vCJD: Potential for a large epidemic - Are dental procedures aiding its spread?

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Abstract

The emergence of *v*CJD, together with the varying incubation period of prion proteins and their resistance to standard sterilisation, raises the possibility that *v*CJD may currently be spreading unnoticed through the population, and routine dental treatments as well as other invasive procedures may be aiding this. Transmissible spongiform encephalopathies (TSEs) are not known to spread by contact from person to person, but transmission can occur during invasive medical interventions. Exposure to infectious material through the use of human cadaveric-derived pituitary hormones, dural and cornea homografts and contaminated neurosurgical instruments have all caused TSEs. The current *v*CJD epidemic in Britain has raised many fears, as no scientist knows the current number of people infected, with estimations ranging from 60 to several million carriers. The potential for iatrogenic spread, via surgical procedures, through the population is also unknown. Few papers have been written on the potential of dentistry to spread *v*CJD. Epidemiological

studies have not revealed any evidence that dental procedures lead to an increased risk of iatrogenic transmission of *v*CJD. However, experimental studies have demonstrated that animals infected by intra-peritoneal inoculation develop a significant level of infectivity in gingival and dental pulp tissues and that TSEs can be transmitted to healthy animals by exposing root canals and gingival abrasions to infectious brain homogenates. Even The World Health Organisation committee on TSEs was, "unable to come to a consensus on the risk of transmission of TSEs through major dental procedures," showing the need for clarification on the issue. This dissertation intends to evaluate the current evidence and ideas on the *v*CJD epidemic, estimate the number of people that could be infected and assess the risk, if any, of these people transmitting *v*CJD via surgical and in particular dental procedures.

Introduction

Creutzfeldt-Jakob disease (CJD) is one of four Transmissible Spongiform Encephalopathies (or prion diseases), which are fatal neurodegenerative disorders effecting humans, with a global incidence of 1 or 2 per million. It, like other TSEs are characterised by spongiform degeneration of the brain, the primary symptoms of which include, severe depression, dementia, ataxia and myoclonus (muscle twitching), death ensues within 6 months of primary symptoms. Bovine spongiform encephalopathy (BSE) and scrapie are examples of prion diseases effecting animals, in this case cattle and sheep respectively. Prion diseases are unique in the fact they are both inherited and infectious.

Prions may be defined as a proteinaceous infectious particle that lacks nucleic acid [Prusiner 1997]. Prion protein (PrP) is encoded by a gene on chromosome 20 and has 253 amino acids [Oesch et al 1985], it is believed to function in the transport of copper ions [Brown et al 1997]. They exists in two isoforms, with identical amino acid sequence, but with drastically different conformations: PrP^c, the conventional isoform, primarily expressed in the central nervous system and the infectious PrP^{sc}, responsible for prion diseases. Prusiner (1998) hypothesised, in his "Prion concept," Prion diseases result from the conformational conversion from PrP^c to PrP^{sc}, which he believes occurs when host PrP^c, either, mutates into PrP^{sc} (somatic, spontaneous or germ-line mutations) or interacts with an exogenous PrP^{sc} to convert into one, his work won him the Nobel prize for Medicine in 1997. The main evidence for the prion concept came from Bueller and Sailor et al in

1993 and 1994 when they showed that transgenic mice devoid of the PrP gene were resistant to scrapie and prion propagation did not occur in these mice, indicating PrP^c must be present for PrP^{sc} to form. Pan et al (1993) showed that a proportion α - helical structure in PrP^c is refolded into β - sheets in the infectious PrP^{sc}. This is the currently accepted theory, as it explains all the current observations on prion diseases. However, it must be noted that definitive evidence of the prion concept - by generating an infective PrP^{sc} by experimentally manipulating recombinant or synthetic PrP^c has not yet been achieved [Kocisko et al 1994], although this does not necessarily disprove the prion concept. PrPs are very resistant to all forms of biological inactivation; heat, surgical sterilization, chemical disinfection, uv light, gamma irradiation and proteolysis have little if any effect on prions, further adding to their novel and intriguing properties. There is currently no treatment for prion diseases.

Prion diseases

The human prion diseases can present as sporadic, genetic or infectious diseases. About 10% of CJD cases (*familial* CJD- *f*CJD) and all cases of Gerstmann-Straussler syndrome and Fatal Familial Insomnia (other prion diseases) are inherited in an autosomal dominant fashion [Young et al 1999]. These cases are all attributable to

germ-line mutations in the gene encoding PrP^c, about 20 different mutations have been found.

Sporadic CJD (*s*CJD) accounts for approximately 85% of cases and mainly occurs in the over seventies, they are believed to arise from somatic mutations in the PrP^c gene or the spontaneous conversion of PrP^c into PrP^{sc}. Infectious prion diseases include, Kuru of the Fore people in New Guinea, where the prion was spread by ritual cannibalism, although the occurrence of kuru drastically decreased with the cessation of cannibalism.

Iatrogenic CJD (*i*CJD), has been spread via various means: Over 90 young adults became infected with CJD after receiving CJD infected human growth hormone, the incubation periods ranged from 3 to more than 20 years [Billette de Villemeur et al 1996]. Dura mater grafts implants in Japan between January 1979 - September 1999 have caused over 57 cases of CJD, 54 of these patients had received the same brand of dura mater graft from the same processor [Hoshi et al 2000], with incubation periods ranged from 1 to more than 14 years.

New variant CJD (*v*CJD) first appeared in 1996 in England, it has killed 83 Britons (as of the 5th January 2001), with a further 5 still alive. *v*CJD differs from other forms of CJD in that the patients tend to be younger (average age is 27), the disease has a protracted course of approximately 16 months and their brains show numerous amyloid plaques surrounded by halo's of intense spongiform degeneration. Its' appearance correlated with the BSE epidemic in cattle.

This, the fact that BSE was able to infect mice and have similar incubation times to *v*CJD in mice [Bruce et al and Hill et al 1997], the observation that BSE produces numerous PrP plaques in macaques similar to those found in *v*CJD infected human brains [Lasmezas 1996] and the fact that PrP^{sc} seen in *v*CJD and BSE are predominantly diglycosylated compared to the predominantly monoglycosylated PrP^{sc} found in *s*CJD [Collinge et al 1996], have lead to the belief that BSE is the causative agent of *v*CJD. Although we are yet to discover why *v*CJD has a predilection for young adults and teenagers, with no cases above 50 reported.

BSE and vCJD

BSE reached epidemic proportions in cattle during the early 1990's, when it is estimated almost 1 million British cattle were infected. Its emergence was believed to be due to a change in the rendering process of the meat and bone meal (MBM) they were fed. MBM was prepared from the offal of chickens, sheep, pigs and cattle. In the late 1970's the hydrocarbon solvent extraction method used in the rendering of MBM was abandoned, resulting in an MBM of much higher fat content, this change is believed to have enabled scrapie prions from sheep to survive rendering and being passed on to cattle [Wilesmith et al 1988].

The mean incubation time for BSE is five years, the major worry is nobody knows the mean incubation time for vCJD, if it is the same then it can be assumed that no new cases of vCJD will arise from the direct transmission from cattle after 2004 (The government declared British beef safe in 1999 after killing more than 2 million cattle). New cases will only arise from the spread of vCJD in already infected individuals, via blood transfusions, tissue transplants or through contaminated surgical instruments. Thus, to assess the possibility of a vCJD epidemic, the major task is to find the incubation time of vCJD and its' ability to spread, unfortunately we can only do this by monitoring the numbers of individuals who are newly diagnosed, at which point it will be too late for them. New spongiform encephalopathies caused by BSE have been noted in animals including cats, monkeys, tigers and bisons, following consumption of contaminated bovine material, thus a further risk to human health from other species cannot be excluded at present.

The Scale of the Epidemic

To evaluate the risk of transmission of vCJD via contaminated surgical instruments or tissue/blood transplants, we must first consider the number of people likely to be infected with vCJD. This question has many estimates, ranging from 63 (already exceeded) to several million cases [Ghani et al 1998], depending on many

factors, including the incubation period, the number of humans infected from one bovine, the stage of incubation at which BSE infection was able to transmit to humans, the height of the species barrier between cattle and human beings, genetic susceptibility, route of exposure and the dose required for infection. However, none of these calculations take into account the fact that vCJD may be spreading through the population via surgical procedures.

Genetic Susceptibility

Collinge et al (1996a) analysed PrP genes from *v*CJD infected individuals and showed they were all homozygous for methionine at the polymorphic 129 codon, suggesting Met homozygosity increases the susceptibility to *v*CJD. Owen et al (1990) estimated about 40% of the Caucasian population in Britain are of this type, however he only used a sample size of 142 people. Worryingly, Hill et al (1997) engineered transgenic mice, which expressed PrP homozygous for valine at codon 129 and infected them with *v*CJD. These mice, on analysis were found to have a different PrP^{sc} type. It is unknown if this different PrP^{sc} (designated type 5) is able to lead to a clinical syndrome, but the concern is that even people without Met homozygosity at codon 129 may be at risk to a different form of *v*CJD. Further experiments to see if Val homozygosity or Met/Val heterozygosity lead to clinical syndromes in animals infected with *v*CJD are in order. Importantly no cases of *v*CJD have been diagnosed in people without homozygosity for Met at codon 129,

however this does not rule out the possibility that cases may arise in the future.

Species Barrier

Transmission of prion diseases between different mammalian species is limited by a so called "species barrier" [Prusiner 1998]. On primary passage of prions from species A to species B, typically not all inoculated animals of species B would succumb; those that did would do so with longer and more variable incubation periods than with transmission of prions within the same species, on which typically, all inoculated animals would succumb with a shorter and consistent incubation period. On second passage of infectivity to further animals of species B, transmission properties resemble within species transmissions [Collinge 1999]. Three factors have been identified that contribute to the species barrier: a) the difference in PrP sequences and conformations between the prion donor and recipient – similar sequences and conformations are believed to transmit more efficiently between species, b) the strain of prion – i.e. BSE and *v*CJD transmits between species more readily than classical CJD [Hill et al 1997], and c) the species specificity of protein X (a protein with a presently undefined role in the conversion of PrP^c to PrP^{sc} –believed to be a cofactor) [Prusiner 1998].

BSE can be readily transmitted to mice, which has enable extensive studies of the species barrier in mice. Collinge et al (1995) conducted a series of experiments in which, transgenic mice expressing, human PrP homozygous for Val at codon 129 and normal mouse PrP were inoculated with BSE. It was noted that only mouse prion replicated when inoculated with BSE, indicating a species barrier between humans and cattle larger than that which exists between mice and cattle. However, the authors noted that expression of human PrP in mouse cells is less efficient than the expression of mouse PrP, which may have contributed the results and led to an invalid conclusion. In further experiments conducted by the group, when transgenic mice only expressing human PrP were inoculated with BSE, only infrequent transmission of BSE occurred at over 500 days. Given the relative ease with which BSE normally transmits to wild type mice (200-400 days), this was taken as further evidence that the cattle – human species barrier was larger than the cattle – mice species barrier. This conclusion had two major faults, firstly the authors used a human PrP gene homozygous for Val at codon 129, which, as explained is the type in humans, which does not cause *v*CJD. In essence the experiments only verified that BSE may be transmissible to humans with Val homozygosity at codon 129. Secondly, it takes the lowest experimental values for the transmission of BSE to wild type mice (200-300 days), where as other authors [Lasmezas et al 1997] have shown the incubation period of BSE in mice is quite variable with incubation periods in excess of one and a half year observed. Further

experiments using human PrP homozygous for Met at 129 must be conducted to get a more accurate idea of the incubation time and species barrier between humans and cattle.

It is important to realise the only way of finding the true species barrier and incubation time will be to chart the number of cases over the next 30-40 years, at which point the epidemic will hopefully be over.

Epidemic estimations

Epidemic estimations have varied from around 60 people becoming infected to several million people becoming infected. The time and source of exposure to BSE of the current cases of *v*CJD are not known. Nor do we know whether the infection was acquired from a single high dose exposure or as a result of accumulation of dose over many years, this together with the small number of cases, have led to massive holes in our knowledge of *v*CJD. A simple test carried out amongst the public, that could detect *v*CJD infection, to determine how many people are incubating the disease, would be a great help in estimating the number of people infected. Unfortunately no such test currently exists, and current prion detection involves the laborious western blotting technique on infected tissue samples (the brain, tonsils and appendices) and even these methods have their limitations; we do not yet know, when in the incubation period *v*CJD become detectable in these tissues.

Much of the current estimates use assumptions on these factors, and as such, as will be seen, no estimation can be taken as a reliable indicator of the true number of *v*CJD cases in the years to come. Better estimations will only emerge after a number of years.

Kuru, the acquired human prion disease in the Fore region of Papua New Guinea, provided the largest experience of a prion epidemic. Rare cases of kuru were recorded in children as young as four and a half years, indicating a minimal incubation period of around this length or shorter (if vertical transmission is ruled out). It should also be noted that occasional cases of kuru are still occurring in the Fore region, consistent with an incubation period of over 60 years. Mean incubation periods have been estimated to be 12 years [Collinge 1999]. Further information on Kuru, could tell us the roughly how many people became infected per eaten body, which may give us an estimation of how many people may die from eating a cow.

Collinge (1999) correlated data for kuru and acquired CJD from the above mentioned tissue transplants, to estimate an incubation range of between 4 and 40 years with a mean of between 10 and 15 years. This, however does not take into account the major effect of the species barrier and is, I believe, probably a better estimation of the incubation period of *v*CJD when transmitted between humans (second passage transmission).

Using experimental data on the species barrier between cattle and mice, Collinge (1997) estimated, (assuming the

species barrier between humans and cattle is the same as mice and cattle) the mean incubation period to be approximately 30 years, with a range between 10 years to longer than a normal human lifespan, the working of this calculation was not published. The data he used for his estimations, did have a number of faults (discussed above), which combined with the relative small number of *v*CJD cases, would suggest caution on the estimation of the size of the epidemic barely five years after the first recorded case.

Ghani et al (1998) proposed a mathematical model to estimate the number of *v*CJD cases that may occur in the future. They found the two most important variables in determining the size of the future epidemic, were the incubation period of *v*CJD and the number of individuals infected from a maximally infectious bovine. There were many questionable assumptions in their model:

1) The BSE epidemic in cattle would have a similar pattern to the future *v*CJD epidemic.
2) *v*CJD only arises via ingestion of infected bovine and no other routes.
3) Only people with 129 codon Met/Met homozygosity are susceptible (even though people with Met/Val or Val/Val maybe susceptible).
4) The 1989 SBO ban was completely ineffective.

Further, for their mathematical model, they only used data from the first two years of the detection of *v*CJD, 1996 and 1997. Using 5 million stimulated epidemic scenarios they

estimated the potential epidemic to be between 29 and ten million cases and that the current small numbers of cases, may still correlate to a huge epidemic. This is shown in the figure below:

Fig. 1. Range of *v*CJD epidemic growth scales according to the number of people infected from a one BSE infected bovine (r), taking case history from 1996 and 1997.

In a more recent study by Ghani et al (2000a), a newer mathematical model using case numbers from 1996-1998, predicted the total number of *v*CJD cases;

Fig. 2 Range of *v*CJD epidemic sizes; using epidemiological data upto 2000. The number of people infected from a one BSE infected bovine = r.

They also hypothesised using their model, that if less than 15 cases are confirmed in 1999, assuming that *v*CJD only effects people homozygous for Met at codon 129, then the maximum epidemic size is reduced to 500,000 and if only 30 cases of *v*CJD are confirmed over the next two years the maximum epidemic size will be limited to 14,000. Unfortunately, at least 16 new cases occurred in 1999 [Andrews et al 2000] and more may come as a result of delays in confirming the diagnosis. 14 people died in the first six months of 2000 compared to 18 people in the whole of 1998, and the incidence appears to be rising.

Fig. 3 shows the observed and expected quarterly onsets and deaths from *v*CJD over the last five years [Andrews et al 2000]:

In a more recent analysis of their model Ghani et al (2000b) proposed that the maximum number of *v*CJD cases to be 136,000, assuming 14 cases of *v*CJD occurred in 1999.

Unfortunately, as stated, 16 new cases have now been confirmed in 1999.

Abnormal prion protein can be detected by western blot in tonsils and appendices [Hill et al 1999]. A recent government study of 3170 archived tonsil and appendix tissues (taken from operations carried out between 1996-1998), revealed no evidence of the abnormal prion protein PrP^{sc} associated with *v*CJD in any samples (as determined by western blot). Using a mathematical model proposed by Ghani et al (2000a), they concluded that the maximum number of cases in the *v*CJD epidemic to be less than 80,000. However there were many frivolous assumptions in this model. They assumed that the test could detect infection in the last 75% of the incubation period with 100% sensitivity and sensitivity. Given, that we do not know when in the incubation period *v*CJD becomes detectable, and the variable specificities and sensitivities of western blotting with *v*CJD [Dobson 2000], this is clearly not the case. The samples were not age categorised, i.e. *v*CJD is known to occur preferentially in the 20-35 year age group, given that the majority of tonsils and appendices are taken from children and young adults (with 50% of tonsils taken from children under 10 years) [Ghani et al 2000a], their value as samples maybe questioned. Also, we do not know the effect the archiving material used to preserve the samples has on prion proteins; it may serve to inactivate them over a period of time, or make the detection of prions harder. Researchers at the National CJD surveillance unit in Edinburgh and Derrisford Hospital in Plymouth are currently analysing some 18,000 appendix and tonsil

samples, using immunohistochemical methods to detect for prions, in order to find a better estimation of the true number of *v*CJD cases that will arise.

Anderson et al (1996) estimated 450,000 BSE infected cattle entered the food chain prior to the specified bovine (SBO) ban in 1989, with a further 280,000 entering between this time and 1999, when the government declared British beef safe. If we assume BSE first infected cattle in 1981, five years (the average incubation time of BSE) before the first clinical case, this means the shortest incubation period for *v*CJD is 11 to 15 years depending on when in the incubation period BSE became infectious to humans. It could, however, be argued that the shortest incubation time is 5 years if we assume that the first clinical case in 1996 occurred as a result of exposure to BSE in 1990, when BSE exposure was maximal, as opposed to 1981 when BSE had just infected cattle. Assuming the mean incubation period of *v*CJD is between 11-15 years, suggested by some authors, then, we may safely assume the epidemic will peak between 2001 and 2005 and only iatrogenic spread via surgical procedures will cause the epidemic to increase substantially after this. The major worry is if the mean incubation period is longer than this, which is more viable, as, 11-15 years is likely to be the shortest in the range of incubation periods and not the mean, the epidemic will increase in severity with time and more people will be at risk of infection from iatrogenic routes. We can also not discount the theory that all currently infected individuals gained the infection from a non-oral route, i.e. via cuts or conjunctiva routes, in which

case the incubation period is likely to be much lower than that of orally acquired *v*CJD.

As can be seen there is a large variation in estimations for the total number of *v*CJD cases in this epidemic. The only certainty is that if the number of cases continue to remain low then, the total number of cases in the epidemic will become lower and lower as time proceeds. As such the number of cases that occur in the next few years will be critical in determining the number of total cases in this epidemic.

Iatrogenic spread

Prion diseases are not transmitted via air, or person to person contact. The transmission via sexual contact or vertical transmission is unknown. The primary route of transmission is by eating contaminated tissue, or tissue contact with that of an infected individual. The main mechanism of iatrogenic spread is via surgical procedures - contaminated instruments or infected tissue transplants. From estimations of the numbers involved in the epidemic, it is clear that possible surgical transmission is a serious mechanism in epidemic propagation. CJD like diseases have arisen following prion transmission via infected, cadaver-derived growth hormone, pituitary gonadotrophins, duramater homografts and corneal grafts. As prions are resistant to conventional chemical, irradiation and sterilisation methods, the question arises whether

contaminated surgical instruments may also be able to spread *v*CJD.

In the only reported case [Bernoulli et al 1977], electrodes had been inserted into the cortex of a patient with unrecognised CJD, after normal disinfection with benzene, 70% ethanol and formaldehyde vapour, after each use, they were employed on two additional patients who subsequently developed CJD. The same electrode was then implanted into a chimpanzee [Gibbs et al 1994], where it again caused lethal spongiform encephalopathy.

The World Health Organisation (WHO) recommended surgical procedures on patients with suspected or confirmed prion diseases, should be managed as much as possible with single use disposable instruments and where non disposable instruments are needed, they should be incinerated along with disposable instruments, if they contact tissues of high infectivity, i.e. those displaying abundant PrPs (brain, spinal cord and eye), those that contact other tissues should be sterilised, using one of the following procedures [WHO Infection Control Guidelines for TSEs 1999]:

A number of controversial points must be made in regards to this report. First, and most importantly it must be acknowledged that the greatest potential for iatrogenic spread is not from those who are suspected of having CJD, but rather, those that are incubating infectious CJD without signs. Since we do not know how many are incubating CJD and when in the incubation periods CJD becomes transmissible, we cannot judge the true risk iatrogenic spread via surgical procedures poses. The WHO made all of these recommendations with respect to experiments, in which sterilisation treatments were carried out on scrapie (strains 22A and 139A) infected brain homogenates or CJD agents (not *v*CJD) [Kimberlin et al 1983, Brown et al 1986, Taguchi et al 1991]. *v*CJD has different biological, physical and chemical properties to these and leads to a different disease in humans [Prusiner 1998], thus it would be expected to behave differently to other prions with respect to sterilisation treatments.

Kimberlin et al (1983) conducted an experiment using mice infected with 22A or 139A strains of scrapie, they removed brain homogenates and subjected them to autoclaving and then inoculated indicator mice intracerebrally. They showed both strains were completely inactivated after autoclaving at 136°C for just 4 minutes, as no mice

developed signs after 500-600 days (100 days more than longest reported incubation period in mice). A study by Taylor et al (1994) similar to the fore mentioned study, in which, approximately 341mg BSE infected bovine brain homogenates were subjected to porous load autoclaving at temperatures between 134-138°C for 18 mins. Homogenates were then inoculated intracerebarally and intraperitoneally into 11 indicator mice. One of these mice developed the disease after 462 days, showing BSE was still infective, even after treatment which had inactivated some strains of scrapie. As *v*CJD is believed to originate from BSE, experiments with BSE may be more indicative, than scrapie, of the threat posed by contaminated (post-sterilised) surgical instruments. Unfortunately, this study did not examine the combined effect of immersion in NaOH and autoclaving (WHO recommendation), and also the time in the autoclave was much less, 18 minutes as opposed to the recommended 60 minutes. Thus, even though BSE is still infectious after the heat treatment stated, it may not be after treatments outlined by the WHO. A more insightful experiment, would involve *v*CJD or BSE infected homogenates subjected to the recommendations of the WHO and then inoculated into indicator mice, unfortunately this is not a quick experiment and results may take 2-3 years to accumulate due to the incubation period of *v*CJD and BSE, in mice. A further experiment by the group, in which hamster brains homogenates infected with different strains of scrapie (ME7 and 263K) were autoclaved at 134°C for 60 mins and then injected intracerebrally into indicator mice and hamsters, revealed that the autoclaving, even for the amount of time prescribed

by WHO was ineffective, as the mice and hamsters inoculated still developed the disease. This indicated some strains of scrapie were more resistant to the sterilisation methods that other strains (used in experiments cited by WHO – Kimberlin et al 1983) were susceptible to. It may be that *v*CJD is similar to these resistant strains rather than the strains used in experiments cited by the WHO. Again, combined treatment with NaOH was not used, thus the WHO guidelines for the sterilisation of *v*CJD contaminated instruments cannot be disproved.

It would be irresponsible to suggest that protocol which leads to the inactivation of some strains of scrapie, would lead to the inactivation of *v*CJD, merely because it is also a prion protein, and as such further experiments using *v*CJD infected brain homogenates must be conducted. It should also be noted all experiments in this field have involved using infected brain homogenates and not prion contaminated instruments. Better experiments would involve using surgical instruments to incise *v*CJD infected mice and then subjecting these to the sterilisation protocol recommended, and further indicator mouse inoculation. This would give a better indication of the risk that contaminated (sterilised) instruments pose. It may be that micro-irregularities on the surface of instruments retain prions and increase the resistance to sterilisation. It could be argued that the current line of experiments are analogous to tissue transplantation after sterilisation and not to the possibility of iatrogenic spreads via contaminated surgical instruments. Also, after reviewing the current experimental evidence (above and not shown) it is apparent that

meaningful conclusions cannot be drawn on the infectivity of autoclaved prion-exposed tissues due to the large variety of experimental protocols used; different autoclaves, different prion strains, different sterilisation treatments, different methods of prion detection, different methods of mouse inoculation, etc.

Collins et al (1999) conducted a epidemiological study in Australia, of the medical histories of 241 patients with *s*CJD (neuropathologically confirmed), between 1970 and 1997 and 784 controls matched by age, sex and area of residence. Information including previous surgery, major dental treatment, blood transfusion, stays in the UK and work on a farm, was obtained. Surgical procedures were found to be significantly associated with the development of *s*CJD, this risk progressively increased with the number of surgical procedures to a maximum to three procedures. They found no significant risk associated with a history of blood transfusion, organ transplantation and major dental treatment, as shown:

Table 1: Risk of *s*CJD by medical and demographic variables:

Table 2: Risk of *s*CJD by surgical procedures

In all patients surveyed history of potential Iatrogenic transmission in the form of dura mater or corneal grafts or exposure to human cadaveric pituitary hormones was sought after and excluded. Major dental work was defined as that beyond fillings and dental hygiene. They concluded that a proportion of *s*CJD cases were likely to be caused by contaminated surgical instruments, instead of the somatic

gene mutation which is believed to cause *s*CJD. An earlier study by Davanipour et al (1985) also came to the same conclusion. A contradictory conclusion came from Harries-Jones et al (1988), who suggested their was no correlation between past surgery and *s*CJD, however, their study was flawed as their controls all came from hospital wards and not the community, and as such most were undergoing or had previously had surgical procedures, which biased the study.

As yet there is still no evidence that surgical instruments can transmit *v*CJD, although it is clear that current surgical instrument sterilisation protocol used across the world - autoclaving at 124-124°C for 15 minutes (1.1-1.25 bar pressure) or at 134-137°C for 3 minutes (2.1-2.3 bar), is clearly not effective against prion proteins. Thus, surgical instruments coming in contact with *v*CJD infected tissues, of undiagnosed patients, present a theoretical risk of *v*CJD transmission. Definitive evidence (by the experiments suggested above) must be gained to assess the real risk posed by surgical instruments.

Iatrogenic Spread via Dental Procedures

In a relatively new development, it has been recognised that dental instruments, which are not routinely sterilised

against prion proteins, maybe aiding the spread of *v*CJD through the population unnoticed. Given the estimations of the numbers of people infected by *v*CJD, in theory if any of these visited a dentist for an invasive procedure, the instruments used would be contaminated with *v*CJD. These would further infect other patients, elevating the numbers in this epidemic. As discussed above surgical instruments are theoretically capable of transmitting *v*CJD (although there is no definitive evidence), the possibility of dental procedures aiding their transmission, is a relatively neglected subject with only 2 papers published [Ingrosso et al 1999 and Grossard et al 2000]. This section intends to evaluate their experiments, to assess the risk of iatrogenic spread via contaminated dental instruments.

Adams and Edgar (1978) preformed a study in which scrapie (ME7 strain) infected mice, had their gingival tissue traumatised by a dental bur, which was then directly (un-sterilised) used, to lacerate the gingival of healthy indicator mice. After 15 months the recipient mice were sacrificed. None of the mice showed neuropathological astrocytosis, characteristic of scrapie infection. However, in a separate experiment by the group, indicator mice injected intra-peritoneally (i.p) with 5mg of infected gingival tissue did develop scrapie. Indicating scrapie was present in gingival tissues. Dental burs weighed before and after traumatising infected tissue, revealed that 0.05-0.1mg of infected tissue had contaminated the bur. The authors estimated that if all of this was transferred to recipient mice, the chance of transmission would be 1 in 50, although, it must be noted that the burs used did not undergo any cleaning, which may

have removed some of the scrapie. *v*CJD, in theory, would be easier to transmit, as the level of infectivity is higher in tissues outside the CNS than of any other prion diseases, including scrapie [Hill et al 1999] and if it displays characteristics similar to BSE, it would also be more resistant to sterilisation.

In a more recent study by Ingrosso et al (1999), indicator hamsters were injected intra-dentally (i.d) - into the lower left incisor pulp, or i.p - into the abdomen, with 236K scrapie infected hamster brain homogenates. The hamsters were then sacrificed, gingival tissues and pulps were removed and inoculated, intra-cerebrally (i.c) into new uninfected recipient hamsters. After i.p inoculation, infectivity was found in the gingiva and pulp, as recipient hamsters inoculated i.c with these tissues, developed scrapie after an average of 79 and 96 days respectively. After i.d inoculation, all hamsters developed scrapie after an average of 152 days, before being sacrificed. These experiments demonstrated that oral tissues bear a substantial level of infectivity, and further scrapie could be efficiently transmitted via tooth pulp inoculation. It must be recognised that these experiments involved scrapie and not *v*CJD, and that tissue, and not contaminated instruments were used to transfer the scrapie. Thus, while scrapie can be transmitted via tooth pulp, when inoculated with infected tissues, there is no evidence to suggest that contaminated dental instruments can transmit *v*CJD. Although, given that *v*CJD carriers have higher levels of PrP^{sc}, and associated infectivity in peripheral tissues than other prion diseases, and that dental instruments are

inadequately sterilised against *v*CJD, it is very plausible that *v*CJD may be able to be transmitted via dental instruments.

Blanqeut-Gossard et al (2000) conducted a study of the detection of prion proteins in the pulp of a *s*CJD infected patient. They detected no trace, although they acknowledged that their detection protocol (western blot) was 3-4 orders less sensitive than that required to detect the amount of infectious PrP^{sc} believed to be present in the pulp (0.04 $\log_{10}LD_{50}$/mg). They also failed to acknowledge that *v*CJD is present in much higher concentrations in peripheral tissues than *s*CJD [Hill et al 1999]. It is important for future studies to determine if *v*CJD is present in the pulp and further, to determine if dental instruments contaminated with *v*CJD are capable of transmitting it.

On the 5th of January 2001 the Government announced that all tonsil operations should be carried out using disposable instruments. Bearing in mind, that there is no evidence that surgical instruments used in tonsil operations can transmit *v*CJD, and that most tonsil operations are carried out in children, who do not generally acquire *v*CJD. Given also, that oral tissues (gingiva and pulp) are more than likely to harbour infectious *v*CJD, a logical argument based around the evidence above may suggest that all invasive dental procedures should also be conducted using disposable instruments. It is important to find out the level of *v*CJD infectivity in dental tissues compared to that of the tonsils, for a balanced opinion on such an argument.

Normal prion protein has not been detected in saliva [WHO Infection Control Guidelines for TSEs 1999 – experimental data not shown], but the saliva of infected people has not been tested for evidence of *v*CJD. In theory, due to the proximity of the tonsils (which are known to harbour infectious *v*CJD [Hill et al 1999]), it maybe envisaged that saliva from a non-symptomatic infected patient may contain the infectious *v*CJD. During a dental procedure, the instruments used may then become contaminated, at which point other patients treated with the same (inadequately sterilised) instruments would become infected. Currently, there is no evidence that saliva is able to transmit *v*CJD and studies to determine this must be carried out.

Epidemiological studies for *s*CJD [Collins et al 1999] have not revealed any link between its incidence and major dental procedures, however, given that scrapie has been found in oral tissues (pulp and gingiva) at infectious concentrations, it is more than likely that *v*CJD maybe present, at infectious levels, in oral tissues. The big question, can *v*CJD be transmitted via dental instruments, still remains unanswered. From the evidence provided, if it is transmissible, it will be at a very low frequency; thus it will not be responsible for increasing the epidemic by a

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