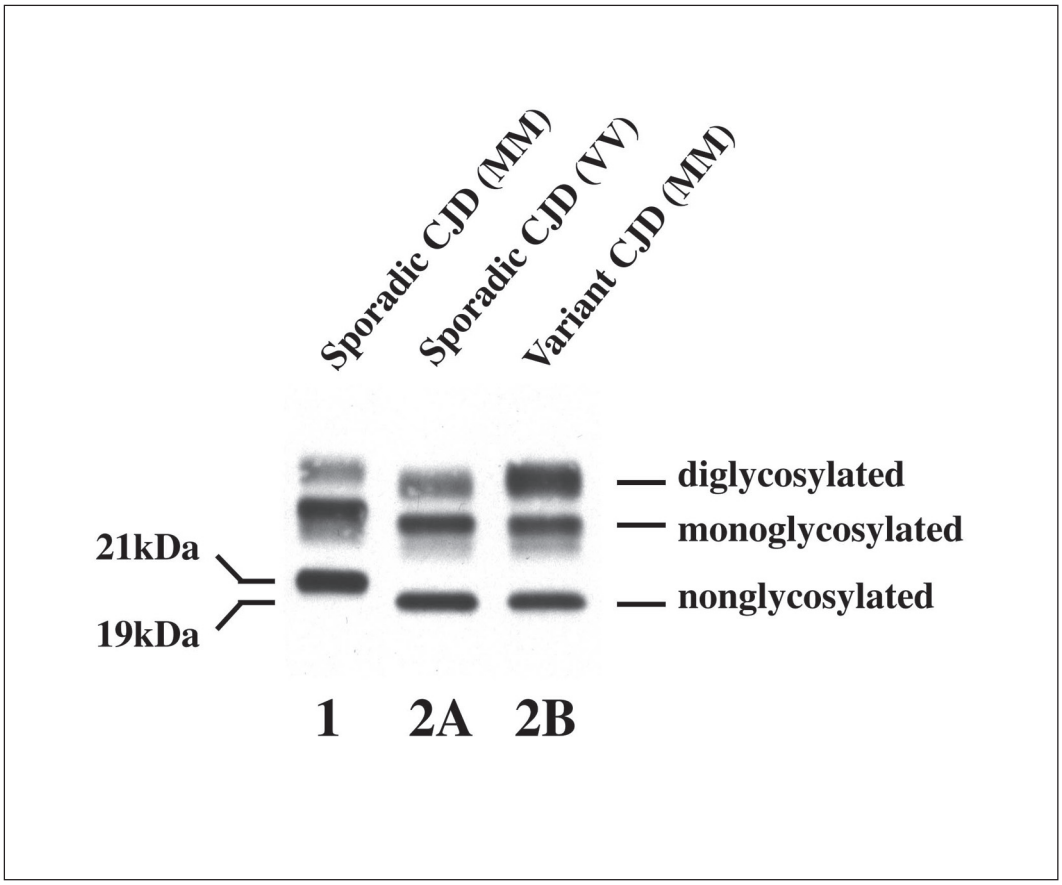

Figure 9.23 Western blot analysis of protease-resistant PrP (PrP^{res}) from post-mortem Creutzfeldt-Jakob disease brain. Specimens of frontal cortex were homogenized, the lysates cleared and digested with proteinase K at 50µg/ml for one hour at 37 ℃. Samples were separated by SDS-PAGE on 12% acrylamide gels, transferred to PVDF membranes and detected using the monoclonal antibodies 3F4 (Dako) or 6H4 (Prionics). The two most commonly occurring forms of sporadic CJD in patients homozygous for methionine (MM) or valine (VV) at codon 129 of the *PRNP* gene are contrasted with that found in a patient with variant CJD (MM at codon 129 of *PRNP*). In sporadic CJD the nonglycosylated (lowest) band has either a relative mobility of 21kDa (termed type 1) or 19kDa (termed type 2). Cases of variant CJD have a type 2 mobility but they are characterized by an over representation of the top (diglycosylated) band. This isotype is termed type 2B to distinguish it from cases of sporadic CJD with the same mobility but a predominance of the monoglycosylated (middle) or nonglycosylated (bottom) band which are referred to as type 2A. The nomenclature used is that described by Parchi et al. *Nature* 1997;386:232–233.