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Title

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Abstract

Prion diseases (or transmissible spongiform encephalopathies; TSE) are a unique group of fatal progressive neurodegenerative diseases of the central nervous system. The infectious agent is hypothesised to consist solely of a highly protease-resistant misfolded isoform of the host prion protein. Prions display a remarkable degree of resistance to chemical and physical decontamination. Many common forms of decontamination or neutralization used in infection control are ineffective against prions, except chaotropic agents that specifically disrupt proteins. Human cadaveric prosection or dissection for the purposes of teaching and demonstration of human anatomy has a distinguished history and remains one of the fundamentals of medical education. Iatrogenic transmission of human prion diseases has been demonstrated from the inoculation or implantation of human tissues. Therefore, although the incidence of human prion diseases is rare, restrictions exist upon the use of tissues from patients reported with dementia, specifically the brain and other central nervous system material. A current concern is the potential for asymptomatic variant Creutzfeldt-Jakob Disease (vCJD) transmission within the UK population. Therefore, despite the preventative measures, the transmission of prion disease through human tissues remains a potential risk to those working with these materials. In this review we aim to summarise the current knowledge on human prion disease relevant to those working with human tissues in the context of anatomical dissection.

Introduction

Prion diseases or transmissible spongiform encephalopathies (TSE) are a group of progressive neurodegenerative disorders with relatively short symptomatic clinical periods when compared to their usually characteristically long asymptomatic incubation periods. While determination of the asymptomatic phase in human prion disease cases can be difficult or impossible to establish, the commencement of clinical symptoms typically precedes death within months to years. Prion diseases have a range of human and animal species as their natural hosts and display limited zoonotic potential. Therefore much of what we know about these diseases has been derived from the study of animal models. Though not all prion diseases have been proven to be transmissible, many of them; including sheep scrapie, bovine spongiform encephalopathy (BSE) and most human prion diseases, have been transmitted to laboratory rodents. From these studies undertaken within controlled laboratory conditions, such concepts as relative infectivity, infectious dose and prion disease incubation period have been determined and subsequently speculated upon for human prion diseases.

Prion diseases are diagnosed clinically according to internationally agreed defined criteria, and pathologically by neuronal loss and spongiform degeneration in the neuropil, reactive glial responses, and deposition of the disease-associated isoform of the prion protein (PrP) primarily within the central nervous system (CNS) (Figure 1). In variant Creutzfeldt-Jakob disease (vCJD) disease-associated PrP deposition also occurs in specific tissues throughout the body, including autonomic and sensory ganglia within the peripheral nervous system and in lymphoid tissues (Figure 1). A fundamental diagnostic marker of these diseases is detection of the post-translational conversion, or refolding, of the labile normal host cellular PrP (PrP^{C}) into the relatively stable, protease-resistant disease-specific isoform

(PrP^{d}), more commonly referred to as PrP^{Sc}, named after scrapie the prototypic prion disease observed in sheep). The prion infectious agent and PrP^{d} are equivalent according to the prion hypothesis (Prusiner, 1982), which proposed that the prion infectious agent may consist solely of abnormally folded host protein. While this hypothesis is still to be proven, it is clear that prions are unique infectious agents which are incredibly resilient to many standard forms of disinfection or decontamination (Brown et al., 1982). As such they may persist within their host after death and within the environment following contamination (Georgsson et al., 2006) and therefore human prion diseases represent a potential risk for prion transmission during anatomical dissection (Figure 2).

Human prion diseases have collectively been identified and described over the previous 100 years and can be classified according to numerous criteria (Table 1). In the 1920's the German neuropathologists Hans Gerhard Creutzfeldt and Alfons Maria Jakob separately identified and described groups of patients with progressive neurodegeneration, lending the name Creutzfeldt-Jakob disease (CJD) to the conditions observed (Creutzfeldt, 1920; Jakob, 1921a, 1921b). In 1936 Josef Gerstmann, Ernst Sträussler and Ilya Scheinker described an inherited form of spinocerebellar ataxia with a distinct neuropathological phenotype that did not appear to be related to CJD, termed Gerstmann-Sträussler-Scheinker (GSS) disease (Gerstmann et al., 1936). Kuru was first described in the 1950's following observation of the Fore people of New Guinea by Australian administrators and anthropologists and was subsequently investigated by Drs Zigas and Gajdusek (Gajdusek and Zigas, 1959). More recently the genetic prion disease fatal familial insomnia was described in the mid 1980's (Lugaresi et al., 1986) and a zoonotic disease, now known as variant CJD (vCJD) identified in the UK in the mid 1990's (Will et al., 1996). In 2008 a novel prion

disease now referred to as variably protease sensitive prionopathy was also described (Gambetti et al., 2008).

In this review we discuss prion diseases grouped by causation as these may have a strong determinant effect upon the tissue distributions of the infectious agent at the end stage of disease.

Causation of Human Prion Diseases

Idiopathic – Idiopathic, or more commonly termed, sporadic prion diseases arise from an unknown cause and account for around 80-85 % of all human prion disease cases. These are explainable within the prion hypothesis either as spontaneous somatic mutations within the *PRNP* gene (which encodes PrP), or stochastic PrP misfolding events or other epigenetic phenomenon which arise due to currently unidentifiable conditions. Sporadic human prion disease are classified according to their clinical and neuropathological features into two groups: sporadic CJD (sCJD) or sporadic fatal insomnia (sFI). In these sporadic diseases infectious prions are largely confined to CNS tissues (Glatzel et al., 2003; Head et al., 2004; Wadsworth et al., 2001), suggestive of a spontaneous internal source of infection. sCJD is the most common of the human prion disease and may account for up to 80% of all cases. Unlike other neurodegenerative conditions, sCJD presents with extensive heterogeneity and has been classified and sub-classified as our knowledge and analysis of the clinical presentation, genetic variation, neuropathology and PrP biochemistry have advanced (Gambetti et al., 2003; Parchi et al., 1999; Parchi et al., 2009). Although the age range of sCJD is very broad (between 16 and 90 years old) the median age at death is around 60-65 years of age and many studies report increased incidences at ages >60 years (Zerr and Poser, 2004). Sporadic fatal insomnia is rare, representing around 2% of all sporadic prion disease cases and has an age range of 36-72 years, mean age of onset is 52 years and a mean duration of 24 months (Gambetti et al., 2003).

Genetic – Genetic prion disease is the second most frequent form of human prion disease, accounting for around 10-15 % of cases. While the familial prion diseases have 3 main phenotypes: familial (f)CJD, GSS and FFI, some rare variants show different

phenotypes such as prion protein cerebral amyloid angiopathy (PrP-CAA) (Ghetti et al., 1996). Disease incidence is rare in terms of the general population, but since genetic human prion diseases are inherited as autosomal dominant disorders, the frequency of carriers of the causative mutations in the prion protein gene (*PRNP*) may be extremely high in affected families. Phenotypic variation occurs in genetic human diseases, in particular the age at disease onset is variable and not all carriers of *PRNP* mutations develop symptoms of genetic prion disease, particularly if they succumb to an unrelated disorder before prion disease onset. A major influence on the phenotypic variability in human prion diseases is the effect of the polymorphism at codon 129 in the *PRNP* gene on the mutant allele, which can encode either methionine or valine. One example of this is the disease FFI, which has an age range of 25-72 years old with a mean age of onset of 51 years of age (Yu et al., 2007). FFI is caused by the D178N mutation when in association with methionine at *PRNP* codon 129 on the mutant allele. Carriers of the D178N mutation with valine at the *PRNP* codon 129 on the mutant allele were originally described as having fCJD on the basis of their clinical and pathological features, which resembled those of sporadic CJD, although phenotypic variability occurs within affected families (Zarranz et al., 2005). The mean disease duration is significantly shorter (12±4 months) in individuals homozygous for the disease causing mutation, than in heterozygous individuals (21±15 months) (Padovani et al., 1998) suggesting a gene dosage effect.

Acquired Zoonotic – Prion disease amongst species other than humans have been well documented and many have been shown to be contagious and can rapidly form epidemics (Detwiler and Baylis, 2003; Ducrot et al., 2008; Miller and Williams, 2004). Historically the oldest and most-studied of these is the disease scrapie in ruminants such as

sheep and goats (McGowan, 1922). Within the Scottish sheep population scrapie was effectively accidentally disseminated by a vaccination programme against louping-ill virus, using a vaccine formulated from tissues including sheep CNS and spleen. This vaccine was deduced later to have contained the causative agent of scrapie, further confounded by the ability of scrapie to become contagious within affected flocks by environmental (Gough and Maddison, 2010; Maddison et al., 2010), horizontal (Evoniuk et al., 2005) and vertical transmission (Foster et al., 2013; Hoinville et al., 2010). Scrapie is considered to represent negligible risk to the human population as it appears not to be infectious to humans (Barria et al., 2014). In North America, both the native and farmed deer and elk populations are currently suffering the natural rapid spread of chronic wasting disease (CWD). Whether CWD has the potential to be infectious to humans is uncertain and is an important current concern (Belay et al., 2004).

Bovine spongiform encephalopathy (BSE) is a prion disease first identified in 1985 in the UK cattle population (Bruce et al., 1997; Hope et al., 1988). BSE reached epidemic proportions within the UK cattle population in the 1980-1990s, resulting in significant contamination of the UK food chain with BSE prions and exposure to the human population via the consumption of BSE contaminated meat products. Following the emergence of BSE, vCJD was first reported in the UK in the 1996, subsequently accounting for around 5% or less of total human prion disease cases in the UK (www.cjd.ed.ac.uk). The evidence from transmission studies to laboratory mice and PrP biochemical analysis is consistent with the hypothesis that vCJD represents BSE infection in humans (Bruce et al., 1997; Collinge et al., 1996). The main distinguishing features of vCJD as opposed to sCJD are the clinical presentation, a young age at clinical onset (mean age of onset = 28 years for vCJD vs. 68

years for sCJD) and the speed at which the disease progresses from clinical symptoms to death (13-14 months for vCJD vs. 4-5 months for sCJD) (Belay and Schonberger, 2002). In keeping with an oral route of transmission, gut-associated lymphoid tissues, the lymphoreticular system and a wide variety of other tissues throughout the body of vCJD infected individuals can reveal appreciable levels of infectious agent (Bruce et al., 2001; Head et al., 2004; Joiner et al., 2005; Notari et al., 2010; Wadsworth et al., 2007; Wadsworth et al., 2001), see also Figure 2 and Table 4.

Acquired – Iatrogenic Iatrogenic CJD is caused by the accidental transmission of prions from person to person during medical or surgical procedures. Several routes of transmission have been implicated such as a corneal transplant from a donor with sCJD (Duffy et al., 1974), or human dura mater grafts from a contaminated commercial source (Thadani et al., 1988) that have resulted in over 200 iatrogenic infections worldwide . The use of human pituitary hormones (growth hormone and gonadotrophin) derived from pituitary glands obtained post-mortem has also resulted in over 200 cases of iatrogenic CJD worldwide, with incubation periods extending over 30 years (see Brown et al. 2012 for review). In contrast, the re-use of neurosurgical instruments and intracerebral electrodes used on CJD affected patients (Bernoulli et al., 1977) has in contrast resulted in smaller numbers of those being exposed, infected or remaining at risk of potential infection. Iatrogenic transmission of vCJD infection via blood transfusion in human patients has recently been reported (Llewelyn et al., 2004; Peden et al., 2004; Wroe et al.), along with transmission via infected blood-derived products in a haemophilia patient in the UK (Peden et al., 2010). Blood transfusion is not associated with sCJD transmission, further highlighting the important differences between vCJD and CJD in terms of the tissue distribution of infectivity.

Acquired – Kuru Kuru was restricted to the Fore people of New Guinea and is hypothesised to be spread by ritual cannibalism, during which as part of their death rites the deceased were consumed by family members. Not only was there potential for oral ingestion of kuru infected material, but epidemiological evidence suggests that women and children experienced higher incidence of kuru due to their consumption of high risk tissues such as brain material from deceased infected individuals (Gajdusek and Zigas, 1959). Kuru is now considered extinct due to changes in these ritual practices adopted in the 1950's, preventing the consumption of the deceased and thereby transfer of infection. However, occasional kuru cases have since been reported and suggest incubation periods may in some cases be over 40 years (Collinge et al., 2006). Following the epidemic there is some indication that genetic selection for natural resistance to kuru infection has occurred within the affected population (Atkins et al., 2013; Mead et al., 2009).

Incidence and disease surveillance

Incidence The incidence of prion diseases is usually extremely rare. Sporadic CJD is the commonest form of the disease, accounting for around 80-85% of all cases. Cases of sCJD are estimated to occur each year at between 0.4 to 2.63 individuals per million people worldwide (Gelpi et al., 2008; Gubbels et al., 2012; Heinemann et al., 2007; Lai and Tseng, 2009; Nozaki et al., 2010; Stoeck et al., 2008) (see also Table 2). Genetic diseases account for 10-15% of all cases and generally occur as autosomal dominant disorders. Acquired prion disease account for less that 5% of all cases (Table 2).

Epidemiological investigations into acquired prion diseases indicate that kuru, whilst restricted to the Fore people of New Guinea, was spread efficiently among families by ritual cannibalism and consumption of brain tissue (Gajdusek and Zigas, 1959). The zoonotic vCJD epidemic is currently under an uneasy hiatus which has left an estimated 1 in 2,000-10,000 of the UK population as potential carriers of vCJD as determined by epidemiological studies and from the retrospective screening of appendix and tonsil tissues (Clarke and Ghani, 2005; Clewley et al., 2009; Gill et al., 2013; Hilton et al., 2004; HPA, 2012). Control measures, combining improved recognition of potentially infected persons with new disinfection methods for fragile surgical instruments and biological products, put in place to prevent the iatrogenic spread of sCJD and vCJD appear to have been successful (Brown et al., 2012). Human prion diseases appear not to be contagious, requiring ingestion or inoculation of infected tissues or contaminated neurosurgical instruments for transmission; some of the genetic prion diseases have still not yet been proven to be experimentally transmissible.

Disease surveillance Human prion diseases are diagnosed according to internationally agreed criteria published by the World Health Organisation (Appendix 1). Patients can be classified as "possible", "probable" or "definite" according to the results of clinical and pathological investigations. A diagnosis of definite prion disease is confirmed via neuropathological analysis. The practices and guidelines for such 'high-risk' neuropathological autopsies have been subjected to critical review in light of the vCJD epidemic in the UK (Ironside and Bell, 1996).

Research into human prion diseases has been made possible via inoculation of brain material from patients into laboratory animals such as non-human primates or rodents (bioassay) and subsequent clinical and neuropathological analysis are used as confirmation of disease transmission. However, around 10% of sCJD and 35% of GSS cases have failed to transmit to laboratory rodents (Bruce et al., 1997; Nonno et al., 2006; Telling et al., 1994). While attempts to transmit FFI to non-human primates failed or were inconclusive (Brown et al., 1995), this disease has been successfully transmitted to laboratory rodents (Tateishi et al., 1995). Clinical investigation, genetic testing for mutations in the *PRNP* gene associated with familial disease, cerebrospinal fluid (CSF) biomarker investigation and brain imaging either magnetic resonance imaging (MRI) or electroencephalogram (EEG) may all inform the diagnostic process. Historically the diagnosis of prion disease in human patients has been confounded by the similar neurological presentations of numerous dementing illnesses, in particular Alzheimer's disease (Haraguchi et al., 2009; Muramoto et al., 1992; Tschampa et al., 2001; Tsuchiya et al., 2004), Pick's disease (Pietrini et al., 1993), Parkinson's disease (Iida et al., 2001), Lewy body disease (Haraguchi et al., 2009; Tartaglia et al., 2012; Tschampa et al., 2001) and other treatable neurologic disorders (Chitravas et al., 2011). It is

therefore imperative that prion disease surveillance continues within the human population worldwide.

The post-mortem use of patients with dementia for anatomical research is heavily restricted. However, asymptomatic patients who may be infected with prions, or patients showing early but unreported clinical signs recorded with other more common causes of death (in particular sudden deaths e.g. stroke or heart failure) represent a potential risk route of exposure of prion-infected human material into anatomical research laboratories. The accuracy of death certificates and reliability of presumptive diagnosis in natural unexpected deaths reveal up to 1/3 of recorded causes of death may be incorrect (Gill and Scordi-Bello, 2010; Nielsen et al., 1991). The discrepancy between death certificate recorded cause of death and actual cause of death may have prompted the falling use of autopsy and pathological examination to be reversed (Roulson et al., 2005). However, hospital autopsy rates have been declining in the UK (Ayoub and Chow, 2008), US (Hoyert, 2011) and Japan (Maeda et al., 2013) and are at an all-time low (< 6% of the US Hospital deaths). Concerns in the US about inadequate infection control and facilities to perform brain autopsy on suspected CJD patients have been suggested as factors discouraging autopsy (Lillquist et al., 2006). In practice within the UK this is not the case and autopsy rates for legal reasons are high, and for suspected cases of human prion disease are exemplary.

For the purposes of anatomy and pathology teaching the UK Department of Health advisory committee on dangerous pathogens (ACDP) TSE subgroup have issued the following guidelines: “Anatomy departments are advised not to accept for teaching or research purposes, bodies, body parts or organs from any patients…” defined in the specific risk categories outlined (Table 3). “Departments should produce local policies to identify

who is responsible for checking whether a potential donor may be in one of the defined categories. The extent of the checks necessary will vary with circumstances, but would normally include checking with those responsible for the donation and the medical staff involved in the care of the donor." Further information regarding human prion diseases, their surveillance and the management of the risks involved in working with them can be found in the online resources detailed in Appendix I.

Tissue distribution of prion infectivity

For human prion diseases, the WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies (WHO, 1999) identified specific risk tissues for CJD. These guidelines were established before it was reported that peripheral organs such as the spleen and tonsils in vCJD patients may harbour significant levels of prion infectivity (Bishop et al., 2013; Bruce et al., 2001; Peden and Ironside, 2004), and that vCJD may have been transmitted by the transfusion of blood to blood-products from affected donors (Llewelyn et al., 2004; Peden et al., 2004; Wroe et al.). The magnitude of prion infectivity may vary however, as a recent case of vCJD has been reported in which extremely low levels of prions were found in lymphoreticular tissues (Mead et al., 2014). The ACDP TSE subgroup have subsequently issued guidelines on risk tissues for both CJD and vCJD summarised in Table 4.

The potential for prion decontamination

Ineffective Prions are notoriously resistant to many common forms of decontamination including ultraviolet light and ionizing radiation (Alper et al., 1967; Field et al., 1969; Gibbs et al., 1978). Treatments with common fixatives such as alcohol, formaldehyde and glutaraldehyde are unlikely to completely reduce prion infectivity in human tissue, even though many of them are effective for more conventional infectious agents. These fixatives generally result in a reduction of less than 3 $\log_{10}$ dose in prion infectivity within tissues.

Effective Treatments for the effective decontamination of prion infectivity from material are based upon chaotropic disruption of proteins such as immersion in sodium hydroxide or sodium hypochlorite. These substances are extremely caustic and hazardous and are handled with great care and used only where absolutely necessary. These are usually most effective when combined with heat or extremes of pH. For the preservation of material for histological (or anatomical) examination the use of formalin-based fixation with a formic acid treatment appears to be the best compromise between reduction in infectious titre and retention of tissue structure for histopathological analysis. Where possible single-use disposable surgical (or dissection) instruments should be used and disposed of accordingly. Further details regarding effective and ineffective decontamination treatments are reviewed in (Weinstein et al., 2001). For further details regarding the WHO decontamination methods for prion contaminated material see Appendix II.

Effects of tissue fixation- While the ACDP guidelines suggest avoidance of embalming procedures following diagnosis of CJD or vCJD, the effect of tissue fixation upon prion transmissibility has been studied experimentally. Formaldehyde fixation cross-links

proteins within the tissues creating a matrix. As such formaldehyde based fixatives generally reduce prion infectivity in tissues but do result in the retention of detectable amounts of infectivity as shown for both formol (Fraser et al., 1992) and periodate-lysine-paraformaldehyde (PLP) fixed (Taylor et al., 1997) tissues. Any apparent effect of tissue fixation is likely to be reversible as breaking the protein cross-links may liberate infectious prion material. Indeed detectable prion infectivity remained even after autoclaving or incineration of formaldehyde fixed infectious material (Brown et al., 1990). It is plausible that the process of embalming terminally affected CJD or vCJD patients represents a risk to the operator and as such is discouraged in the current WHO guidelines. Alternative routes of embalming prion disease patients post mortem are recommended to minimise the risk of prion transmission. The embalming of patients not categorised in Table 3, even accounting for potential asymptomatic human prion disease patients, are considered to represent a negligible risk for the transmission of prion disease to the operator.

Case studies

The occurrence of sCJD within health professionals has been assessed and reviewed (Alcalde-Cabero et al., 2012). This study determined that despite reports of sCJD in health professionals they occurred at expected rates when compared to the population as a whole. These studies suggest health professionals were at no greater risk of developing sCJD. However this review equally adopted the viewpoint that some professions are associated with an increased occupational risk of exposure to prion disease in specific circumstances. In these instances, appropriate care and preventative measures should continue to be taken to avoid increasing the risk of prion disease transmission. For further details regarding the WHO working practices for healthcare laboratories see Appendix III. For the purposes of anatomy teaching, the adoption of the general protective measures or similar Good Laboratory Practice guidelines should be sufficient so long as the sourcing of materials for teaching purposes avoids those patients categorised in Table 3.

Concluding Remarks

In this review we have attempted to present a balanced and critical review of the currently available data regarding the potential risks of transmission of prion disease from human cadaveric material to personnel working with or handling these materials. These diseases are extremely rare and the current working practices of avoidance of using cadavers with known dementia and avoidance of specific highly infectious tissues appear to be sufficient preventative measures. However the potential risk of prion disease transmission is still present and continued screening and adherence to current working practices should be maintained at least at their current level to reduce this risk.

Disclosure

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Administration determination or policy.

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Appendix I

Online references for information regarding human prion diseases

World Health Organisation (WHO)

http://www.who.int/zoonoses/diseases/prion_diseases/en/

USA

http://www.cdc.gov/ncidod/dvrd/prions/index.htm

http://www.cdc.gov/ncidod/dvrd/cjd/qa_cjd_infection_control.htm

http://www.cjdsurveillance.com/

UK – The national CJD research and surveillance unit (NCJDRSU)

http://www.cjd.ed.ac.uk/

https://www.gov.uk/government/publications/guidance-from-the-acdp-tse-risk-management-subgroup-formerly-tse-working-group

Europe

http://www.eurocjd.ed.ac.uk/

A more detailed review of human prion disease and their various pathologies is available in Greenfield's Neuropathology (Love et al., 2015)

Appendix II WHO Decontamination methods for Transmissible Spongiform Encephalopathies (WHO, 1999)

The following recommendations are based on the best available evidence at this time and are listed in order of more to 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) and heat in a gravity displacement autoclave at 121°C for 30 min; clean; rinse in water and subject to routine sterilization.

2. Immerse in NaOH or sodium hypochlorite for 1 hr; transfer instruments to water; heat in a gravity displacement autoclave at 121°C for 1 hr; clean and subject to routine sterilization.

3. Immerse in NaOH or sodium hypochlorite for 1 hr.; remove and rinse in water, then transfer to open pan and heat in a gravity displacement (121°C) or porous load (134°C) autoclave for 1 hr.; clean and subject to routine sterilization.

4. Immerse in NaOH and boil for 10 min 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 hr; clean; rinse in water and subject to routine sterilization.

6. Autoclave at 134°C for 18 minutes.

Chemical methods for surfaces and heat sensitive instruments

1. Flood with 2N NaOH or undiluted sodium hypochlorite; let stand for 1 hr.; 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 another of the partially effective methods.

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 hr.

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 hr.

Appendix III WHO Working Practices for healthcare laboratories (WHO, 1999)

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.

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 re-usable items must be either cleaned using methods set out in Annex III, or destroyed as per Section 7.

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 and Annex III.

9. All contaminated materials, specimens and cultures must be either incinerated, or decontaminated using methods described in Section 6 and Annex III and Section 7 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.

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 labelled ‘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 and Annex III or adsorbed onto materials such as sawdust and disposed of by incineration as a hazardous material.

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.

References

ACDP. 2012 revised. Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection: Part 4. Guidance from the ACDP TSE Risk Management Subgroup (formerly TSE Working Group).

Alcalde-Cabero E, Almazán-Isla J, Brandel JP, Breithaupt M, Catarino J, Collins S, Haybäck J, Höftberger R, Kahana E, Kovacs GG, Ladogana A, Mitrova E, Molesworth A, Nakamura Y, Pocchiari M, Popovic M, Ruiz-Tovar M, Taratuto AL, van Duijn C, Yamada M, Will RG, Zerr I, de Pedro Cuesta J. 2012. Health professions and risk of sporadic Creutzfeldt–Jakob disease, 1965 to 2010. Eurosurveillance 17.

Alper T, Cramp WA, Haig DA, Clarke MC. 1967. Does the Agent of Scrapie Replicate without Nucleic Acid ? Nature 214:764-766.

Atkins KE, Townsend JP, Medlock J, Galvani AP. 2013. Epidemiological mechanisms of genetic resistance to kuru. Journal of The Royal Society Interface 10.

Ayoub T, Chow J. 2008. The conventional autopsy in modern medicine. Journal of the Royal Society of Medicine 101:177-181.

Barria MA, Balachandran A, Morita M, Kitamoto T, Barron R, Manson J, Knight R, Ironside JW, Head MW. 2014. Molecular Barriers to Zoonotic Transmission of Prions. Emerging Infectious Diseases 20:88-97.

Belay ED, Maddox RA, Williams ES, Miller MW, Gambetti P, Schonberger LB. 2004. Chronic wasting disease and potential transmission to humans. Emerging Infectious Diseases 10:977-984.

Belay ED, Schonberger LB. 2002. Variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. Clinics in Laboratory Medicine 22:849-862.

Bernoulli C, Siegfried J, Baumgartner G, Regli F, Rabinowicz T, Gajdusek DC, Gibbs Jr CJ. 1977. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. The Lancet 309:478-479.

Bishop MT, Diack AB, Ritchie DL, Ironside JW, Will RG, Manson JC. 2013. Prion infectivity in the spleen of a PRNP heterozygous individual with subclinical variant Creutzfeldt–Jakob disease. Brain.

Brown P, Brandel J-P, Sato T, Nakamura Y, MacKenzie J, Will RG, Ladogana A, Pocchiari M, Leschek EW, Schonberger LB. 2012. Iatrogenic Creutzfeldt-Jakob Disease, Final Assessment. Emerging Infectious Diseases 18:901-907.

Brown P, Gibbs CJ, Amyx HL, Kingsbury DT, Rohwer RG, Sulima MP, Gajdusek DC. 1982. Chemical Disinfection of Creutzfeldt–Jakob Disease Virus. New England Journal of Medicine 306:1279-1282.

Brown P, Kenney K, Little B, Ironside J, Will R, Cervenáková L, Bjork RJ, San Marchtin RA, Safr J, Roos R, Haltia M, Gibbs CJ, Gajdusek DC. 1995. Intracerebral distribution of infectious amyloid protein in spongiform encephalopathy. Annals of Neurology 38:245-253.

Brown P, Liberski PR, Wolff A, Gajdusek DC. 1990. Resistance of Scrapie Infectivity to Steam Autoclaving after Formaldehyde Fixation and Limited Survival after Ashing at 360°C: Practical and Theoretical Implications. Journal of Infectious Diseases 161:467-472.

Bruce ME, McConnell I, Will RG, Ironside JW. 2001. Detection of variant Creutzfeld-Jakob disease infectivity in extraneural tissues. Lancet 358:208-209.

Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ. 1997. Transmissions to mice indicate that `new variant' CJD is caused by the BSE agent. Nature 389:498-501.

Chitravas N, Jung RS, Kofskey DM, Blevins JE, Gambetti P, Leigh RJ, Cohen ML. 2011. Treatable neurological disorders misdiagnosed as Creutzfeldt-Jakob disease. Annals of Neurology 70:437-444.

Clarke P, Ghani AC. 2005. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. Journal of The Royal Society Interface 2:19-31.

Clewley JP, Kelly CM, Andrews N, Vogliqi K, Mallinson G, Kaisar M, Hilton DA, Ironside JW, Edwards P, McCardle LM, Ritchie DL, Dabaghian R, Ambrose HE, Gill ON. 2009. Prevalence of disease related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey. BMJ 338.

Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. 1996. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 383:685-690.

Collinge J, Whitfield J, McKintosh E, Beck J, Mead S, Thomas DJ, Alpers MP. 2006. Kuru in the 21st century—an acquired human prion disease with very long incubation periods. The Lancet 367:2068-2074.

Creutzfeldt HG. 1920. A peculiar localised disease of the central nervous system (Preliminary announcement). Zeitschrift Fur Die Gesamte Neurologie Und Psychiatrie 57:1-18.

Detwiler LA, Baylis M. 2003. The epidemiology of scrapie. Revue Scientifique Et Technique-Office International Des Epizooties 22:121-143.

Ducrot C, Arnold M, de Koeijer A, Heim D, Calavas D. 2008. Review on the epidemiology and dynamics of BSE epidemics. Veterinary Research 39.

Duffy P, Wolf J, Collins G, Devoe AG, Streeten B, Cowen D. 1974. Possible Person-to-Person Transmission of Creutzfeldt-Jakob Disease. New England Journal of Medicine 290:692-693.

Evoniuk JM, Stoltenow CL, O'Rourke KI, Moore BL, Redmer DA. 2005. Assessment of the genetic risk and impact of lateral transmission in a valine-associated scrapie outbreak in sheep. American Journal of Veterinary Research 66:1302-1307.

Field EJ, Farmer F, Caspary EA, Joyce G. 1969. Susceptibility of Scrapie Agent to Ionizing Radiation. Nature 222:90-91.

Foster JD, Goldmann W, Hunter N. 2013. Evidence in Sheep for Pre-Natal Transmission of Scrapie to Lambs from Infected Mothers. PLoS ONE 8.

Fraser H, Bruce ME, Chree A, McConnell I, Wells GAH. 1992. Transmission of bovine spongiform encephalopathy and scrapie to mice. J. Gen. Virol. 73:1891-1897.

Gajdusek DC, Zigas V. 1959. Kuru: Clinical, pathological and epidemiological study of an acute progressive degenerative disease of the central nervous system among natives of the Eastern Highlands of New Guinea. The American journal of medicine 26:442-469.

Gambetti P, Dong Z, Yuan J, Xiao X, Zheng M, Alshekhlee A, Castellani R, Cohen M, Barria MA, Gonzalez-Romero D, Belay ED, Schonberger LB, Marder K, Harris C, Burke JR, Montine T, Wisniewski T, Dickson DW, Soto C, Hulette CM, Mastrianni JA, Kong Q, Zou W-Q. 2008. A novel human disease with abnormal prion protein sensitive to protease. Annals of Neurology 63:697-708.

Gambetti P, Kong Q, Zou W, Parchi P, Chen SG. 2003. Sporadic and familial CJD: classification and characterisation. British Medical Bulletin 66:213-239.

Gelpi E, Heinzl H, Höftberger R, Unterberger U, Ströbel T, Voigtländer T, Drobna E, Jarius C, Lang S, Waldhör T, Bernheimer H, Budka H. 2008. Creutzfeldt-Jakob Disease in Austria: An Autopsy-Controlled Study. Neuroepidemiology 30:215-221.

Georgsson G, Sigurdarson S, Brown P. 2006. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. J. Gen. Virol. 87:3737-3740.

Gerstmann J, Sträussler E, Scheinker I. 1936. Über eine eigenartige hereditär-familiäre Erkrankung des Zentralnervensystems. Zugleich ein Beitrag zur Frage des vorzeitigen lokalen Alters. Z Neur Psychiat 154:736-762.

Ghetti B, Piccardo P, Spillantini MG, Ichimiya Y, Porro M, Perini F, Kitamoto T, Tateishi J, Seiler C, Frangione B, Bugiani O, Giaccone G, Prelli F, Goedert M, Dlouhy SR, Tagliavini F. 1996. Vascular variant of prion protein cerebral amyloidosis with tau-positive neurofibrillary tangles: the phenotype of the stop codon 145 mutation in PRNP. Proceedings of the National Academy of Sciences of the United States of America 93:744-748.

Gibbs CJ, Gajdusek DC, Latarjet R. 1978. Unusual resistance to ionizing-radiation of the viruses of Kuru, Creutzfeldt-Jakob disease, and Scrapie. Proceedings of the National Academy of Sciences of the United States of America 75:6268-6270.

Gill JR, Scordi-Bello IA. 2010. Natural, Unexpected Deaths: Reliability of a Presumptive Diagnosis*. Journal of Forensic Sciences 55:77-81.

Gill ON, Spencer Y, Richard-Loendt A, Kelly C, Dabaghian R, Boyes L, Linehan J, Simmons M, Webb P, Bellerby P, Andrews N, Hilton DA, Ironside JW, Beck J, Poulter M, Mead S, Brandner S. 2013. Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: large scale survey. BMJ 347.

Glatzel M, Abela E, Maissen M, Aguzzi A. 2003. Extraneural Pathologic Prion Protein in Sporadic Creutzfeldt–Jakob Disease. New England Journal of Medicine 349:1812-1820.

Gough KC, Maddison BC. 2010. Prion transmission: Prion excretion and occurrence in the environment. Prion 4:275-282.

Gubbels S, Bacci S, Laursen H, Høgenhaven H, Cowan S, Mølbak K, Christiansen M. 2012. Description and analysis of 12 years of surveillance for Creutzfeldt–Jakob disease in Denmark, 1997 to 2008. Eurosurveillance 17.

Haraguchi T, Terada S, Ishizu H, Sakai K, Tanabe Y, Nagai T, Takata H, Nobukuni K, Ihara Y, Kitamoto T, Kuroda S. 2009. Coexistence of Creutzfeldt-Jakob disease, Lewy body disease, and Alzheimer's disease pathology: An autopsy case showing typical clinical features of Creutzfeldt-Jakob disease. Neuropathology 29:454-459.

Head MW, Ritchie D, Smith N, McLoughlin V, Nailon W, Samad S, Masson S, Bishop M, McCardle L, Ironside JW. 2004. Peripheral Tissue Involvement in Sporadic, Iatrogenic, and Variant Creutzfeldt-Jakob Disease: An Immunohistochemical, Quantitative, and Biochemical Study. The American journal of pathology 164:143-153.

Heinemann U, Krasnianski A, Meissner B, Varges D, Kallenberg K, Schulz-Schaeffer WJ, Steinhoff BJ, Grasbon-Frodl EM, Kretzschmar HA, Zerr I. 2007. Creutzfeldt–Jakob disease in Germany: a prospective 12-year surveillance. Brain 130:1350-1359.

Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW. 2004. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. The Journal of Pathology 203:733-739.

Hoinville LJ, Tongue SC, Wilesmith JW. 2010. Evidence for maternal transmission of scrapie in naturally affected flocks. Preventive Veterinary Medicine 93:121-128.

Hope J, Reekie LJD, Hunter N, Multhaup G, Beyreuther K, White H, Scott AC, Stack MJ, Dawson M, Wells GAH. 1988. Fibrils from brains of cows with new cattle disease contain scrapie-associated protein. Nature 336:390-392.

Hoyert DL. 2011. The changing profile of autopsied deaths in the United States, 1972–2007. NCHS data brief, no 67. Hyattsville, MD: National Center for Health Statistics.

HPA. 2012. http://www.hpa.org.uk/hpr/archives/2012/hpr0612.pdf. Health Protection Report 6.

Iida T, Doh-ura K, Kawashima T, Abe H, Iwaki T. 2001. An atypical case of sporadic Creutzfeldt–Jakob disease with Parkinson's disease. Neuropathology 21:294-297.

Ironside JW, Bell JE. 1996. The 'high-risk' neuropathological autopsy in AIDS and Creutzfeldt-Jakob disease: principles and practice. Neuropathology and Applied Neurobiology 22:388-393.

Jakob A. 1921a. On a peculiar disease process of the central nervous system in a chronic Psychosis with catatonic symptoms. Zeitschrift Fur Die Gesamte Neurologie Und Psychiatrie 66:178-207.

Jakob A. 1921b. Unusual diseases of the central nervous system with striking anatomic results (Spastic pseudosclerosis - Encephalomyelopathy with disseminated focal degeneration). Zeitschrift Fur Die Gesamte Neurologie Und Psychiatrie 64:147-228.

Joiner S, Linehan JM, Brandner S, Wadsworth JDF, Collinge J. 2005. High levels of disease related prion protein in the ileum in variant Creutzfeldt-Jakob disease. Gut 54:1506-1508.

Lai CH, Tseng HF. 2009. Population-Based Epidemiological Study of Neurological Diseases in Taiwan: I. Creutzfeldt-Jakob Disease and Multiple Sclerosis. Neuroepidemiology 33:247-253.

Lillquist PP, Thomas N, Belay ED, Schonberger LB, Morse D. 2006. Barriers to Autopsy: Creutzfeldt-Jakob Disease in New York State. Neuroepidemiology 26:207-211.

Llewelyn CA, Hewitt PE, Knight RSG, Amar K, Cousens S, Mackenzie J, Will RG. 2004. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. The Lancet 363:417-421.

Love S, Perry A, Ironside J, Budka H. 2015. Greenfield's Neuropathology, Ninth Edition.

Lugaresi E, Medori R, Montagna P, Baruzzi A, Cortelli P, Lugaresi A, Tinuper P, Zucconi M, Gambetti P. 1986. Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. New England Journal of Medicine 315:997-1003.

Maddison BC, Baker CA, Terry LA, Bellworthy SJ, Thorne L, Rees HC, Gough KC. 2010. Environmental Sources of Scrapie Prions. Journal of Virology 84:11560-11562.

Maeda S, Kamishiraki E, Starkey J, Ikeda N. 2013. Why are autopsy rates low in Japan? Views of ordinary citizens and doctors in the case of unexpected patient death and medical error. Journal of Healthcare Risk Management 33:18-25.

McGowan JP. 1922. Scrapie in sheep. Scottish Journal of Agriculture 5:365-375.

Mead S, Wadsworth JF, Porter M, et al. 2014. VAriant creutzfeldt-jakob disease with extremely low lymphoreticular deposition of prion protein. JAMA Neurology.

Mead S, Whitfield J, Poulter M, Shah P, Uphill J, Campbell T, Al-Dujaily H, Hummerich H, Beck J, Mein CA, Verzilli C, Whittaker J, Alpers MP, Collinge J. 2009. A Novel Protective Prion Protein Variant that Colocalizes with Kuru Exposure. New England Journal of Medicine 361:2056-2065.

Miller MW, Williams ES. 2004. Chronic wasting disease of cervids. Mad Cow Disease and Related Spongiform Encephalopathies 284:193-214.

Muramoto T, Kitamoto T, Koga H, Tateishi J. 1992. The coexistence of Alzheimer's disease and Creutzfeldt-Jakob disease in a patient with dementia of long duration. Acta Neuropathologica 84:686-689.

Nielsen GP, Björnsson J, Jonasson JG. 1991. The accuracy of death certificates. Virchows Archiv A 419:143-146.

Nonno R, Di Bari MA, Cardone F, Vaccari G, Fazzi P, Dell'Omo G, Cartoni C, Ingrosso L, Boyle A, Galeno R, Sbriccoli M, Lipp HP, Bruce M, Pocchiari M, Agrimi U. 2006. Efficient transmission and characterization of Creutzfeldt-Jakob disease strains in bank voles. Plos Pathogens 2:112-120.

Notari S, Moleres FJ, Hunter SB, Belay ED, Schonberger LB, Cali I, Parchi P, Shieh W-J, Brown P, Zaki S, Zou W-Q, Gambetti P. 2010. Multiorgan Detection and Characterization of Protease-Resistant Prion Protein in a Case of Variant CJD Examined in the United States. PLoS ONE 5:e8765.

Nozaki I, Hamaguchi T, Sanjo N, Noguchi-Shinohara M, Sakai K, Nakamura Y, Sato T, Kitamoto T, Mizusawa H, Moriwaka F, Shiga Y, Kuroiwa Y, Nishizawa M, Kuzuhara S, Inuzuka T, Takeda M, Kuroda S, Abe K, Murai H, Murayama S, Tateishi J, Takumi I, Shirabe S, Harada M, Sadakane A, Yamada M. 2010. Prospective 10-year surveillance of human prion diseases in Japan. Brain 133:3043-3057.

Padovani A, D'Alessandro M, Parchi P, Cortelli P, Anzola GP, Montagna P, Vignolo LA, Petraroli R, Pocchiari M, Lugaresi E, Gambetti P. 1998. Fatal familial insomnia in a new Italian kindred. Neurology 51:1491-1494.

Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, Zerr I, Budka H, Kopp N, Piccardo P, Poser S, Rojiani A, Streichemberger N, Julien J, Vital C, Ghetti B, Gambetti P, Kretzschmar H. 1999. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Annals of Neurology 46:224-233.

Parchi P, Strammiello R, Notari S, Giese A, Langeveld JM, Ladogana A, Zerr I, Roncaroli F, Cras P, Ghetti B, Pocchiari M, Kretzschmar H, Capellari S. 2009. Incidence and spectrum of sporadic Creutzfeldt–Jakob disease variants with mixed phenotype and co-occurrence of PrPSc types: an updated classification. Acta Neuropathologica 118:659-671.

Peden A, McCardle L, Head MW, Love S, Ward HJT, Cousens SN, Keeling DM, Millar CM, Hill FGH, Ironside JW. 2010. Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. Haemophilia 16:296-304.

Peden AH, Head MW, Diane LR, Jeanne EB, James WI. 2004. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. The Lancet 364:527-529.

Peden AH, Ironside JW. 2004. Review: pathology of variant Creutzfeldt-Jakob disease. Folia neuropathologica / Association of Polish Neuropathologists and Medical Research Centre, Polish Academy of Sciences 42 Suppl A:85-91.

Pietrini V, Danieli D, Bevilacqua P, Lechi A. 1993. Panencephalopathic type of Creutzfeldt-Jakob Disease with neuropathologic features similar to Picks Disease. Clinical Neuropathology 12:1-6.

Prusiner SB. 1982. Novel proteinaceous infectious particles cause scrapie. Science 216:136-144.

Roulson J, Benbow EW, Hasleton PS. 2005. Discrepancies between clinical and autopsy diagnosis and the value of post mortem histology; a meta-analysis and review. Histopathology 47:551-559.

Stoeck K, Hess K, Amsler L, Eckert T, Zimmermann D, Aguzzi A, Glatzel M. 2008. Heightened incidence of sporadic Creutzfeldt-Jakob disease is associated with a shift in clinicopathological profiles. Journal of Neurology 255:1464-1472.

Tartaglia MC, Johnson DY, Thai JN, Cattaruzza T, Wong K, Garcia P, DeArmond SJ, Miller BL, Geschwind MD. 2012. Clinical Overlap between Jakob-Creutzfeldt Disease and Lewy Body Disease. Canadian Journal of Neurological Sciences 39:304-310.

Tateishi J, Brown P, Kitamoto T, Hoque ZM, Roos R, Wollman R, Cervenakova L, Gajdusek DC. 1995. First experimental transmission of fatal familial insomnia. Nature 376:434-435.

Taylor DM, Brown JM, Fernie K, McConnell I. 1997. The effect of formic acid on BSE and scrapie infectivity in fixed and unfixed brain-tissue. Veterinary Microbiology 58:167-174.

Telling GC, Scott M, Hsiao KK, Foster D, Yang SL, Torchia M, Sidle KC, Collinge J, DeArmond SJ, Prusiner SB. 1994. Transmission of Creutzfeldt-Jakob disease from humans to transgenic mice expressing chimeric human-mouse prion protein. Proceedings of the National Academy of Sciences 91:9936-9940.

Thadani V, Penar PL, Partington J, Kalb R, Janssen R, Schonberger LB, Rabkin CS, Prichard JW. 1988. Creutzfeldt-Jakob disease probably acquired from a cadaveric dura mater graft. Journal of Neurosurgery 69:766-769.

Tschampa HJ, Neumann M, Zerr I, Henkel K, Schröter A, Schulz-Schaeffer WJ, Steinhoff BJ, Kretzschmar HA, Poser S. 2001. Patients with Alzheimer's disease and dementia with Lewy

bodies mistaken for Creutzfeldt-Jakob disease. Journal of Neurology, Neurosurgery & Psychiatry 71:33-39.

Tsuchiya K, Yagishita S, Ikeda K, Sano M, Taki K, Hashimoto K, Watabiki S, Mizusawa H. 2004. Coexistence of CJD and Alzheimer's disease: An autopsy case showing typical clinical features of CJD. Neuropathology 24:46-55.

Wadsworth JDF, Joiner S, Fox K, Linehan JM, Desbruslais M, Brandner S, Asante EA, Collinge J. 2007. Prion infectivity in variant Creutzfeldt–Jakob disease rectum. Gut 56:90-94.

Wadsworth JDF, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J. 2001. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. The Lancet 358:171-180.

Weinstein RA, Rutala WA, Weber DJ. 2001. Creutzfeldt-Jakob Disease: Recommendations for Disinfection and Sterilization. Clinical Infectious Diseases 32:1348-1356.

WHO. 1999. WHO infection control guidelines for transmissible spongiform encephalopathies. Report of a WHO consultation, Geneva, Switzerland, 23-26 March 1999. http://www.who.int/csr/resources/publications/bse/WHO_CDS_CSR_APH_2000_3/en/.

Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347:921-925.

Wroe SJ, Pal S, Siddique D, Hyare H, Macfarlane R, Joiner S, Linehan JM, Brandner S, Wadsworth JDF, Hewitt P, Collinge J. 2006. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. The Lancet 368:2061-2067.

Yu S, Zhang Y, Li S, Sy M-S, Sun S, Tien P, Xiao G. 2007. Early onset fatal familial insomnia with rapid progression in a Chinese family line. Journal of Neurology 254:1300-1301.

Zarranz JJ, Digon A, Atarés B, Rodríguez-Martínez AB, Arce A, Carrera N, Fernández-Manchola I, Fernández-Martínez M, Fernández-Maiztegui C, Forcadas I, Galdos L, Gómez-Esteban JC, Ibáñez A, Lezcano E, López de Munain A, Martí-Massó JF, Mendibe MM, Urtasun M, Uterga JM, Saracibar N, Velasco F, de Pancorbo MM. 2005. Phenotypic variability in familial prion diseases due to the D178N mutation. Journal of Neurology, Neurosurgery & Psychiatry 76:1491-1496.

Zerr I, Poser S. 2004. Epidemiology and risk factors of transmissible spongiform encephalopathies in man. Contributions to microbiology 11:98-116.

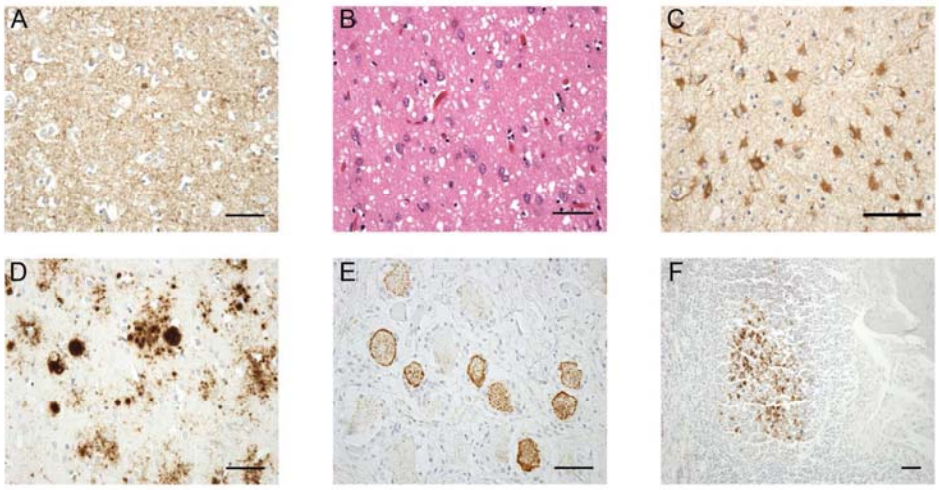
Figure Legends

Figure 1. Neuropathological hallmarks of human prion disease

Photomicrographs depicting the common neuropathological hallmarks of prion disease in the brains (A-D) and peripheral tissues (E & F) of affected humans: A, Granular/synaptic PrP deposition in sporadic Creutzfeldt-Jakob disease (sCJD) detected using 12F10 anti-prion protein antibody (brown); B, Microvacuolar spongiform change in the cerebral cortex in sporadic CJD, haematoxylin and eosin stain; C, Reactive astrocytosis in the thalamus in variant CJD, glial fibrillary acidic protein antibody; D, PrP deposition in the cerebral cortex in variant CJD, 12F10 anti-prion protein antibody; E, PrP deposition in variant CJD dorsal root ganglion, 12F10 anti-prion protein antibody; F, PrP deposition in follicular dendritic cells in a lymphoid follicle in the tonsil in variant CJD, 12F10 anti-prion protein antibody. Scale bars = 50 μm.

Figure 2. The potential for transmission of human prion disease during anatomical dissection

The relative distribution of prion infectivity within the body of affected humans. Although potential exists for the transmission of human prion disease during anatomical dissection, human prion diseases are extremely rare and the risks involved can be effectively managed by the following: 1, screening of patients and materials used for dissection; 2, identification and avoidance of handling high risk materials; 3, fixation and potential for decontamination of prions.



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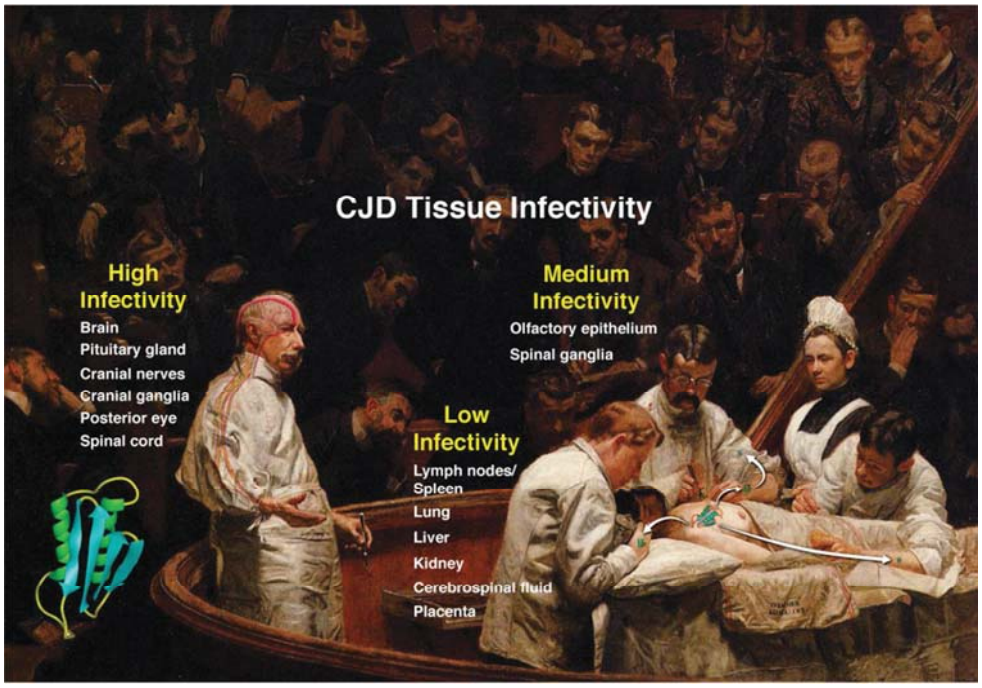

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Table 1 Human prion diseases

Disease	Acronym	Occurence
Sporadic Creutzfeldt-Jakob disease	sCJD	Idiopathic
Sporadic fatal insomnia	sFI	Idiopathic
Variably protease-sensitive prionopathy	VPSPr	Idiopathic
Familial Creutzfeldt-Jakob disease	fCJD	Genetic
Gerstmann-Sträussler-Scheinker disease	GSS	Genetic
Fatal familial insomnia	FFI	Genetic
Kuru		Acquired - via consumption of kuru infected material
Iatrogenic Creutzfeldt-Jakob disease	iCJD	Acquired - via inadvertent transmission of sCJD (or vCJD) as a result of medical treatment
Variant Creutzfeldt-Jakob disease	vCJD	Acquired - primarily via consumption of BSE contaminated meat products, secondary iatrogenically, blood transfusion

Table 2 Incidence of human prion diseases as reported by active surveillance

Region/Period	Total Referrals	Prion Disease	Sporadic	Genetic	Iatrogenic	vCJD
US 1990-1999	377	228	198	28	2	0
US 2000-2009	3118	1817	1552	258	4	3
US 2010-present	1586	955	789	166	0	0
UK 1990-1999	1130	595	455	48	36	56
UK 2000-2009	1277	884	675	71	26	112
UK 2010-present	583	421	361	38	13	9
Total (% of total)	**8071**	**4900**	**4030 (82.3 %)**	**609 (12.5 %)**	**81 (1.6 %)**	**180 (3.6 %)**

Data from the US National Prion Disease Pathology Surveillance Center, and UK National CJD Research & Surveillance Unit.

Table 3 Categorisation of patients by risk (ACDP, 2012 revised)

Patient groups	
Symptomatic patients	• Patients who fulfil the diagnostic criteria for definite, probable or possible CJD or vCJD • Patients with neurological disease of unknown aetiology, who do not fit the criteria for possible CJD or vCJD, but where the diagnosis of CJD is being actively considered
Patients "at increased risk" from genetic forms of CJD	• Individuals who have been shown by specific genetic testing to be at significant risk of developing CJD. • Individuals who have a blood relative known to have a genetic mutation indicative of genetic CJD; • Individuals who have or have had two or more blood relatives affected by CJD or other prion disease.
Patients identified as "at increased risk" of CJD/vCJD through iatrogenic exposures	• Recipients of hormone derived from human pituitary glands, *e.g.* growth hormone, gonadotrophin, are "at increased risk" of transmission of sporadic CJD. In the UK the use of human-derived gonadotrophin was discontinued in 1973, and use of cadaver-derived human growth hormone was banned in 1985. However, use of human-derived products may have continued in other countries after these dates. • Individuals who underwent intradural brain or intradural spinal surgery† before August 1992 who received (or might have received) a graft of human-derived dura mater are "at increased risk" of transmission of sporadic CJD (unless evidence can be provided that human-derived dura mater was not used). • Individuals who have had surgery using instruments that had been used on someone who went on to develop CJD/vCJD, or was "at increased risk" of CJD/vCJD; • Individuals who have received an organ or tissue from a donor infected with CJD/vCJD or "at increased risk" of CJD/vCJD; • Individuals who have been identified prior to high risk surgery as having received blood or blood components from 80 or more donors since January 1980; • Individuals who have received blood from someone who went on to develop vCJD; • Individuals who have given blood to someone who went on to develop vCJD; • Individuals who have received blood from someone who has also given blood to a patient who went on to develop vCJD; • Individuals who have been treated with certain implicated UK sourced plasma products between 1980 and 2001

Table 4. Distribution of prion infectivity in CJD and vCJD (ACDP, 2012 revised)

Tissue	Infectivity in CJD (other than vCJD)	Infectivity in vCJD
Brain	High	High
Spinal cord	High	High
Cranial nerves, specifically the entire optic nerve and only the intracranial components of the other cranial nerves	High	High
Cranial ganglia	High	High
Posterior eye, specifically the posterior hyaloid face, retina, retinal pigment epithelium, choroid, subretinal fluid, optic nerve	High	High
Pituitary gland	High (?)	High (?)
Spinal ganglia	Medium	Medium
Olfactory epithelium	Medium	Medium
Dura mater	Low	Low
Tonsil	Low	Medium
Lymph nodes and other organised lymphoid tissues containing follicular structures	Low	Medium
Gut-associated lymphoid tissue	Low	Medium
Appendix	Low	Medium
Adrenal gland	Low	Medium
Spleen	Low	Medium
Thymus	Low	Medium
Anterior eye and cornea	Low	Medium
Peripheral nerve	Low	Low
Skeletal muscle	Low	Low
Dental pulp	Low	Low
Gingival tissue	Low	Low
Blood and bone marrow	Low	Low
CSF	Low	Low
Placenta	Low	Low
Urine	Low	Low
Other tissues	Low	Low