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The Risk of Prion Infection through Bovine Grafting Materials

Yeoungsug Kim, DDS, MSD;* Angel Emmanuel Rodriguez, DDS;† Hessam Nowzari, DDS, PhD‡

ABSTRACT

Background: Bovine-derived grafting materials are frequently used in a variety of bone augmentation techniques. The aim of this paper is to assess the unique safety issue of bovine-derived grafting materials that is rarely addressed in dental literature: risk of bovine spongiform encephalopathy (BSE).

Methods: The validity of the current BSE diagnostic methods, surveillance and epidemiological trends in affected countries, and BSE infectivity in bovine bone before and after manufacturing processing were reviewed and analyzed.

Results: Prion screening has significant limits. Humans are not safe from the infection of prion disease of other species. Prions can and do break the species barrier. There is evidence there may be tens of thousands of infectious carriers in the western countries alone. This raises concern about the potential for perpetuation of infection via medical procedures.

Conclusion: The limited ability to screen prions within the animal genome, along with a long latency period to manifestation of the disease (1 to over 50 years) in infected patients, provides a framework for discussing posible long-term risks of the xenografts that are used so extensively in dentistry. We suggest abolishing the use of bovine bone.

KEY WORDS: cCJD, EU, FDA, IHC, PMCA, PK-sensitive, PrPC' PrPSc, PrP 27–30, SAF, sCJD, WB

INTRODUCTION

Bovine bone was first introduced in surgery by Orell in 1934.[1] Xenografts have been hardly validated in orthopedics,[2] whereas bovine-derived bone graft materials are commonly used in dentistry as an alternative to autogenous tissue. The unique safety issue of using these materials is rarely addressed in dental literature and appears to be ignored by practitioners: risk of bovine spongiform encephalopathy (BSE) transmission from bovine xenograft. BSE is a type of transmissible spongiform encephalopathies (TSE) or prion diseases, a group of fatal neurodegenerative diseases affecting humans and a broad spectrum of animal species.[3]

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Creutzfeldt–Jakob disease (CJD) was first described in the 1920s by Creutzfeldt and Jakob as human TSE with long periods of incubation. Sporadic CJD, the most common form of CJD, has been estimated to affect 1 to 1.5 persons per million annually around the world (Table 1). An epidemic variant of CJD appeared in the 1990s in the United Kingdom and public and scientific attention was attracted to the understanding of the propagation and pathogenesis.[4–8]

Different types of prion diseases share common molecular mechanisms in which the misfolding of PrP^{C} leads to the pathologic version of the protein called PrP^{Sc}. The "protein-only" hypothesis suggests that once PrP^{Sc} is formed, it serves as a template to propagate this version of the protein, promoting the misfolding of PrP^{C}.[9,10]

Humans had been believed to be safe from the infection of prion diseases of other species for over two hundred years because of the "species barrier," the relative lack of disease transmission between species (cited by Bruce and colleagues[11]). However, epidemiological[4,12] and laboratory studies[11,13]evidenced the causal association between BSE epidemics and variant Creutzfeldt–Jakob disease (vCJD), the human prion disease.

TABLE 1 Types of Creutzfeldt–Jakob Diseases

Type	Cause and clinical features
Kuru	Acquired by cultural cannibalism (legally prohibited in the 1960's). Particular to Papua New Guinea people, prevalence is about 1%. Affects more women and children. Accompanied by tremor, choreiform, and athetoid movements.
Sporadic CJD	Cause Unknown Not common but found worldwide Generally found in people over 50 years old, onset on elder people. Duration <2 years. Short period of illness, ataxia, dementia, myoclonus.
Familial CJD	Genetically caused as result of mutation of PrP gene (PRNP), also called genetic CDJ. Found in approximately 10% of all CJD cases Slightly younger onset than sCJD Longer period of illness: various symptoms dependent on type of mutation.
Iatrogenic CJD	Acquired: transmitted through medical procedures including surgery, transplantation, and blood transfusion. Younger onset than sCJD: ataxia rather than dementia.
Variant CJD	Acquired: assumed to be caused by the consumption of BSE-infected beef (Was initially called new variant CJD [nvCJD]). Duration >6 months. Younger onset: psychiatric features and longer period of illness.

Manufacturers of anorganic bovine bone products claim that they are completely devoid of organic materials, because the presence of organic materials in bovine products indicates the possible existence of pathological prion (PrP^{Sc}) protein, the causative agent of prion diseases. However, plastic surgeons detected proteins including collagens in Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland, blocks following uneventful patient recovery after orthognathic surgery.[14]

Our review of the literature was conceived with the safety of patients in mind. Four questions were put forth for investigation by available literature. The questions asked were those we feel should appropriately be asked and welcomed by manufacturers who prepare bovine bone substitutes. Examination and questioning of these areas should be considered helpful for securing ongoing information that may assist in keeping products safe and the risks to patients as low as possible.

1. Does BSE prion infectivity exist in raw bovine bone?
2. If present, will the infectivity be inactivated by the treatment used for anorganic bovine bone substitutes manufacturing process?
3. Can deproteinization processes remove proteins in anorganic bovine bone substitutes completely?
4. Are current BSE diagnostic tests reliable and valid?

In our paper, we considered the above four questions with references to literature and summarized our search with the caution appropriate to the scientific methods.

Wenz and colleagues reported no protein detection in Bio-Oss® and Osteograf/N[15], DENTSPLY Corporate, PA, USA and Sogal and Tofe suggested the risk of BSE transmission from bovine bone graft substitutes should be negligible.[16]

However, our systematic review pointed out that their methodologies were erroneous.[17] The Lowry protein assay is used to estimate the content of proteins already in a solution or easily-soluble in dilute alkali,[18,19] therefore bone matrix should have been extracted and solubilized via defatting and decalcification procedures.[20] In addition, prion inactivation should be assessed by bioassay, not by WB because of apparent discrepancies between residual PrP^{Sc} levels in WB and prion inactivation levels.[21–23]

Although we concluded that bovine-derived graft biomaterials may carry a risk of prion transmission to patients,[17] the risk could not be quantified because of many variables and uncertainties in BSE prion and prion disease. In this paper, additional information will be presented to help understand the risk of BSE prion infection through bovine bone graft materials.

BSE Epidemiology

Control Measures in UK and EU Member States. The origin of BSE remains unknown; however after the first BSE case registered in 1986 epidemiological studies identified contaminated meat and bone meal (MBM) as the most likely vehicle of infection, which led to a series of countermeasures in the United Kingdom (UK).[24] In 1988, adding of MBM to ruminants was banned, in 1990 the use of specified bovine offal (SBO; infectious parts such as brain, spinal cord, spleen, and intestines) for all MBM was prohibited,[19]and total ban, prohibiting feeding mammalian protein to any farmed animals, was placed in 1996.[25] Approximately 4.1 million cattle were slaughtered between 1996 and 2000 under the Over Thirty Months Scheme (OTMS—the purchase and destruction of all UK cattle over thirty months of age *without testing*).[26]

Similar control measures were placed in other EU member states at different points after the British BSE outbreak and they were reinforced gradually over time. A total ban was placed throughout the EU in July 2001,[25] and EU cofounded OTMS was placed in EU member states for 6 months before the implementation of the active surveillance with rapid testing in July 1, 2001.[27]

The first case of vCJD in 1996 showed that prions could overcome the species barrier between cattle and humans and research linking the BSE prions being transmitted to humans in UK increased the interest in its pathogenesis.[4] The results of these efforts began with establishment of the EuroCJD in 1993 and NeuroCJD in 1998 by the European commission.[27]

Import restrictions against countries with BSE cases were placed in some EU member states in 1996, but trades within the EU community were not mainly affected.[28] Conversely, prohibition of the MBM use within a country increased animal product exports to other countries where MBM use was still allowed. When the UK placed the ruminant feed ban in 1988, the export of UK animal feeds doubled the following year, mostly to France. Also, the ruminant feed ban in the EU member states in the early 1990s substantially increased MBM exports from the EU countries to outside the EU, especially into Eastern Europe.[25,29]

It is claimed that bovine material is obtained from a country with negligible BSE risk, from selected and certified abattoirs, and from the extremity bone with no BSE infectivity. Our investigation shows that there is no country with negligible BSE risk, contamination of carcasses in abattoirs, and BSE infectivity in various bone tissue.

BSE Surveillance and Epidemiological Trends in UK and Other Affected Countries

Active surveillance was mandated by the EU commission in July 2001.[19] Under the scheme of active surveillance system, animals of target populations (usually, bovines over 30 months of age for the routinely slaughtered healthy population and over 24 months of age for the risk stock) were tested using one of EU-validated 'rapid' tests for abnormal prion protein (PrP^{Sc}), and a positive or an inconclusive case was confirmed by Western blot or immunohistochemistry.[30]

The size of the BSE epidemic was estimated at 3.5 million for Great Britain[31] and 0. 3 million for France[32] using back-calculation modeling based on the data of active surveillance, but the number of BSE cases reported in UK were only 180,000 since 1986.[19]

Although it was not operationally implemented in every country, active surveillance detected indigenous BSE cases in 11 countries previously considered as BSE-free (Austria, Czech Republic, Finland, Greece, Italy, Japan, Slovakia, Slovenia, Israel, Poland, Canada, USA, and Sweden).[19] Table 3 shows national prion disease pathology surveillance center cases examined in the United States in 2015.

In addition, atypical forms of BSE were discovered under the system. Since 2003, two types of variants (H-type and L-type) of the classical BSE were detected in several countries including Sweden and the United States with low or unlikely exposure to BSE.[33] Neuropathological and molecular phenotypes of the two atypical BSE differ from each other and the classical BSE. They appeared to occur spontaneously and H-type BSE is associated with mutation E211K within the prion protein gene,[34] which is heritable.[35]

Molecular patterns and brain pathologies of L-type BSE were identical to those of sporadic CJD, a type of human CJD with unknown etiology.[36,37] Furthermore, serial passages of L-type BSE to non-transgenic mice generated a prion strain similar to that of the classical BSE, implicating the origin of the BSE.[38] Taken together, BSE transmission may not be

prevented by control measures such as a meat and bone meal ban.

Many countries do not test cattle for BSE on a regular basis and there are substantial variations in BSE surveillance programs between countries. The EU member states test target populations, while Japan tests all cattle slaughtered for human consumption. Japan detected 31 BSE cases out of 6 million cattle tested between October 2001 and December 2006,[39] whereas two US native born cases were detected out of 787. 711 cattle tested during the enhanced surveillance from June 2004 to September 2006.[40] Considering the numbers of cattle population slaughtered annually (37 million in the US versus 1.26 million in Japan), the proportion of cattle tested in the USA is significantly lower than in Japan.[41]

In addition, the age threshold at which cattle had to be tested for BSE has been changing. In January 2009, the age limit of all healthy slaughtered cattle was raised from 30 months to 48 months and in July 2011, from 48 months to 72 months in EU member states including the UK. From January 1, 2013, a minimum sample of healthy slaughtered cattle aged over 72 months will be tested in the UK.[42]

Most of these control measurements were focused on stopping the spread of the disease to avoid a bigger epidemic event. Prions diseases have a long latency period. The duration varies depending on the type of the disease, but distinction among them is not always clear.[43–47]

Archived appendix tissues from UK surveys approximate the prevalence of asymptomatic vCJD infection of 1 in 2,000 persons born during 1941 to 1985.[48] Gill and colleagues suggested that depending on the genetic genotypes some people may harbor the pathologic prion and never develop clinical signs. Therefore, prion transmission through iatrogenic transmission such as blood transfusions, organ transplant, and surgical instrumentation is a constant possibility. [39,45,48,49]

NeuroPrion was founded in 2003 to facilitate communication between the most important prion research groups, private and public, including 52 research institutes in 20 different countries and federates representing 90% of the public and private research teams in Europe.[50] (Table 2)

TABLE 2 Current Surveillance Programs

Country	Surveillance program
UK	National CJD Surveillance Unit
EU	European CJD Surveillance Network
Germany	The CJD Surveillance Unit
Argentina	Epidemiologic Surveillance Committee for CJD
USA	National Prion Disease Pathology Surveillance Center
Japan	CJD Surveillance System
Canada	CJD Surveillance Committee
Korea	Center for Infectious Disease Control

Prion and BSE Diagnostic Tests

Prion. Pathological prion (PrP^{Sc}) is an abnormal isomer of a host-encoded prion protein (PrP^{C}).[3] The prion hypothesis states that PrP^{Sc} is the causative agent of a group of TSE or prion diseases[51] accumulating in individuals affected with most forms of TSE.[3]

PrP^{C} was completely degraded with the treatment of 50 μg/mL proteinase K (PK) for 30 minutes at 37°C, whereas PrP^{Sc} from prion-infected Syrian hamster was partially resistant to this treatment, leaving N-terminally truncated 142 amino acid residues (PrP27–30, molecular mass of 27–30 kDa) because of the high β-sheet content.[51,52]

PrP27–30 and validity of Current BSE Diagnostic Tests. PrP27–30, amino acid residues of PrP^{Sc} after PK treatment, has been used as a surrogate marker for prion infectivity and most TSE diagnostic tests currently available rely on detection of PrP27–30. However, a number of studies have shown that prion infectivity does not always correlate with the presence of PrP^{Sc} or PK27-30. A recent study by Balkema-Buschmann and colleagues. indicates there is no concurrence between PrP^{Sc} detection and BSE infectivity.[53] None of the TSE diagnostic tests used (IHC, SAF immunoblot, and PMCA) detected PrP^{Sc} in peripheral nerves, tongue, and nasal mucosa, while 30 to 92% of transgenic mice developed the disease after tissues homogenates inoculation.

In Creutzfeldt–Jakob disease (CJD)-infected mice, PK27-30 levels of CNS microglia were 50 fold less than those of undiluted brain homogenates with WB, but the infectivity titers of the two tissue types were similar to each other.[54] None or only traces of PK27-30 were detected in 263K scrapie infected hamster brain fractions containing high infection titers[55] and

TABLE 3 National Prion Disease Pathology Surveillance Center Cases Examined in US* (August 25, 2015)

Year	Prion disease	Sporadic CJD	Familial CJD	Iatrogenic CJD	Variant CJD
2000	101	89	12	0	0
2001	118	110	8	0	0
2002	144	125	17	2	0
2003	159	138	21	2	0
2004	180	162	17	0	1†
2005	179	157	21	1	0
2006	182	163	17	1	2‡
2007	206	187	19	0	0
2008	223	207	16	0	0
2009	233	212	20	1	0
2010	247	218	29	0	0
2011	239	216	23	0	0
2012	241	218	23	0	0
2013	257	222	34	1	0
2014	210	187	21	1	1§
2015	137	118	8	0	0
TOTAL	3056¶	2729	306**	9	4

*Listed based on the year of death or, if not available, on year of referral

†Disease acquired in the United Kingdom

‡Disease acquired in the United Kingdom in one case and in Saudi Arabia in the other.

§Disease possibly acquired in a Middle Eastern or Eastern European country

¶Includes 12 (11 from 2015) cases with type determination pending in which the diagnosis of vCJD has been excluded. The sporadic cases include 2853 cases of sporadic Creutzfeldt-Jakob disease (sCJD), 54 cases of Variably Protease-Sensitive Prionopathy (VPSPr) and 22 cases of sporadic Fatal Insomnia (sFI).

**Total excludes 194 familial blood only cases.

PrP^{Sc} levels were extremely low or undetectable by WB, IHC, CDI, or immunoprecipitation in either hamster 263K scrapie or human GSS (Gerstmann Sträussler Scheinker; a type of human TSE) disease-infected transgenic mice brain tissues containing high infectivity titers.[56]

Conversely, PK-sensitive PrP^{Sc} has been discovered in prion-infected humans and animals[57,58] and abnormal PrP^{Sc} (~6 kDa), which was not detected with standard diagnostic procedures was also discovered in a novel human prion disease.[59,60] Furthermore, several studies showed discrepancies in tissue PrP^{Sc} distribution when different diagnostic tests were used, which may be attributed to uneven PrP^{Sc} distribution within tissues or organs,[61] different diagnostic sensitivity between tests,[60,62] relatively low PrP^{Sc} accumulation in non-CNS tissues in cattle, and so forth. Conflicting results were also found in some CNS tissue samples of spinal cord and rostral medulla. In addition, when different antibodies (R145; F99) were applied for IHC, the test results differed in multiple samples of trigeminal ganglion and dorsal root ganglion.[63]

The exact portion and the size of brain samples for BSE diagnostic tests or the antibody for IHC may vary among laboratories. However, inconsistent PrP^{Sc} detection test results may be problematic in the context of the BSE confirmatory tests because WB or IHC are used to confirm a positive or an inconclusive case with a rapid test for the large screening.[64]

BSE Infectivity in Bovine Bone Before and After Manufacturing Processing. BSE infectivity and PrP^{Sc} were detected in sternal bone marrow in experimental and serum samples from BSE-confirmed cases using flow cytometry.[65] In spite of BSE regulations such as prohibition of the use of particular types of stunning methods and SRM (specified risk materials that most likely contain BSE agents such as brain, spinal cord, intestine, tonsil, etc.) removal, carcass contamination with CNS tissue occurred at slaughter houses and cutting plants. For example, spinal cord removal prior to carcass splitting reduced the carcass contamination level but could not prevent carcass contamination.[66,67] Under the simulated commercial abattoir condition, less than 1.5 mg (maximum 9 mg) spinal cord tissue from a previous carcass was recovered in four subsequent carcasses.[68] Contamination or cross-contamination of bovine carcasses by CNS tissue, which frequently occurred in abattoirs, was not removed by various washing procedures.[67]

Proteins including collagens were detected in some anorganic bovine bone substitutes including Tutoplast®, Zimmer Dental, CA, USA (bovine), Bio-Oss®, and tibia samples treated at the similar condition for Bio-Oss® deproteinization process,[68–70] and most importantly, whether BSE prion is inactivated by anorganic bovine bone manufacturing processes has not been proven yet.[17]

Prions are well known for their resistance to conventional chemical and physical decontamination methods, and the heat treatment used for anorganic bovine bone material preparation (300°C for Bio-

Oss®,1100°C for PepGen P-15®, DENTSPLY Corporate, PA, USA)[69] has not proven to inactivate BSE prion. Brain tissue from scrapie-infected hamsters transmitted the disease after exposure to dry heat at 600°C for 15 minutes, but no transmission at 1000°C for 5 min.[70,71] Whether BSE prion has similar resistance to inactivation as scrapie prion at the same heat treatment condition is unknown.

CONCLUSION

The limited ability to screen prions within the animal genome along with a long latency period to manifestation of the disease (1 to over 50 years) in infected patients provides a framework for discussing possible long-term risks of the xenografts that are used so extensively in dentistry. Patient counseling should include a clear description of the origin of the graft. A close evaluation of published papers in favor of bovine bone reveals wide variation in study designs, creating conditions for erroneous conclusions from the methods used. Often, use of broad or vague definitions and errors in analytical models can be noted. With the advancements made in bone biology, the available alternatives should be considered. We suggest abolishing the use of the bovine bone.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest.

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