THE DENTAL CLINICS OF NORTH AMERICA

Dent Clin N Am 47 (2003) 493–516

Prions and the human transmissible spongiform encephalopathies

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Transmissible spongiform encephalopathies (TSEs) are a group of lethal degenerative brain diseases (encephalopathies) characterized by the appearance of microscopic vacuoles in the brain gray matter, giving a spongelike (spongiform) appearance. TSEs were originally termed *slow virus infections*, although no virus has yet been associated with the disease. Current evidence points to the specific association of an abnormal form of a host-encoded protein termed *prion* (proteinaceous infectious particle). Prions are composed of a cell surface glycoprotein designated PrP (protease-resistant protein) [1,2]. Prion diseases appear to have a common mechanism of pathogenesis, namely, the accumulation of a protease-resistant isoform (PrP^{sc}) in the brain. Recognized in animals and humans, all TSEs have prolonged incubation periods of months to years, gradually increase in severity, and lead to death over months or years, with varying amounts of PrP^{sc} accumulated in the central nervous system (CNS). The classic example of a human TSE is the progressive brain disease Creutzfeldt-Jakob disease (CJD), first recognized in the 1920s; its transmissibility to primates was shown in 1968 [3]. There are several well-recognized animal TSEs including bovine spongiform encephalopathy (BSE; also known as "mad cow disease") in cattle and scrapie in sheep and goats [4].

The human TSEs present several significant challenges to health care workers, including those in dentistry. At present, there is no form of

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doi:10.1016/S0011-8532(03)00017-X

treatment or prophylaxis for this invariably fatal disease. Furthermore, a unique feature of the abnormal prion protein is its remarkable resistance to inactivation by conventional methods, which presents significant infection control problems when patients with TSEs undergo medical or dental interventions.

Nature of the TSE infectious agents

Historically, there have been a number of hypotheses to explain the nature and behavior of the TSE agents. To account for the behavior of the infectious agent by conventional concepts such as strain variation, it has been proposed that the agent comprises a conventional genome with a small amount of nucleic acid (ie, virinos or unconventional viruses) [5]. Alternatively, the TSE agents may be composed entirely of proteins (ie, they are prions, as discussed previously). The protein-only hypothesis suggests that the infectious agent (the prion—PrP^{sc}) is an abnormally shaped form of the normal prion protein (PrP^{c}) [6]. The two forms of the prion protein are chemically identical and differ only in their three-dimensional shape. PrP^{sc} is known to contain a higher β-sheet content than that of PrP^{c}. Prion replication requires the conversion of PrP^{c} into the abnormal form. Relatively little is known about the molecular state of the protein that constitutes the self-propagating infectious unit. A single infectious unit corresponds to approximately 10^5 PrP molecules [6]. It is unknown, however, whether this large aggregate is necessary for infectivity or whether a single molecule alone is infectious. The abnormal prions accumulate in brain tissue, sometimes forming amyloidlike deposits. In man, the normal prion protein (PrP) is encoded by a single-copy host PrP gene on chromosome 20 [7,8]. The normal or cellular form of the prion protein (PrP^{c}) exists as soluble protease-sensitive, cell surface protein on many cells, especially in the CNS and lymphoreticular tissue. PrP^{c} is rich in α-helical structures and is continuously recycled through endocytosis, but its function is currently unknown. In humans, the prion protein gene on chromosome 20 codes for both the normal (PrP^{c}) and abnormal (PrP^{sc}) forms of the protein (the latter is an altered form of prion protein that is partially resistant to proteinase K digestion) [1]. The influence of the host genetic background became apparent after investigators demonstrated that deletion of the PrP^{c} gene protects mice from experimental scrapie following their exposure to scrapie prions. Deletion of the PrP^{c} gene did not affect murine development, viability, or life expectancy [9]. The discovery of doppel, a new PrP^{c}-like protein coded for by the *Prnd* gene, may shed some light on the precise role of PrP^{c} [10].

PrP^{sc} seems to be composed mainly of β-pleated sheet and is postulated to act as a conformational template that promotes the conversion of PrP^{c} to further PrP^{sc}. This conversion continues until a stable seed is formed. This unit may continue to grow by accretion and divide by breaking into smaller infectious units, eventually leading to the accumulation of further insoluble

PrP^{sc} in neural cells, disrupting function, and leading to vacuolization and cell death. Prion replication, with recruitment of PrP^{c} into the aggregated PrP^{sc} isoform, may be initiated by a pathogenic mutation (resulting in a PrP^{c} predisposed to form PrP^{sc}) in inherited prion diseases [familial CJD (fCJD), Gerstmann-Straussler-Scheinker syndrome, and fatal familial insomnia) by exposure to a seed of PrP^{sc} in acquired disease [variant CJD (vCJD) or iatrogenic CJD (iCJD)] or as a result of the spontaneous conversion of PrP^{c} to PrP^{sc} (and subsequent formation of aggregated material) as a rare event in sporadic CJD (sCJD) [11].

Prions do not evoke a protective immune response, although attempts at vaccine production are underway [12]. In all species affected by TSEs, infectivity is highest in brain tissue and is also present in some peripheral tissues but appears generally absent from most body fluids including saliva [13]. This area has been extensively reviewed [1,2].

Animal forms of TSEs

There are a number of groups of animal TSE agents. One group consists of scrapie, which has a worldwide distribution, and BSE, first recognized in the United Kingdom. Chronic wasting disease of mule deer and Rocky Mountain elk occurs only in Colorado and Wyoming [14]. Transmissible mink encephalopathy exists as two strains and was first described in Wisconsin in 1947 [15]. BSE was first recognized in United Kingdom cattle in 1986, although cattle were probably infected a decade earlier than this. The disease has accounted, directly or indirectly, for the deaths of over 4 million cattle. The infective agent of BSE was spread by the use of meat and bonemeal in cattle food. The meat and bonemeal was infective due to the rendering of contaminated offal from cattle with BSE or those incubating the disease. In June 1988, the United Kingdom government made BSE a notifiable disease, and a month later banned the supply and use of ruminant-derived protein in ruminant feed. The peak incidence of BSE occurred in 1992, when 36,597 confirmed cases were reported. In 1996, a ban on the use of cattle over 30 months for human consumption was implemented. As part of the over-30-month scheme, 3.3 million cattle were slaughtered and destroyed, although subsequent disposal of this volume of carcasses has proved problematic. The primary origin of BSE is unclear at present because the BSE agent does not resemble any scrapie agents so far examined [16], although the full extent of scrapie strain variation from the United Kingdom flock is unknown.

In 1990, TSE appeared in domestic cats in the United Kingdom, suggesting transmission of TSE in pet foods and that the causative agent was not species specific. Domestic cats were the sixth species in which a scrapielike spongiform encephalopathy was diagnosed; other affected species included a number of captive animals (eg, Arabian oryx, scimitar horned oryx, greater kudu, eland, puma, cheetah, ocelot, and tiger) [4,17].

In 1996, a variant of sporadic CJD (vCJD) was observed in humans in the United Kingdom, and linked with the consumption of infected bovine offal [18]. Some TSEs have clearly spread across species barriers.

Animal studies showing prions in the trigeminal nerve, tooth pulp, gingiva, and salivary glands

Several investigators have demonstrated immunoreactivity to PrP^{sc} in the trigeminal ganglion and peripheral nerves. For example, Wells et al [19] observed infectivity in the trigeminal ganglion during preclinical phases of BSE in cattle exposed orally to BSE agent. In experimental scrapie in sheep and hamsters, PrP^{sc} has been detected in the trigeminal ganglion [20]. The normal cellular isoform of PrP has been shown to move by rapid anterograde axonal transport [21]. Some investigators have failed to detect prions in dorsal root ganglia of mice infected intraperitoneally [22]. In Syrian hamsters, however, infectivity appeared in trigeminal ganglion, dental pulpal tissue, and gingival tissue [23]; in scrapie-affected goats, infectivity appeared in salivary glands in some animals [24]. PrP^{sc}, however, has not consistently been detected in salivary gland tissue, with some studies failing [22] and others demonstrating infection [25,26]. No infectivity in saliva has been detected to date using the mouse inoculation test [13,27].

Animal studies of prion transmission by way of the dental route

Even before the widespread acceptance of the prion hypothesis, some investigators were suggesting the possibility of transmission of the causative agent of scrapie by way of dental procedures. As long ago as 1978, Adams and Edgar [28] used a mouse model to assess the transmissibility of this agent by way of dental instruments. The gingival tissues of scrapie-infected mice were traumatized using a slow dental bur, which was then used without cleaning to traumatize the gingivae of healthy mice. Following sacrifice after 15 months, none of the recipient mice demonstrated clinical signs of scrapie and histologic examination of neural tissue was normal. When gingival tissue from scrapie-infected mice was injected intraperitoneally, however, infection was transmitted with a 5 mg inoculum but not with a 0.5 mg inoculum. In a later study using a similar mouse model, Carp [29] showed that all (26/26) mice in which he scarified the gingival tissue became infected compared with 71% (17/24) of nonscarified mice, the former also demonstrating a shorter incubation period. In a further experiment, Carp [29] injected 31 mice intracerebrally with oral lavage fluid obtained from scrapie-infected mice that had gingival scarification, and 3 became infected. In a more recent study, scrapie material was inoculated into the pulpal cavity of the mandibular incisor of Syrian hamsters: each of the six animals developed scrapie disease, with spread of the agent to the trigeminal ganglion on the inoculated side alone [23].

Human forms of TSEs

The human TSEs, or prion diseases, occur in inherited, acquired, or sporadic forms (Table 1). These diseases are frequently referred to collectively as CJD. The most common form of CJD is the sporadic form (sCJD), which affects approximately one per million of the population per annum across the world and accounts for about 85% of all cases of CJD. The incidence of sCJD is the same in countries where scrapie has been eliminated from the sheep population, such as Australia and New Zealand. The disease commonly arises in middle-to-late life [30], with a peak age of onset between 60 and 65 years of age, presenting as a rapidly progressive multifocal dementia. Up to a third of patients have nonspecific prodromal symptoms that may include insomnia, fatigue, depression, weight loss, headache, general malaise, and ill-defined pain. Over a period of weeks, there is rapid progression to akinetic mutism, with mental deterioration, myoclonus, extrapyramidal and pyramidal signs, cerebrellar ataxia, and cortical blindness [31]. The most useful specific investigation of sCJD is electroencephalography (EEG), which shows characteristic changes. There is spongiform change, neuronal loss, and astrocytosis but no amyloid plaques in affected brain. The causative prion is not present in lymphoid tissue [31,32].

The underlying cause of sCJD is unknown. Some investigators, however, have suggested a significant association between risk of sCJD and previous surgery, and with residence or employment on a farm or market garden for longer than 10 years [33]. Another study did not confirm the association of sCJD with a history of surgery or occupational exposure to animals or leather [34]. There is no evidence for an infective basis for sCJD. Thus, consumption of brains and offal or lifetime vegetarianism do not alter the risk; there is no seasonal variation; there is no evidence for geographic clustering; and the incidence in Australia and New Zealand (countries that

Table 1
Types of CJD

Type	Abbreviation	Cause	Other comments
Sporadic	sCJD	Somatic mutation in PrP gene	—
Familial	fCJD	Germ-line PrP mutation	Autosomal dominant
Acquired-Iatrogenic	iCJD	Contaminated surgical instruments or human graft material	Dura mater grafts or pituitary hormones
Variant	vCJD	Consumption of BSE-infected material	Sometimes termed nvCJD
Kuru	—	Ritualistic cannibalism	Isolated to members of Fore tribe, Papua New Guinea

Abbreviations: BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt-Jakob disease; nvCJD, new variant CJD; PrP, protease-resistant protein.

are free of scrapie) is similar to that in the United Kingdom where scrapie is endemic. Surgeons, pathologists, butchers, abattoir workers, and cooks exposed to blood and uncooked animal products do not have an increased risk of sCJD.

The inherited or familial form of CJD (fCJD) accounts for around 10% of cases. The inherited forms are all rare autosomal dominant disorders that do not manifest until early-to-middle adult life [35]. Typically, fCJD has an earlier age of onset and a more prolonged course than sCJD. The typical EEG changes are often absent and the prevalence of amyloid plaques varies, but the essential changes in vacuolization of neuronal cells and neuronal loss are generally present. More than 20 different mutations in the PrP gene have now been described. Several mutations lead to phenotypes that have been regarded as distinct diseases. Fatal familial insomnia is characterized by progressive insomnia, dysautonomia, disruption of circadian rhythms, motor dysfunction, and deterioration in cognition. The usual age of onset is between 40 and 60 years, and death occurs between 7 and 18 months after onset. There is deposition of modified amyloid protein and neuronal depletion, particularly within the anteroventral and mediodorsal thalamic nuclei. Polymorphism of codon 129 of the PRNP determines clinical presentation and, hence, within affected families, disease can present like fatal familial insomnia or CJD [36–40]. Gerstmann-Straussler-Scheinker syndrome is an autosomal-dominant illness in which neuropathologic findings are distinct, with many PrP^{sc}-positive plaques throughout the brain. There are a variety of mutations different to those of fatal familial insomnia. Clinical features include cerebellar ataxia, pyramidal features, and dementia. The usual age of onset is 20 to 30 years and the clinical course is about 5 to 11 years—much longer than any form of CJD—yet, the mean age at death is only 48 years [41,42].

The acquired forms of CJD can be divided into kuru, iCJD, and vCJD. Kuru is an acquired spongiform encephalopathy that was first described in the 1950s and was the first recognized human transmissible prion disease [43]. Kuru is a fatal disease of cerebellar degeneration that was endemic among the Fore ethnic group in the eastern highlands of Papua New Guinea and spread by cannibalism. Most cases were in children and women, who ritually prepared the tissues and consumed brain tissue from deceased relatives. Typically, there was progressive cerebellar ataxia but, unlike CJD, dementia was not common. The transmissible nature was recognized [44]. Because the cannibalistic practice ceased about 40 years ago, kuru has gradually disappeared, but there are still occasional new cases in patients over 40 years of age, suggesting a very long incubation time [43,45].

iCJD can be transmitted mainly by surgery or pituitary extracts [46]. CJD-like disease has arisen following exposure to cadaver-derived growth hormone, pituitary gonadotropins, dura mater homografting, corneal grafts, or inadequately sterilized neurosurgical equipment [47–52]. iCJD varies clinically, ranging from an sCJD-like disease to a more slow-onset

disease reminiscent of kuru. The causative prion does not occur in lymphoid tissue. In 1985, CJD developed in four patients, all under 40 years old, who had received human growth hormone. Injection of the hormone, which was derived from pooled cadaver human pituitary glands, had been discontinued 4 to 15 years before the onset of disease. In the United Kingdom, approximately 1% of recipients have been affected, with a mean incubation period of 12 years. Recombinant growth hormone was licensed in 1985 and is now used. Contaminated human pituitary gonadotrophin has also been linked with CJD [53]. Over the past decade, at least 114 cases of CJD have been recognized 1.6 to 17 years after neurosurgical placement of human cadaver dura mater grafts [53]. A number of surgical procedures involving nerve tissue have been associated with inadvertent transmission of CJD. The first suspected case was a woman who developed rapidly progressive neurologic disease 18 months after a corneal transplant [54]. Contaminated neurosurgical instruments may also be a mode of transmission, the most convincing instances being reported after two patients underwent neurosurgical procedures with electrodes that had previously been implanted into a patient with CJD. The electrodes had been treated with 70% alcohol and formaldehyde vapor, yet 2 years later, these electrodes, when retrieved and implanted into a primate, resulted in CJD [55]. Small clusters of CJD possibly connected by intracranial surgery for treatment of trigeminal neuralgia have also been reported [56]. Of concern over routes of iatrogenic transmission is the experimental transmission of scrapie agent while bound to a stainless steel surface [57].

vCJD

In 1996, the CJD Surveillance Unit (Edinburgh, UK) identified in patients with CJD a previously unrecognized but consistent disease pattern that varied from classic CJD. This disease pattern suggested that the most likely explanation was exposure to BSE. There are a number of alternative theories proposed for the source of BSE and CJD, including the use of organophosphate pesticides, an autoimmune reaction, endocrine poisoning, and methyl bromide poisoning.

Persons with this type of CJD (originally termed *new variant*, or nvCJD) displayed prominent early psychiatric and behavioral manifestations together with persistent paraesthesias and dysaesthesias. The disease was subsequently termed *vCJD*. vCJD has affected mainly teenagers or young adults, the mean age of onset being 24 years [18,58,59].

Unlike sCJD or iCJD, vCJD typically presents with severe depression or other mental illness or with sensory disturbances (dysaesthesia or paraesthesia). Sensory problems have been prominent. Some patients have presented with oral dysaesthesia [60–62] or abnormal taste sensation [63]. These neuropsychiatric symptoms persist for several months and are followed by dementia, cerebellar, and other neurologic signs, myoclonus or

other involuntary movements and, finally, akinetic mutism [60,61]. The clinical course of vCJD is much longer than that of sCJD, and affected patients do not have the same typical EEG changes. In contrast to sCJD, there is extensive plaque deposition in brain tissue of vCJD patients, and magnetic resonance (MR) imaging scans show a characteristic bilateral symmetric high signal in the thalamic pulvinar nuclei. An important difference from other human TSEs is the demonstration of prions in lymphoid tissue of patients with vCJD [64].

The first patient diagnosed with vCJD died in 1995. All cases thus far have been in Europe (mainly the United Kingdom) or in persons who have lived in Europe (Table 2). To date, one vCJD case has been observed in the United States. As of August 2002, there have been 92 deaths from definite vCJD and 23 from probable vCJD in the United Kingdom. In addition, there are another 10 probable cases of vCJD still alive. Currently, the incidence is rising by an estimated 20% to 30% per annum, but it is still too early to know whether this trend is likely to be sustained or to forecast the ultimate size of the epidemic.

Pathologic examination of the CNS in vCJD shows prominent and diffuse plaques of an abnormal prion protein (PrP^{sc}), similar to that found in other TSEs. Laboratory experiments have provided compelling evidence that the causative agents of vCJD and BSE have a common origin [16,65,66]. Glycosylation patterns of PrP^{sc}, susceptibility studies in mice, and patterns of disease in brain tissue from vCJD patients and BSE are similar but distinct from the patterns associated with scrapie, sCJD, and fCJD. Because it has become possible to strain type the different prions based on animal inoculation studies or Western blot analysis, it has become clear that the prion associated with BSE is similar to the strain causing vCJD [4,16,35]. There also is a specific genetic predisposition to the disease, with all vCJD patients analyzed to date being homozygous for methionine at codon 129 of the PrP gene. Other risk factors for the development of vCJD are age and residence in the United Kingdom.

Table 2
Countries reporting patients with vCJD as of August 2002

Country	Previous residence in United Kingdom	Number of cases (definite/probable)
United Kingdom	+	122
Republic of Ireland	?	1
Hong Kong	+	1
France	?	6
Italy	?	1
United States	+	1
Canada	+	1

Note. + = residence in United Kingdom; ? = unknown if ever resident in United Kingdom.

Factors that are important in determining the probability of developing vCJD include genetic susceptibility, incubation period, infective dose, and route of exposure. Attempts by statistical modeling to predict the eventual scale of any vCJD epidemic only serve to emphasize the uncertainties, with estimates ranging from a few hundred to many hundreds of thousand cases. Data from the various types of human TSEs suggest that incubation periods of prion diseases in humans, after peripheral or oral exposure, range from at least 4 to 40 years, with a mean of 10 to 15 years.

The pathogenesis of prion diseases varies with different host species and prion strain combinations. It is difficult to extrapolate the animal experimental data directly to humans. In vCJD, however, postmortem studies have shown that abnormal PrP accumulates in CNS and lymphoreticular tissues, including the tonsils and appendix. It is likely that patients who are developing vCJD will have potentially infective peripheral tissues in the preclinical phase of their illness.

This finding has raised the theoretic risk of iatrogenic transmission of the infective agent of vCJD. Currently, it is assumed that CNS, posterior orbit, and lymphoreticular tissue are (in descending order of risk) the tissues most likely to be infective [67].

In cases of iCJD where the infectious agent is introduced into or near the brain, the incubation period is typically measured in months. Peripheral inoculation (intravenous, intramuscular, or oral) results in incubation periods of years or decades [46,68]. For scrapie, it has been shown that inoculation through non-neural, peripheral routes involves an initial phase of replication in the lymphoreticular system, followed by spread to the thoracic spinal cord by way of visceral autonomic nerves, and then to the brain [69].

There is no current evidence to suggest that vCJD has been caused by iatrogenic transmission, but any transmission may have been masked by the long incubation period. The infective dose required to cause vCJD is also unknown. Much of the information is extrapolated from other nonhuman TSEs. A recent report, however, has established some working levels of infectivity for a risk assessment of vCJD transmission by surgical treatment (Table 3). These figures suggest that the oral tissues containing highest infectivity will be located in the oral lymphoreticular tissue. Within the oropharynx, the largest collections of lymphoid tissue are located in the posterior third of the tongue, termed the *lingual tonsillar tissue*. This region contains a number of nodules of lymphoid tissue that are embedded in the submucosa and collectively represent the lingual tonsil. These tissues are likely to contain levels of infectivity of 10^5 $\log ID_{50}/g$ but are unlikely to contaminate dental instruments during routine dental procedures.

Transfusions of whole blood, component blood, or blood derivatives have not been shown to transmit the classic CJD agent. To avoid the theoretic possibility of transmission of CJD by transfused blood, however, recipients of human growth hormone were excluded from blood donation in 1989, and recipients of other human-derived pituitary hormones have been

Table 3
Infectivity ranges for various tissues

Transmission route	Duration within incubation period	Range/value of infectivity ($logID_{50}$/g for specified route)
CNS to CNS (or posterior eye)	0%–60%	0
CNS to CNS (or posterior eye)	Remainder of incubation period	8
Anterior eye to anterior eye	As CNS	0 then 5–6
LRS to LRS (or equivalent-risk peripheral tissue)	All	5–6
Remaining tissues	All	0 (up to 4 in sensitivity analysis)

Abbreviations: CNS, central nerrous system; LRS, lymphoreticular system.

From Department of Health. Risk assessment for transmission of vCJD via surgical instruments: a modelling approach and numerical scenarios. 2001.

excluded since 1993. People receive blood products in transfusions, vaccines, diagnostic tests, and in vitro fertilization cultures. The Centers for Disease Control and Prevention is currently conducting a study to determine whether CJD is transmitted to humans by blood (http://members.aol.com/larmstr853/cjdvoice/cdcprog.htm). The infectious agent has been found in blood, but there have been no documented cases of blood transmission in humans. Blood from a patient with Gerstmann-Straussler-Scheinker syndrome, however, did give rise to infection in mice, the infectivity occurring mainly in the plasma, leucocytes, and cryoprecipitate [70]. As a precaution, blood relatives of CJD victims and people at risk for iCJD or fCJD should not donate blood. Blood product withdrawals due to theoretic CJD risk have resulted in many people receiving blood-product withdrawal notifications. These notifications can cause a great deal of anxiety for recipients.

There also may be other potential sources of infection, such as products produced from cell lines grown in the presence of fetal bovine serum, some vaccines, and bovine products such as collagen [71], but there were no demonstrable TSE-related safety issues arising from the use of relevant animal-derived vaccines, including United Kingdom bovine materials (from cattle) in the manufacture of vaccines currently available in the United Kingdom market. The benefit from vaccines far exceeds any theoretic risk of transmission of TSE. Concern has also been expressed over the use of human dura mater grafts and bovine products for periodontal reconstruction [72–74].

It is unknown how the agent of vCJD reaches the CNS in humans. It is likely that it follows a similar route to that described for experimental TSEs in animal models. Thus, following oral ingestion, prions are found first in Peyers patches. The PrP^{sc} accumulates in follicular dendritic cells and is possibly transported by these cells to the enteric lymph nodes and the spleen.

The lymphoreticular organs, especially the spleen, serve as amplification sites. The PrP^{sc} may move from the spleen and lymph nodes through retrograde axonal transport to the spinal cord or the brain stem [69]. When PrP^{sc} has reached the brain, it replicates at an exponential rate and may spread from the CNS to the periphery by way of the peripheral nervous system The vCJD PrP^{sc} occurs within $CD35^+$ $CD21^+$ follicular dendritic cells, within all sites of the body [64]. Although it has yet to be demonstrated, it is likely that the PrP^{sc} will be present within lingual tonsillar tissue. It is unclear why lymphoid tissue of vCJD contains PrP^{sc} infectivity, as opposed to the other human TSEs, but lymphoreticular involvement also occurs in scrapie (although not in BSE). It may be that there is lymphoreticular invasion before neural involvement because PrP^{sc} was found in the appendix of a patient 8 months before onset of neurologic vCJD [75] and, in BSE and scrapie, there is early infection of the gut lymphoreticular system [25,76,77]. The dendritic cells may be a site of prion replication, whereas B lymphocytes may be important in the transportation of prions [78].

Treatment

There is no effective treatment for prion diseases. In vitro, some success has been achieved with quinacrine and chlorpromazine [79].

TSEs and dentistry

The data examining the relationship between TSEs and dentistry have been reviewed [80] and are now updated (see http://www.fdi.org.uk/assets/pdf/statements/tse.pdf.).

Orofacial manifestations of human TSEs

Orofacial manifestations of human TSEs comprise dysphagia and dysarthia (due to pseudobulbar palsy) and, in vCJD patients, there may be orofacial dysaesthesia or paraesthesia [60–62] or abnormal taste sensation [63].

Association of CJD with dental treatment

To date (August 2002), conventional dental treatment has no proven association with transmission of any form of CJD. A preliminary analysis of previous dental treatment in vCJD cases (H. Ward, R.G. Will, personal communication, 2002) has not revealed any consistent pattern that suggests past dental treatment as a risk factor for vCJD, but collecting appropriate data is difficult.

Animal studies indicate that prions might be present in neural, gingival, pulpal, lymphoid, and salivary tissues. In patients with sCJD, fCJD, or

iCJD, any levels of PrP^{sc} in oral tissues are likely to be very low compared with the levels in the CNS.

There are no current published data for levels of PrP^{sc} in oral tissues of vCJD cases, but evidence from studies investigating other peripheral tissues suggests that it is likely to be low. In contrast, PrP^{sc} has been reported in human trigeminal ganglion tissue from vCJD cases [81]. Procedures involving dura mater may carry a risk [82–84], and there may be risks associated with the use of bovine collagen (eg, lip augmentation) [85].

There have been two reports of clusters of CJD cases with a suspected dental connection. The first report described the cases of two patients from England who died in 1965 and 1968, followed by their dentist who died in 1980, all of sCJD [55]. There is no strong evidence of a link with dental treatment for these cases. The second cluster occurred near Fukuoka City, Japan and involved three patients who died of sCJD within 4 months of each other. Although two of the three patients had attended the same dentist, the link with dental treatment was entirely circumstantial [86]; however, transmission cannot be excluded.

Many series and case-control studies have searched for risk factors such as diet, exposure to animals, surgical treatment including dentistry, and occupational exposures. A retrospective case-control study [87] of 60 definite cases of sCJD occurring in Japan between 1975 and 1977 found no association with dental extractions; however, the authors reported a significant association with physical injuries including surgical operations but not including dental treatment, blood transfusions, or lumbar punctures. More recently, an Australian study reported that surgical procedures were significantly associated with the development of sCJD, although these researchers found no significant risk associated with a history of major dental treatment [33]. An analysis of 26 CJD cases and 40 matched controls from the United States [88] failed to discover a significant odds ratio for endodontic surgery, although these investigators noted statistically significant odds ratios for intraocular pressure testing, injury to or surgery on the head, face, or neck, and trauma to other parts of the body.

Prions in human trigeminal nerve and oral tissues

Deposits labeled by polyclonal antibodies to scrapie-associated fibril/prion protein have been detected in the trigeminal ganglion of two patients with CJD (one fCJD and one sCJD) [89]. Specific immunostaining of dystrophic axons in the nerve roots and around degenerating ganglion cells suggested centripetal or centrifugal extension along the axons, similar to that seen in brain tissues of chimps and spider monkeys experimentally infected with kuru [90–92].

A study of the levels of prion protein in dental pulp tissue from eight patients with sCJD [93] was unable to detect any prion protein, but the authors suggested that because the method they used was relatively

insensitive, the potential for transmission of CJD by way of dental procedures, although low, could not be dismissed.

Human TSEs, infection control, and dental treatment

One of the unique features of PrP^{sc} is its remarkable resistance to inactivation, and it is this property that challenges infection control in the health care setting, especially in the context of surgical instruments. Ionizing, ultraviolet, and microwave radiation have little effect [94], and prions are resistant to most disinfectants used in dental offices, although concentrated bleach appears to achieve inactivation of all strains. Gases such as ethylene oxide and formaldehyde and most chemical disinfectants such as alcohols, formalin, aldehydes, β propiolactone, hydrogen peroxide, iodophors, peracetic acid, sodium hydroxide, and phenolics are ineffective. Prions are also heat resistant and may survive autoclaving at 134∘C for 18 minutes. The PrP^{sc} is more resistant to inactivation by autoclaving when infected tissue becomes dried onto glass or metal surfaces or following prior fixation of tissue in ethanol or formaldehyde. Despite these findings, a recent report has highlighted the importance of effective cleaning (to remove adherent tissue) coupled with autoclaving (to reduce infectivity levels by several log fold) to produce a significant reduction in infectivity levels on contaminated instruments [67].

A formal risk assessment for the risk of transmission of vCJD by surgical procedures has stated that the risk of onward transmission by way of surgical procedures cannot be excluded as a risk to public health [67]. The risk assessment further stated that the most pragmatic way to reduce the risk of transmission was to ensure that all instruments for every patient should be thoroughly cleaned and sterilized before reuse. This procedure is particularly important in relation to vCJD where peripheral tissues may harbor considerable levels of infectivity in asymptomatic individuals (see Table 3).

Fortunately, CJD is not highly transmissible or infectious and, although there are records of CJD being transmitted accidentally by way of contaminated instruments or pituitary hormones, there are no confirmed cases of occupational transmission. It is prudent, however, to take a precautionary approach. Critical devices, such as surgical instruments, and semicritical devices contaminated with high-risk tissues (brain, spinal cord, and eye tissue) from high-risk patients (those with known or suspected infection with prions) require special treatment [95].

For the management of patients with TSEs, it is useful to divide patients into three main groups, as follows (Table 4).

Confirmed cases of CJD

A diagnosis of CJD is usually confirmed post mortem. An antemortem diagnosis is difficult but a number of diagnostic criteria based on a detailed

Table 4
Decontamination of dental instruments for patients with Creutzfeldt-Jakob disease (CJD)

Patient category	Tissue category[a]	Decontamination
Confirmed cases of CJD of any type	High/low infectivity	Use single use instruments where feasible or destroy instruments by incineration after use
Suspected cases of CJD of any type	High/low infectivity	Use single use instruments where feasible and quarantine remainder instruments pending definitive diagnosis
Persons with known prior exposure to human pituitary-derived hormones, cornea or dura mater grafts	High infectivity	Use single use instruments where feasible or destroy instruments by incineration after use
Persons with known prior exposure to human pituitary-derived hormones, cornea or dura mater grafts	Low infectivity	Routine cleaning and decontamination procedures
Members of families with heritable forms of CJD (familial CJD)	High infectivity	Use single use instruments where feasible or destroy instruments by incineration after use
Members of families with heritable forms of CJD (familial CJD)	Low infectivity	Routine cleaning and decontamination procedures

Adapted from World Health Organization. WHO infection control guidelines for transmissible spongiform encephalopathies. Geneva: WHO; 2000; with permission.

[a] High infectivity tissue is classed as brain, spinal cord, and eye for sporadic CJD, familial CJD, variant CJD and iatrogenic CJD. Lymphoreticular tissue is classed as high infectivity for variant CJD cases. Dental tissues are classed as low infectivity.

clinical history of the nature of the symptoms, pattern of progression, and the duration of the illness and diagnostic tests can lead to a diagnosis with a reasonable degree of accuracy [96]. A number of tests and investigations can contribute to a diagnosis:

Blood tests

In addition to excluding other forms of illness, blood tests collected with informed consent can be performed for any of the genetic mutations already identifiable in inherited prion diseases. Blood can also be tested for met/met homozygosity at codon 129.

EEG

EEG can be a useful test for the diagnosis of sCJD. In other forms of CJD, the EEG may be normal or there may be nonspecific abnormalities.

MR imaging

An MR imaging scan can be useful in assisting a diagnosis. In vCJD, abnormal changes can be observed in the thalamic area (pulvinar sign) and, in sCJD, there are occasional changes in the basal ganglia.

Psychometric tests

These tests of reading, writing, and other activities can be helpful in highlighting which part of the brain is most affected by disease.

Cerebrospinal fluid

Analysis of cerebrospinal fluid is useful to exclude some types of infection. An increase in the specific cerebrospinal fluid proteins 14-3-3 can help in the differential diagnosis [97].

Biopsy

Previously, a brain biopsy was required to confirm a diagnosis of CJD. In vCJD, PrP^{sc} also replicates in lymphoreticular tissue, so biopsy of tonsillar tissue can provide a means of diagnosis. Within the United Kingdom, the use of tonsil biopsy for diagnosis of vCJD has been developed and validated at St. Mary's Hospital in London [64].

In sCJD, fCJD, and iCJD it is thought that infectivity is primarily concentrated in the brain, spinal cord, and ocular tissues; therefore, it is these tissue groups that are classified as "high risk." In vCJD, in addition to the sites already mentioned, PrP^{sc} is present in the lymphoreticular system, which is also treated as high risk in vCJD cases.

There are a number of national [27] and international infection control guidance documents [98] for the management of patients with CJD (Table 5, Box 1). The consensus for the management of patients with a confirmed diagnosis of CJD is that all instruments used for invasive procedures should be destroyed after use. Normal social and clinical contact with CJD patients and noninvasive investigations such as radiographs do not present a risk to health care workers, relatives, or the community. There is no reason to defer, deny, or in any way discourage the admission of a person with a TSE into any health care setting. Because the disease is usually rapidly progressive, however, the patient will develop high-dependency needs and may require specialist dental input. It is essential that care is coordinated and dental input should be sought at the earliest opportunity to provide advice on supportive oral care.

When providing dental treatment for confirmed cases of CJD, contamination by body fluids such as saliva or blood poses no greater hazard than for any other patient. Although there is uncertainty about the risk of transmission from blood, adherence to standard infection control precautions should minimize any risk that may exist. Some investigators, however, have adopted a more precautionary approach and recommend that such patients be referred to specialist units. A further precaution suggested is that

Table 5
Identification of patients with CJD, suspected of having CJD or at risk of developing CJD-like disease[a]

Known or suspect patients	At-risk patients
Patients diagnosed as having CJD or a related disorder[b]	Asymptomatic patients who are potentially at risk of developing CJD or a related disorder[b]:
Patients suspected of having CJD or a related disorder[b] (ie, have clinical symptoms that are suggestive of CJD but the diagnosis has not yet been confirmed)	Recipients of hormone derived from human pituitary glands (eg, growth hormone and gonadotrophin but not those only given recombinant hormone)
	Recipients of human dura mater grafts
	People with a family history of familial CJD (ie, close blood-line relatives: parents, brothers, sisters, children, grandparents, and grandchildren of FFI, GSS, and familial CJD).

Abbreviations: CJD, Creutzfeldt-Jakob disease; FFI, Fatal familial insomnia; GSS, Gerstmann-Straussler-Scheinker syndrome.

[a] United Kingdom guidelines.

[b] Infective CJD: sporadic, iatrogenic or variant CJD; inherited CJD: FFI, GSS, and other familial CJD.

the dental handpiece should not be connected to the waterline of the dental unit because there is a chance, albeit remote, of clinical material being sucked into the waterlines, which are very unlikely to withstand the recommended method of chemical inactivation of prions. Water for cooling of the tissues should be provided from a separate source (eg, infusion or syringe). When the patient has CJD or is at risk of developing CJD, the handpiece should be discarded. In addition, in view of the difficulties of disinfection, the dental unit aspiration system cannot be used; instead, a stand-alone unit is suggested. Ideally, the reservoir of the unit should be disposable. The spittoon of the dental unit should not be used, and patients should expectorate into a disposable bowl, which should be discarded directly into the clinical waste bin for incineration. These latter precautions

Box 1. Recommended methods of inactivation of human and TSE agents

- 20,000 ppm available chlorine of sodium hypochlorite for 1 hour
- 2 M sodium hydroxide for 1 hour
- nonporous load steam sterilizer 134°C to 137°C for a single cycle of 18 minutes, or 6 successive cycles of 3 minutes each (but this is known not to be completely effective)

are controversial and are not universally routinely adopted [99]; some centers use no special precautions other than the disposal of invasive instruments for confirmed cases. In summary, units must adopt a local protocol based on current evidence and adopt a pragmatic approach.

Suspected cases of CJD

These are patients who present with a clinical history that is suggestive of CJD, but a confirmed diagnosis is unavailable. In this situation, instruments used for invasive procedures should be quarantined pending a confirmation of diagnosis. At the completion of the procedure, single-use instruments should be separated and disposed of by incineration; reusable instruments should be washed to remove gross soil. Care should be taken to avoid splashing and generating aerosols by holding instruments below the water surface in a sink into which water is running and draining continuously. Instruments should not be held directly under a flowing tap because this is likely to generate splashes. Operatives should wear protective gloves and eye and face protection (either a visor or goggles), and care must be taken to avoid injuries from sharp instruments. Instruments should be placed in a disposable instrument tray and allowed to air dry. They should then be placed in an impervious rigid plastic container with a close-fitting lid. The lid should be sealed with heavy-duty tape (eg, autoclave tape) and labeled with the patient's identification (name, date of birth, and hospital number if appropriate), the type of procedure for which the instruments were used, and the name of the responsible person (dentist or theater superintendent if appropriate). The sealed box should be stored indefinitely in a suitable designated place until the outcome of any further clinical investigations is known. The instrument tray should be disposed of by incineration. If the patient is confirmed as suffering from CJD of any type, the box and its contents should be incinerated without further examination. If an alternative diagnosis is confirmed, the instruments may be removed from the box by the responsible person (or deputy) and reprocessed in the usual way. Records must be kept of all decisions.

Cases at risk of developing CJD

Individuals at risk of developing CJD but who are clinically well include those at risk by virtue of an inheritable defect in the prion protein gene producing fCJD and those who have been exposed to infectious material through the use of human cadaveric–derived pituitary hormones and dural and corneal homografts (iCJD).

The majority of guidelines recommend that surgical instruments used to operate on high-risk tissues (CNS, spinal cord, and eye) in patients at risk of developing CJD should be disposed of by incineration. For interventions such as routine dental treatment, which do not involve high-risk tissues, some national guidelines recommend the use of stringent instrument

decontamination. It is difficult, however, to accurately identify such groups of patients, and the facilities for stringent decontamination are not readily available in general dental practice. World Health Organization guidelines [98] suggest that no additional precautions are necessary for routine dental procedures for this group of patients. Family members of vCJD cases are not classified in the at-risk category and, therefore, no special infection control protocols are necessary for these patients.

Percutaneous exposure to tissue/fluids from patients with CJD or at risk of developing CJD

Previously, investigators have suggested a number of postexposure prophylaxis treatments such as surgical excision and antilymphocytic treatment following percutaneous exposure to infectious material [100]. More recently, researchers have suggested the use of oligodeoxynucleotides to stimulate innate immunity and delay onset of disease [101]. There is limited clinical data to support these approaches, particularly those injuries likely to occur during routine dental treatment. The authors recommend that units involved in the treatment of confirmed cases of CJD consider, in advance, the risks and benefits of any prophylaxis treatment. In addition, units should follow their local protocols for the management of injuries from sharp instruments arising during dental treatment to prevent transmission of blood-borne viruses. Splashes into the eye or mouth should be dealt with by thorough irrigation. All accidents should be appropriately reported and recorded.

Dental health care clinical procedures

TSEs are not known to spread by contact from person to person or by the air-borne route. Contamination by saliva poses no greater hazard than for any other patient. Dental treatment, therefore, requires no additional precautions other than the destruction of instruments used in the treatment of confirmed cases of CJD and the quarantining of instruments used to treat cases suspected of suffering from CJD.

Spillages

Disposable gloves and an apron should be worn when removing spillages and disposed of by incineration. Spillage of potentially CJD-infectious materials should be removed using absorbent material, the surface disinfected with an appropriate disinfectant (see Box 1), and any waste disposed of as clinical waste by incineration.

Biopsy samples

Biopsy samples from known, suspected, or at-risk patients should only be taken by experienced clinicians who are aware of the hazards

involved. Samples should be marked with a biohazard label and particular consideration should be given to the need to maintain patient confidentiality. The pathology laboratory, however, must be notified specifically of any risk.

Maxillofacial surgery involving the brain or eyes of patients with CJD or at risk of developing CJD

Invasive procedures involving the eyes or brain (eg, after profound facial injury) necessitate using disposable instruments as much as possible and destroying all instruments by incineration. Expensive items of equipment (eg, drills) may be protected from contamination by using shields, guards, or coverings so that the entire items do not need to be destroyed. The drill bit, other parts in contact with high-risk tissue, and the protective coverings must be incinerated. When possible, the patient should be last on the day's operating list. No other discrimination should be permitted.

Follow-up of 74 (54%) of 137 patients treated for orbital floor fractures between 1988 and 1992 confirmed the biocompatibility and stability of allogeneic dura mater used in orbital floor restoration, but the potential problems of CJD transmission were highlighted [84]. There is no evidence to suggest that any patients who received dura mater allografts for the management of maxillofacial defects have developed iCJD. Cases of infectious disease transmission from inadequately screened donors of allogeneic tissues and cases related to improper sterilization and cataloging of these tissues have been reviewed [83]. The reviewers concluded that good judgment and attention to good science on the part of the tissue bank and the surgeon can maximize the ability to place contamination-free specimens, thereby avoiding such complications [83]. Concerns exist about the theoretic risk of the use of animal-derived graft materials and heterologous human graft materials in oral or periodontal surgery. Unless and until these products have been certified as not carrying potentially infectious doses of prions, the Federation Dentaire International recommends they should not be used (www.fdi.org.uk/assets/pdf/statements/tse.pdf + CJD + and + dentistry&hl = en&ie = UTF-8).

Dental health care staff infected or potentially infected with prions

Dental health care staff who are infected or potentially infected with prion disease should not practice invasive clinical procedures in view of the risk of motor and cognitive dysfunction.

Summary

The last 5 years have seen the emergence of a new disease in humans (vCJD), mainly in the United Kingdom. This emergence has been accompanied by an explosion of scientific data on a novel group of the responsible

infectious agents called prions and has profound implications for infection control and transfusion policies. Also of concern is the finding of prions in neural, gingival, pulpal, and salivary tissue in animal models and significant titers of infectivity from extraneural organs (particularly, in cases of vCJD, in lymphoreticular tissues). There is limited information on the presence of prion proteins in the oral tissues from human studies. Because of the differences in patterns of disease in animal models and in strains of prion protein, it is difficult to extrapolate directly these findings to humans, but it illustrates a potential for transmission by way of the dental route. High levels of infectivity may be present in tissues early in the incubation period and before clinical signs and symptoms.

The dental profession must turn its attention to the routine decontamination of dental instruments to ensure that these procedures are performed to the highest regulatory standard. Clinicians and manufacturers must work closely together to develop instruments that are either single use or can be presented in a form that can be more easily decontaminated. Clinicians must pay close attention to manufacturers' decontamination instructions and must not reuse items designated as single use, such as endodontic files. Improvements in compliance with these requirements will not only reduce the risk of transmission of TSEs but also other less tenacious infectious agents.

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