

## Prions (bovine spongiform encephalopathy)

Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease of cattle. It is caused by proteinaceous infectious particles known as prions. BSE is the only transmissible spongiform encephalopathy (TSE) of animals that is known to be infectious to humans through the consumption of contaminated meat. The human form of the disease is known as variant Creutzfeldt-Jakob (vCJD) disease.

### Description of the infective agent

BSE and other TSEs are caused by a mis-folded isoform of the prion protein (PrP), a widely expressed glycoprotein. PrP is a normal constituent of cell membranes in vertebrates, and is encoded by the prion protein gene *PRNP*. The mis-folded pathogenic isoform protein is often referred to as a 'prion', a term made up from the contraction of the words 'proteinaceous' and 'infectious' (Prusiner 1982). By convention, the normal cellular isoform of PrP is represented as PrP<sup>C</sup>. The C superscript refers to the cellular form. The prion form has the same amino acid sequence as the normal form and is represented as PrP<sup>Sc</sup>. The Sc superscript is a reference to scrapie, a disease of sheep that is the prototypical animal prion disease. The prions replicate themselves by binding to the normal PrP<sup>C</sup> protein and acting as a template that coerces the PrP<sup>C</sup> molecule to refold into the abnormal PrP<sup>Sc</sup> form (Gains and LeBlanc 2007; Cobb and Surewicz 2009).

PrP<sup>C</sup> has been identified in mammals, birds, reptiles, amphibians, fish and yeasts. In mammals, the protein is expressed in a wide variety of tissues including spleen, lymph nodes, kidney, pancreas, salivary gland, adrenal gland, liver, thymus, and bone marrow; and is highly expressed in the nervous system (Gains and LeBlanc 2007; Linden et al. 2008; Brown and Mastrianni 2010). However, the physiological function of PrP<sup>C</sup> remains obscure and a number of strains of mice bred not to express PrP<sup>C</sup> show only subtle, non-lethal differences in physiologic and locomotor activity when compared to wild-type mice (Cobb and Surewicz 2009; Chakrabarti et al. 2009).

Within some prion diseases, including BSE and scrapie, strains exist which exhibit distinct disease phenotypes. Differences between strains include patterns of protein deposition in the brain and lymphoid tissues, incubation times after experimental infection of animals, histopathology and clinical manifestation. For example, with scrapie some strains preferentially propagate in the central nervous system while others are characterised by substantial infectivity in lymphoid organs (Aguzzi and Calella 2009). There are three strains of BSE which have been identified. Only one strain, classical BSE, was responsible for the BSE epidemic which started in the United Kingdom (UK) and spread to other countries, and the associated epidemic of vCJD in humans (Harman and Silva 2009). The atypical strains, known as H (high) and L (low) type, are diagnosed rarely, typically in cattle of 8–20 years of age, and appear to be sporadic and arise spontaneously (Seuberlich et al. 2010; Konold et al. 2012).

### Stability characteristics

Prions are notoriously resistant to inactivation with conventional sterilization procedures used for preparation of surgical instruments and materials. PrP<sup>Sc</sup> is resistant to UV irradiation at 254 nm, 70% alcohol treatment, gamma irradiation, and conventional autoclaving (121°C for 20 minutes). PrP<sup>Sc</sup> can be inactivated by a number of measures including severe

autoclaving conditions (134°C for 8-18 minutes) in conjunction with detergents and hydrogen peroxide gas plasma sterilization (Aguzzi and Calella 2009; Sakudo et al. 2011).

A number of procedures that modify or hydrolyse proteins can reduce the infectivity of prions (Aguzzi and Calella 2009). However, while PrP<sup>C</sup> is protease-sensitive and soluble in non-denaturing detergents, PrP<sup>Sc</sup> is insoluble in detergents and contains a protease-resistant core (Gains and LeBlanc 2007).

## Symptoms and clinical signs of disease

### *Cattle*

Field data suggest that susceptibility to BSE infection peaks around 12 months of age in cattle, although there have been BSE cases in cattle that were not fed meat-and-bone meal (MBM) until they were over 2 years of age. The incubation period in cattle is estimated to be from 30 months to 8 years (mean of 4.5–5.5 years), but the course of the clinical disease is short from the onset of clinical signs, with animals generally dying or requiring euthanasia within 6 months (USDA FSIS 2005; St Rose et al. 2006; Harman and Silva 2009). Among 124,000 UK cattle for which the age of onset of clinical signs was known, 7% were 3 years old, 31% were 4 years old, 33% were 5 years old (generally thought to be the average age of onset) and 29% were 6 years old or older (Harman and Silva 2009).

Clinical signs in cattle include changes in temperament, such as nervousness or aggression, abnormal posture, incoordination and difficulty in rising, decreased milk production, or loss of body weight despite continued appetite (USDA FSIS 2005; Seuberlich et al. 2010).

### *Humans*

Over 200 human cases of vCJD have been reported (Aguzzi and Calella 2009). Patients have ranged in age from 17–42 years (Brown and Mastrianni 2010). The great majority of patients were UK residents during the period 1985–1996 (Mackay et al. 2011). Variant CJD is invariably fatal, and generally runs a course of approximately 18 months from the onset of symptoms. Symptoms and clinical signs include both cognitive and motor dysfunction. Early in the illness, patients usually experience psychiatric or sensory symptoms. Reported psychiatric symptoms include depression, apathy, agitation, insomnia, poor concentration, paranoid delusion, recklessness, aggression, withdrawal or anxiety. Approximately one third of the patients reported unusual persistent and painful sensory symptoms. Neurological signs develop as the illness progresses, including cerebellar ataxia, muscle spasms and involuntary movements. Late onset signs include urinary incontinence, progressive immobility, and akinetic mutism. Death is often due to opportunistic infections (Imran and Mahmood 2011a).

The great majority of cases of prion disease occurring in humans are spontaneous occurrences of sporadic Creutzfeld-Jakob disease (sCJD). In comparison to vCJD, sCJD typically affects people between 55 and 70 years old (Mackay et al. 2011). Cerebellar ataxia or progressive dementia predominate in the first few months of sCJD, which contrast with vCJD (Imran and Mahmood 2011a). Also, vCJD features distinctive histopathology (Mackay et al. 2011). Besides classical BSE, the only other TSE known to be infectious to humans by the oral route is the now nearly extinct disease known as kuru, which occurred in a small group of communities of indigenous Papua New Guinean people who practised cannibalism (Aguzzi and Calella 2009).

## Pathogenic mechanism

The pathogenesis of BSE in cattle has been studied extensively although there are still a number of knowledge gaps. After oral exposure of calves to infective material, PrP<sup>Sc</sup> is first observed in Peyer's patches of the ileum, and also detected in gut-associated lymphoid tissue (GALT) of the ileocaecal junction and the jejunum. The infectivity is located in macrophages and follicular dendritic cells (FDC). Later, infectivity can be identified in the enteric nervous system, although it is not clear how infectivity moves from the cells of the lymphoreticular system to those of the nervous system (Hoffmann et al. 2011). It is possible that after crossing the mucosal barrier of the intestine, prions infect the nervous tissue when they come into contact with the fine nerve fibres directly under the intestinal mucosa (van Keulen et al. 2008). Once the nervous system is infected, infectivity then ascends to the brain via both the sympathetic (e.g. splanchnic nerve) and parasympathetic (e.g. vagus nerve) nervous systems (Cobb and Surewicz 2009). Involvement of GALT is less extensive in BSE than in ovine scrapie (van Keulen et al. 2008). It has been proposed that orally acquired prion diseases can also reach the brain through the bloodstream (Caughey et al. 2009; Gough and Maddison 2010), but infectivity is not detectable in the blood of BSE-affected cows (van Keulen et al. 2008). This is in contrast to experimental BSE in sheep and human vCJD, in which GALT shows high levels of infectivity and the blood also contains prions (Gough and Maddison 2010).

The role of replication of PrP<sup>Sc</sup> in FDCs of the spleen in propagation of the agent is unclear, and may vary between species. Studies of scrapie have provided evidence that depletion of FDCs prevents or delays neuroinvasion, that increased nerve supply to the spleen promotes neuroinvasion and that denervation of the spleen delays or prevents neuroinvasion (Gains and LeBlanc 2007). Splenic PrP<sup>Sc</sup> is found in BSE infection of mice expressing the ovine prion protein (Baron et al. 2010). However, splenic PrP<sup>Sc</sup> was detected in only one of three cattle terminated in the advanced clinical stage of BSE (Murayama et al. 2010).

Once a cell is infected with PrP<sup>Sc</sup>, spread of infection to adjacent cells may occur by transfer of PrP<sup>Sc</sup>-containing membrane microparticles. Consistent with this hypothesis, it has been shown that PrP<sup>Sc</sup> can be released from infected cells *in vitro* in association with exosomes. Exosomes are small membrane-bound vesicles that can be secreted by cells and can fuse with other cells. However, although exosome production by lymphoid cells has been demonstrated, exosomes have not been shown to be produced by neurons. Another possible route by which PrP<sup>Sc</sup> could be transferred between adjacent cells is via tunnelling nanotubes, thin membranous bridges that can form between cells and allow the transfer of organelles, plasma membrane components, cytoplasmic molecules and pathogens (Caughey et al. 2009). Other proposed pathways of propagation within the nervous system include axonal transport, sequential infection of Schwann cells (cells that support and insulate peripheral nerves) and via the flow of lymph in the vicinity of neurons (Kovacs and Budka 2008).

The molecular pathways leading to cerebral damage are largely unknown, although various theories have been advanced. Depletion of PrP<sup>C</sup> does not appear to be a cause, as mice that have been genetically engineered to lack PrP<sup>C</sup> altogether, and those in which PrP<sup>C</sup> expression is turned off in adulthood, do not develop clinical signs of TSE. In fact depletion of PrP<sup>C</sup> in mice with established prion infection has been shown to reverse early spongiform degeneration and prevent progression to clinical disease. These findings suggest that the toxicity of PrP<sup>Sc</sup> depends on some PrP<sup>C</sup>-dependent process (Aguzzi and Calella 2009). It has been suggested that PrP<sup>C</sup> is neuroprotective and its conversion to PrP<sup>Sc</sup> interferes with this function and allows neurodegeneration (Caughey et al. 2009; Solomon et al. 2009). Another possibility is that binding of PrP<sup>Sc</sup> to PrP<sup>C</sup> triggers a signal transduction pathway leading to neuronal damage (Soto and Satani 2011).

Other theories of PrP<sup>Sc</sup> pathogenicity, based around *in vitro* observations, include impairment of breakdown of cellular waste by lysosomes, up-regulation of genes involved in endoplasmic reticulum function, and reduced degradation of proteins by the proteasome system (Kovacs and Budka 2008; Chakrabarti et al. 2009). These various theories are not mutually exclusive.

## Infectivity

There is no robust evidence that BSE can be transmitted between cattle by routes other than consumption of feed contaminated with certain tissues from BSE-infected cattle. This is in marked contrast to the horizontal infectivity of scrapie in sheep and chronic wasting disease (CWD) in deer. CWD prions are found in saliva, urine, faeces, placenta or decomposed carcasses (Gough and Maddison 2010; Haley et al. 2011; Imran and Mahmood 2011b). PrP<sup>Sc</sup> from scrapie-infected sheep is found in faeces, milk, saliva, nasal secretions and placental tissues. Scrapie and CWD prions have also been shown to persist in the environment, bound to soil or other fomites, but there is no evidence that BSE has been transmitted between cattle by this route, or via exposure to excreta or secretions (Gough and Maddison 2010).

There is no evidence that vCJD has been transmitted between humans except due to medical intervention such as blood transfusion (Brown and Mastrianni 2010).

## Modes of transmission

The epidemic of BSE, first recognized in 1986 in the UK, was propagated by the rendering of dead cattle infected with BSE to produce MBM which was then included in feed for cattle (Harman and Silva 2009). Ingestion of infectious material in MBM made from BSE-infected animals was the only known route of transmission of the agent between cattle.

Consumption of beef contaminated with infected bovine central nervous system tissue also led to an epidemic of vCJD in humans. Although the majority of vCJD cases have been attributed to consumption of such contaminated beef, four cases of person-to-person vCJD transmission by blood or plasma transfusion have been reported in the UK (PHE 2009; Imran and Mahmood 2011a).

Rare cases of transmission of sCJD between humans have resulted from corneal grafts, dura mater grafts and growth hormone injections (Brown and Mastrianni 2010). Similar transmission of vCJD remains a concern because retrospective analysis of tonsil and appendix specimens led to the estimation that up to 1 in 4,000 persons exposed during the UK epidemic may be a sub-clinical carrier (Harman and Silva 2009; Collinge 2012).

There is no evidence that sCJD is a TSE of animal origin because this disease develops even among lifelong vegetarians (Harman and Silva 2009).

## Incidence of illness and outbreak data

### Animals

More than 184,000 cases of classical BSE have been diagnosed in cattle, and at the peak of the epidemic 1,000 cases were being diagnosed each week in the UK (Imran and Mahmood 2011b; OIE 2013). The epidemic is believed to have been amplified from a single common source (Aguzzi and Calella 2009). The infection was spread elsewhere in Europe and the

world by exports of infected cattle and MBM from the UK (Seuberlich et al. 2010). The feeding of MBM to cattle was banned in the UK in 1988, and in 1996 it became illegal in the UK to prepare any feed containing any mammalian protein for any farm animal. However, because of the long incubation period of BSE cases continued to occur, peaking in 1992. The incidence of new cases has steadily declined since then, and the disease is now very rare (Hueston and Bryant 2005).

A number of zoo and domestic animals developed TSEs at the same time as the BSE epidemic in cattle. All the species affected belonged to either the *Bovidae* or *Felidae* family, with the exceptions of a small number of non-human primates (Imran and Mahmood 2011b). All cases in zoo animals were attributed to ingestion of infective material derived from bovine BSE cases, as were two cases in domestic goats (Spiropoulos et al. 2011). A number of domestic cats developed TSE concurrently with the bovine BSE epidemic, and these cases were attributed to consumption of infective material in beef or beef-derived pet food (Harman and Silva 2009; Imran and Mahmood 2011b).

Epidemics of TSEs were not observed in other domestic species at the same time. Dogs and horses express PrP<sup>C</sup> with a very stable structure that is resistant to mis-folding, and these species are resistant to infection with PrP<sup>Sc</sup> (Harman and Silva 2009; Zhang 2011). Pigs are highly resistant to oral infection with the BSE prion, but may be infected by parenteral challenge (Harman and Silva 2009).

The original source of the classical BSE epidemic has been the subject of much conjecture but remains unknown. Surveillance in some countries has shown that sporadic cases of BSE occur in cattle, although to date only two strains distinct from 'classical' BSE, the L- and H-type strains, have been observed. However, it is possible that classical BSE may also occur spontaneously and that the epidemic represented amplification of infective material originating from a single sporadic case. It is also possible that the BSE epidemic originated from material from another species also rendered to produce MBM. It has been suggested that the BSE epidemic arose from rendering of scrapie-infected sheep. However, although sheep scrapie samples can cause a TSE when inoculated intracerebrally into cattle, the disease does not resemble BSE, and experimental BSE in sheep does not resemble scrapie. In fact cattle are resistant to oral infection with scrapie or CWD (Harman and Silva 2009).

## Humans

The first 10 human patients with vCJD were reported in April 1996 in the UK (Mackay et al. 2011; Imran and Mahmood 2011a). As of December 2012, 227 vCJD cases have been reported in total. Current data may be found on the UK National Creutzfeldt-Jakob Disease Research & Surveillance Unit website, <http://www.cjd.ed.ac.uk>. The majority of cases (176) occurred in the UK (Imran and Mahmood 2011a).

There is strong evidence to show that vCJD is caused by ingestion of BSE infective material. Classical BSE prions from affected cows and vCJD prions from the brains of infected humans produce the same lesions in mice. The biochemical properties of BSE prions from cattle and vCJD prions from humans are indistinguishable. Furthermore the great majority of vCJD cases have occurred in the UK, with a few cases in other countries including France, Ireland and Italy (Aguzzi and Calella 2009).

A two-fold difference was seen between the prevalence of vCJD in the north versus the south of the UK. Contemporaneous National Dietary Surveys showed that consumption of mechanically recovered beef products was much higher in the north of the UK (Mackay et al. 2011). Mechanically recovered beef is more likely to be contaminated with infective material

in the spinal cord, and recovery of beef by this method is no longer permitted for human foodstuffs across Europe.

### Occurrence in food

The incidence of classical BSE in cattle has declined markedly since the 1990s through prevention measures based on knowledge of how the disease is transmitted between cattle. There are now very few cases of BSE reported in cattle worldwide (OIE 2013).

A key component of prevention of both BSE in cattle and vCJD in humans is the prohibition on feeding ruminant derived protein to ruminants. This measure was enacted in 1994 in the UK when it became illegal to feed ruminants with mammalian proteins, with specific exceptions such as dairy proteins. The feed ban was further extended in 1996 by a ban on feeding any farmed livestock, including fish and horses, with mammalian MBM. Feeding of mammalian derived proteins was prohibited throughout the European Union (EU) in 1994 (EC No. 94/381). This was further extended in EU Regulation (EC No. 999/2001) which introduced further EU-wide controls to combat the spread of BSE, including a ban on the feeding of processed animal proteins to any animals kept, fattened or bred for the production of food.

With regards to infectivity in food, the risk of exposure of humans to BSE can essentially be removed by withholding the particular lymphoid and central nervous tissues known to harbour infectivity, termed specific risk materials (SRM), from the food supply. The list of SRM, and the ages of cattle from which they must be removed, have been modified over time with advancing knowledge, but currently include brain, eyes, tonsils, spinal cord and intestines, as well as the entire spinal column of older cattle. Bans on the use of SRM from bovine carcasses above specified ages have been implemented throughout Europe and other countries to ensure the safety of beef and beef products. Slaughter procedures of cattle are designed to prevent contamination of the carcass with SRM, and SRM are rendered and incinerated to destroy infectivity in countries with BSE risk factors. In addition, mechanical recovery of meat from bones is prohibited in order to prevent inclusion of dorsal root ganglia, which may contain infectivity. Beef and beef products from countries with these and animal feed control systems are therefore considered to be safe for human consumption. Countries assessed as being of negligible risk of BSE in the cattle population are not required to practise these precautions.

### Human host factors that influence disease

A polymorphism at position 129 of the PrP<sup>C</sup> amino acid sequence has been identified in humans, with different genotypes exhibiting different susceptibilities to TSEs. Approximately 40% of Caucasians are homozygous for methionine (Met) at this position, 10% are homozygous for valine (Val) and 50% are Met/Val heterozygotes. To date, all confirmed clinical cases of vCJD have been homozygous for Met at codon 129 (Mackay et al. 2011). A presumptive clinical case in a Met/Val heterozygote was reported in 2009, but an autopsy was not done and the MRI findings were not typical of vCJD (Kaski et al. 2009; Mackay et al. 2011). A confirmed pre-clinical infection in a Met/Val heterozygote was identified in a patient who died of a ruptured aortic aneurysm five years after receiving a blood transfusion from a person who subsequently developed vCJD. The Met/Val heterozygote had PrP<sup>Sc</sup> in the spleen but not in the central nervous system, and there was no evidence of central nervous pathology typical of vCJD (Gains and LeBlanc 2007; Mackay et al. 2011). The three known clinical cases of vCJD infection by blood transfusion were all Met/Met homozygotes (Mackay et al. 2011). Two of three PrP<sup>Sc</sup>-positive samples in an anonymous postsurgical study of appendices were from Val/Val homozygotes. This indicates that lymphoid tissue, at least, of

all three genotypes may become infected (Harman and Silva 2009; Will 2010; Mackay et al. 2011).

It is not yet clear whether the Met/Val and Val/Val genotypes are protective against neurological infection with vCJD, or whether onset is delayed rather than prevented (Mackay et al. 2011). Some authors have predicted a multiphasic vCJD epidemic with a late peak of cases affecting people heterozygous at codon 129 (Aguzzi and Calella 2009; Will 2010). Besides vCJD, the only other orally acquired prion disease known in humans is kuru, a historical disease of a small number of communities in Papua New Guinea who propagated the disease through the practice of funerary cannibalism. The mean incubation period of kuru is 12 years, but the incubation period has exceeded 50 years in some individuals (Imran and Mahmood 2011a). Retrospective analysis of blood samples from kuru patients shows an age stratification of codon 129 genotype. The young kuru patients were mainly Met/Met or Val/Val homozygotes, whereas the elderly patients were mostly Met/Val heterozygotes. Eight of eleven of the more recent cases of kuru were Met/Val heterozygotes, which supports the hypothesis that the Met/Val genotype delays but does not prevent the onset of kuru in all individuals, because exposure of these individuals almost certainly ended more than 40 years ago when funerary cannibalism was outlawed (Mackay et al. 2011).

The majority of vCJD cases in the UK affected people less than 40 years of age. Possible explanations include a higher rate of dietary exposure, increased susceptibility to infection or a reduced incubation period in this age group. Greater susceptibility could be conferred by the volume of GALT, which declines with age (Mackay et al. 2011). In addition, Peyer's patches that are thought to be involved in intestinal uptake of prions, decline during adulthood (St Rose et al. 2006).

### Infective dose

It appears that ingestion of less than 1 mg of infected brain material may be sufficient to transmit infection between cattle (Harman and Silva 2009). Transmission of BSE to macaques has been accomplished by oral administration of 5 g of infective brain homogenate, but the infective dose of bovine PrP<sup>Sc</sup> to human beings is unknown (Mackay et al. 2011).

### Recommended reading and useful links

European Commission Food and Feed Safety page on BSE:  
[http://ec.europa.eu/food/food/biosafety/tse\\_bse/index\\_en.htm](http://ec.europa.eu/food/food/biosafety/tse_bse/index_en.htm)

World Health Organization Media Centre page on vCJD:  
<http://www.who.int/mediacentre/factsheets/fs180/en/>

FSANZ Consumer Information on BSE  
<http://www.foodstandards.gov.au/industry/bse/Pages/default.aspx>

UK National Creutzfeldt-Jakob Disease Research & Surveillance Unit website  
<http://www.cjd.ed.ac.uk>

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