

Risk of Prion Disease Transmission through Bovine-Derived Bone Substitutes: A Systematic Review

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ABSTRACT

Background: Despite the causal association between variant Creutzfeldt – Jakob disease and bovine spongiform encephalopathy (BSE), bovine origin graft materials are widely used during dental surgical procedures. The aim of this study was to assess the risk of BSE transmission through anorganic bovine bone substitutes.

Methods: Electronic database of MEDLINE was searched to identify relevant studies regarding our focused questions, presence of BSE prion infectivity in raw bovine bone, BSE prion inactivation by bone substitute manufacturing process, protein contents in anorganic bovine bone substitutes, and validity of current BSE diagnostic methods. Search terms yielded 1,704 titles. After title/abstract screening and duplicates removal, 36 full-text articles were screened for inclusion.

Results: A total of 16 studies were included in the final analysis. No eligible studies were identified regarding the efficacy of BSE prion inactivation by the treatments used for anorganic bovine bone manufacturing. BSE infectivity and PrP^{Sc}, pathological prion, were detected in bovine bone marrow and serum samples. Proteins were detected in Tutoplast® (bovine), Bio-Oss®, and tibia samples treated at the similar condition for Bio-Oss deproteinization. Inconsistent results of different BSE diagnostic tests were not unusual findings (Iwata et al. 2006; Arnold et al. 2007; Murayama et al. 2010), and a study by Balkema-Buschmann and colleagues showed an apparent discrepancy between BSE infectivity and detection of PrP(27-30), the current surrogate marker for prion disease infectivity.

Conclusion: This review indicates that bovine-derived graft biomaterials may carry a risk of prion transmission to patients.

KEY WORDS: anorganic bovine bone substitutes, BSE diagnostic test, BSE prion inactivation, BSE prion infectivity, protein, PrP(27-30), PrP^{Sc}

INTRODUCTION

As an alternative to autogenous tissue, bovine-derived biomaterials are frequently used for grafting during oral surgical procedures. Epidemiological evidence¹

and laboratory studies^{2,3} have indicated the link between variant Creutzfeldt – Jakob disease, fetal prion disease in humans, and bovine spongiform encephalopathy (BSE) epidemics, but the safety of using bovine-derived grafting materials has rarely been addressed in dental literature.

In 1999, Sogal and Tofe⁴ suggested that the risk of BSE transmission from bovine bone graft substitutes should be negligible and attributed the risk to sourcing and processing of raw bovine bone.

However, active surveillance, testing target cattle populations for BSE, mandated by the European Union (EU) commission since July 2001, revealed important epidemiological BSE trends in UK and other affected countries. The size of the BSE epidemic was estimated at 3.5 million for Great Britain⁵ and 0.3 million for France⁶ using back-calculation modeling. Indigenous BSE cases were detected in 11 countries, previously

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considered as BSE-free.⁷ Atypical BSE cases, the spontaneous form, were detected in several countries including Sweden and the USA with low or unlikely exposure to BSE,⁸ implying that BSE transmission may not be prevented by control measures, such as a meat and bone meal ban. In addition, in spite of the fact that BSE case ascertainment was significantly low before the implementation of active surveillance, 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 2006,⁹ whereas two U.S. native-born cases were detected out of 787,711 cattle tested during the enhanced surveillance from June 2004 to September 2006.¹⁰ Also, the proportion of cattle tested in the USA is not comparable with Japan considering the numbers of cattle population slaughtered annually (37 million vs 1.26 million).¹¹

Despite the European Commission and the Food and Drug Administration regulations including prohibition on particular types of stunning methods and specified risk material (such as central nervous system [CNS] tissue and intestine with high prion infectivity) removal, contamination or cross-contamination of carcasses by CNS tissue of potentially high prion infectivity frequently occurred in abattoirs and was not removed by various washing procedures.¹²

According to the prion hypothesis, pathological prion (PrP^{Sc}), an abnormal isomer of a host-encoded prion protein (PrP^C), is the causative agent of transmissible spongiform encephalopathies (TSEs) or prion diseases and usually accumulates in affected individuals.¹³ Manufacturers of anorganic bovine bone mineral products claim that they are completely devoid of organic materials. However, plastic surgeons detected proteins including collagens in Bio-Oss® blocks following uneventful patient recovery after orthognathic surgery,¹⁴ and more recently, Bannister and Powell¹⁵ reported foreign body reaction consisting of multinucleated giant cells within anorganic bovine bone particles and fibrous encapsulation in histologic specimens harvested from a guided bone regeneration site.

On the other hand, studies have suggested that proteinase K (PK)-resistant PrP27-30 may not be fully

responsible for TSE.^{16–21} PrP27-30, amino acid residues of PrP^{Sc} after PK treatment because of the partial resistance to PK, has been considered as the structural component of infectious prion, and most current TSE diagnostic tests rely on detection of PrP27-30.

Therefore, the aim of this manuscript was to assess the risk of BSE transmission through bovine bone substitutes by systematic literature review.

MATERIALS AND METHODS

Focused Questions

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

Search Strategy

A systematic literature search on the electronic database of National Library of Medicine (PubMed-MEDLINE) was conducted using search terms to identify relevant articles regarding the focused questions. The search was restricted to articles published in English between January 1998 and March 2011, and references of the retrieved articles were also searched.

Search terms included “Encephalopathy, Bovine Spongiform” [MeSH]; “Encephalopathy, Bovine Spongiform/diagnosis” [MeSH]; “Bone Substitutes” [MeSH] and “Cattle” [MeSH]; “Prions” [MeSH] and “Decontamination” [MeSH]; and “Sterilization” [MeSH] and “Prions” [MeSH].

Study Selection

Inclusion criteria selected for the current systematic review are: (1) studies on BSE infectivity in bovine tissues using bioassays; (2) studies on PrP^{Sc} distribution in bovine tissue using any currently available diagnostic tests; (3) studies using either natural BSE cases or experimentally BSE-infected bovines; (4) studies on protein content in anorganic bovine bone substitutes; and (5) studies on BSE prion inactivation by the treatment used for anorganic bovine bone manufacturing, assessed by bioassay.

Outcome Measures

Primary Outcomes

1. Detection of either BSE infectivity or PrP^{Sc} distribution in BSE-infected bovine tissues and organs to evaluate BSE prion infectivity in raw bovine tissue before processing
2. Protein detection in anorganic bovine bone substitutes after processing
3. Efficacy of BSE prion inactivation by the treatment used for anorganic bovine bone substitute manufacturing

Secondary Outcome. Agreement between the results of different BSE diagnostic tests and between BSE infectivity and PrP^{Sc} detection in BSE-infected bovine tissues to evaluate the validity of current BSE diagnostic tests.

Data Extraction and Analysis

Data of selected studies were extracted by using the standardized data abstraction form for each outcome. There was a substantial heterogeneity in protocols of the studies selected regarding the dose and the route of experimental BSE infection, the stages of the disease of BSE-infected cattle, the number of BSE-infected cattle tested, the number of tissue samples tested for BSE infectivity or PrP^{Sc} distribution, the type and the number of bioassay animals, and the interval between BSE inoculation and bioassay animal sacrifice. Because the details on anorganic bovine bone substitute processing methods were not described by manufacturers, the information from references^{22,23} were used to evaluate outcomes for protein content and BSE prion inactivation.

RESULTS AND DISCUSSION

Search terms identified 1,704 studies. Reviews were excluded. Title/abstract screening and duplicates removal yielded 36 potentially relevant articles. Following full-text evaluation, a total of 16 studies were selected including one additional study retrieved from the references.

Studies excluded after full-text evaluation are listed in Table 1. Exclusion criteria were based on the information that resistances of mouse-passaged BSE prion and scrapie prion to inactivation were significantly lower than BSE prion,²⁴ PrP^{Sc} tissue involvement varied in different host genotypes,²⁵ and disappearance of PrP^{Sc} in Western blot (WB) did not verify the efficacy prion inactivation.^{24,26,27}

TABLE 1 Reasons for Exclusion of Publications

Authors, Year	Reasons for Exclusion
Dickinson et al. (2009)	Mouse-passaged BSE prion strains (301 V prions) were used to test BSE prion inactivation
Grobben et al. (2004)	
Grobben et al. (2005)	
Grobben et al. (2006a)	
Grobben et al. (2006b)	
McLeod et al. (2004)	
Taylor (2002)	Mouse-passaged BSE prion strains (6PB1 prions) were used to test BSE prion inactivation
Taylor et al. (2002)	
Fichet et al. (2007)	
Cardone et al. (2006)	Scrapie strains were used to test BSE prion inactivation
Thomzig et al. (2006)	301 V prions were inoculated into rodents to test BSE PrP ^{Sc} distribution in cattle
Langeveld et al. (2003)	Western blot was used to test BSE prion inactivation.
Wenz et al. (2001) ⁴⁹	
Bannister and Powell (2008) ¹⁵	Case report
Hönig et al. (1999) ¹⁴	
Sohn et al. (2009)	Overlapping of previous study
Adam (2001)	Not relevant
Brown (2000) ⁵³	
Figueiredo et al. (2010) ²³	
Foster et al. (2001)	
Wells et al. (2005)	

Tissue BSE Infectivity and PrP^{Sc} Distribution

Table 2 shows BSE infection types of the cattle and test methods for BSE infectivity and PrP^{Sc} detection used in the studies included in the current review. Results derived from 13 studies indicate wide distribution of PrP^{Sc} and BSE infectivity in peripheral nerve system and other peripheral tissues examined (Table 3).^{28–40} Wells and colleagues²⁹ detected BSE infectivity in sternal bone marrow from cattle at 38 months following experimental oral exposure to BSE, assessed by conventional mouse bioassay; however, sternal marrow samples from cattle at 32, 36, and 40 months after oral exposure were not infectious. Trieschmann and colleagues³² detected PrP^{Sc} in all serum samples from six BSE-confirmed cases using flow cytometry, whereas neither Espinosa and

TABLE 2 Studies on BSE Tissue Infectivity and PrP^{Sc} Detection

Reference	Type of Infection	Test Method
Wells et al. (1998) ²⁸	Experimental oral exposure	Conventional mouse assay
Wells et al. (1999) ²⁹	Experimental oral exposure	Conventional mouse assay
Terry et al. (2003) ³⁰	29 natural cases and 3 experimental oral exposure	IHC
Buschmann et al. (2005) ³¹	3 natural cases with clinical signs	Transgenic (Tgbov XV) and conventional mouse assay
Trieschmann et al. (2005) ³²	6 natural cases with clinical signs	Flow cytometry
Iwata et al. (2006) ³³	3 natural cases with no clinical signs	IHC and WB
Arnold et al. (2007) ³⁴	Experimental oral exposure	IHC, WB, Bio-Rad (ELISA)
Masujin et al. (2007) ³⁵	5 natural cases and experimental oral exposure	WB
Espinosa et al. (2007) ³⁶	3 experimental oral exposure	Transgenic (BoPrP-Tg110) mouse assay
Kimura and Haritani (2008) ³⁷	1 natural case, a 7-year- and 10-month-old cow	IHC
Murayama et al. (2010) ³⁸	4 experimental oral exposure and i.c. inoculation	WB, DSP-PMCA
Yokoyama et al. (2010) ³⁹	5 experimental i.c. inoculation	WB
Balkema-Buschmann et al. (2011) ⁴⁰	2 natural cases and 2 experimental oral exposure	SAF immunoblot, IHC, PMCA, Transgenic (Tgbov XV) mouse assay

DSP-PMCA, potassium dextran sulfate-protein misfolding cyclic amplification; PMCA, protein misfolding cyclic amplification; SAF, scrapie-associated fibrils; IHC, immunohistochemistry; WB, Western blot; BSE, bovine spongiform encephalopathy; i.c., intracerebral; ELISA, enzyme-linked immunosorbent assay.

colleagues³⁶ nor Murayama and colleagues³⁸ detected BSE infectivity or PrP^{Sc} in BSE-infected bovine blood samples using different tests. Different test results may be related to the stage of the disease⁴¹ or different diagnostic sensitivity between tests.^{42,43} Following intraperitoneal inoculation of 263 K scrapie prion into hamsters, PrP^{Sc} was detectable in blood samples of infected animals during the early incubation period and the symptomatic phase but disappeared in the late incubation period. The authors suggested that it might be related to different proportion of circulating lymphocytes carrying PrP^{Sc} in blood.⁴¹

Evaluation of the Validity of Current BSE Diagnostic Tests. Three studies used more than one BSE diagnostic test for PrP^{Sc} detection. Discrepancies in test results on PrP^{Sc} involvement of bovine tissues were not infrequent findings when different detection methods were used (Table 4). Inconsistent test results may be attributed to uneven PrP^{Sc} distribution within tissues or organs,⁴² different diagnostic sensitivity between tests,^{42,43} relatively low PrP^{Sc} accumulation in non-CNS tissues in cattle, etc. However, 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 immunohistochemistry (IHC), the test results were different in multiple samples of trigeminal

ganglion and dorsal root ganglion.³⁴ 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 is used to confirm a positive or an inconclusive case with a rapid test for large screening.⁴⁴

A study by Balkema-Buschmann and colleagues⁴⁰ (Table 5) indicates there is no concurrence between PrP^{Sc} detection and BSE infectivity. None of the TSE diagnostic tests used (IHC, scrapie-associated fibrils immunoblot, and PMCA) detected PrP^{Sc} in peripheral nerves, tongue, and nasal mucosa, while 30–92% of transgenic mice, inoculated with the tissues homogenates, developed the disease.

Although PK27-30, PK-resistant amino acid residues of PrP^{Sc}, is used as a surrogate marker for TSE diagnosis, a number of studies have shown that prion infectivity does not always correlate with the presence of PrP^{Sc}(PK27-30). 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.¹⁷ None or only traces of PK27-30 were detected in 263 K scrapie-infected hamster brain fractions containing high infection titers

TABLE 3 BSE Tissue Infectivity and PrP Detection

	References
Central nervous system	Wells et al. (1998); Iwata et al. (2006); Kimura and Haritani (2008); Murayama et al. (2010); Yokoyama et al. (2011) ^{28,33,37–39}
Peripheral nervous system	Dorsal root ganglion: Wells et al. (1999); Iwata et al. (2006); Arnold et al. (2007); Masujin et al. (2007); Kimura and Haritani (2008) ^{29,33–35,37} Coeliac ganglion: Kimura and Haritani (2008) ³⁷ Trigeminal ganglion: Wells et al. (1998); Arnold et al. (2007); Masujin et al. (2007); Kimura and Haritani (2008); Yokoyama et al. (2011); Balkema-Buschmann et al. (2011) ^{28,34,35,37,39,40} Cranial cervical ganglion: Masujin et al. (2007); Yokoyama et al. (2011); Balkema-Buschmann et al. (2011) ^{35,39,40} Cervical ganglion: Masujin et al. (2007); Murayama et al. (2010); Balkema-Buschmann et al. (2011) ^{35,38,40} Optical nerve: Buschmann and Groschup (2005); Yokoyama et al. (2011); Balkema-Buschmann et al. (2011) ^{31,39,40} Facial nerve: Buschmann and Groschup (2005); Balkema-Buschmann et al. (2011) ^{31,40} Vagus nerve: Masujin et al. (2007); Murayama et al. (2010); Yokoyama et al. (2011) ^{35,38,39} Retina: Kimura and Haritani (2008) ³⁷ Suprascapular nerve: Yokoyama et al. (2011) ³⁹ Accessory nerve: Yokoyama et al. (2011) ³⁹ Brachial nerve plexus: Yokoyama et al. (2011) ³⁹ Phrenic nerve: Masujin et al. (2007); Yokoyama et al. (2011) ^{35,39} Median nerve: Yokoyama et al. (2011) ³⁹ Femoral nerve: Iwata et al. (2006) ³³ Tibial nerve: Yokoyama et al. (2011) ³⁹ Lumber nerve: Iwata et al. (2006) ³³ Radial nerve: Murayama et al. (2010); Yokoyama et al. (2011) ^{38,39} Sciatic nerve: Buschmann and Groschup (2005); Masujin et al. (2007); Espinosa et al. (2007); Murayama et al. (2010) ^{31,35,36,38} Splanchnic nerve: Masujin et al. (2007) ³⁵ Enteric nerve system: Terry et al. (2003); Iwata et al. (2006) ^{30,33}
Digestive system	Intestine: Wells et al. (1998); Terry et al. (2003); Buschmann and Groschup (2005); Iwata et al. (2006); Kimura and Haritani (2008); Murayama et al. (2010) ^{28,30,31,33,37,38} Pancreas: Kimura and Haritani (2008) ³⁷
Lymphatic system	Thymus: Kimura and Haritani (2008) ³⁷ Subiliac lymph node: Kimura and Haritani (2008) ³⁷ Peyer's patches: Espinosa et al. (2007) ³⁶ Palatine tonsil: Espinosa et al. (2007); Murayama et al. (2010) ^{36,38} Spleen: Murayama et al. (2010) ³⁸ Mesenteric lymph node: Murayama et al. (2010) ³⁸ Rouviere lymph node: Murayama et al. (2010) ³⁸
Urogenital system	Kidney: Kimura and Haritani (2008) ³⁷
Endocrine system	Adrenal gland: Masujin et al. (2007); Murayama et al. (2010); Yokoyama et al. (2011) ^{35,38,39} Pituitary gland: Yokoyama et al. (2011) ³⁹
Body fluid	Salivary gland and saliva: Murayama et al. (2010) ³⁸ Cerebrospinal fluid: Murayama et al. (2010) ³⁸ Blood serum: Trieschmann et al. (2005) ³²
Bone marrow	Sternum: Wells et al. (1999) ²⁹
Muscles	Musculus semitendinosus: Buschmann and Groschup (2005); Murayama et al. (2010) ^{31,38} Triceps brachii muscle: Murayama et al. (2010) ³⁸
Tongue	Murayama et al. (2010); Balkema-Buschmann et al. (2011) ^{38,40}
Nasal mucosa	Balkema-Buschmann et al. (2011) ⁴⁰

BSE, bovine spongiform encephalopathy.

TABLE 4 PrP^{Sc} Distribution in BSE-Infected Bovine Tissues Using Different Detection Methods

	Tissue or Organ	WB	IHC	DSP-PMCA
Iwata et al. (2006) ³³	Occipital cortex, peripheral nerves, distal ileum	+	–	
Arnold et al. (2007) ³⁴	Rostral medulla, cervical spinal cord, thoracic spinal cord, lumbar spinal cord	–	+	
Murayama et al. (2010) ³⁸	Cerebral spinal fluid, spleen, lymph nodes, palatine tonsils, muscular tissues, ileocecal region	–		+

WB, Western blot; IHC, immunohistochemistry; DSP-PMCA, potassium dextran sulfate-protein misfolding cyclic amplification, BSE, bovine spongiform encephalopathy.

in WB,¹⁸ and PrP^{Sc} levels were extremely low or undetectable by WB, IHC, conformation-dependent immunoassay (CDI), or immunoprecipitation in either hamster 263 K scrapie or human Gerstmann – Sträussler – Scheinker (a type of human TSE) disease-infected transgenic mice brain tissues containing high-infectivity titers.¹⁹

On the other hand, PK-sensitive PrP^{Sc} (PrP^{Sc}) has been discovered in prion-infected humans and animals. PrP^{Sc} constituted >80% of total PrP^{Sc} in brain regions from sporadic CJD-infected patients⁴⁵ and abnormal PrP^{Sc} (~6 kDa), which was not detected with standard diagnostic procedures, was discovered in a novel human prion disease.^{20,46} In natural cases of scrapie-infected

TABLE 5 PrP^{Sc} Detection Using BSE Diagnostic Tests and BSE Infectivity Using Mouse Bioassay (Balkema-Buschmann et al. 2011)

	IHC	PMCA	SAF Immunoblot	Infectivity Test using Transgenic Bioassay
Peripheral nerves, tongue, and nasal mucosa	–	–	–	30–92% transmission rates

IHC, immunohistochemistry; PMCA, protein misfolding cyclic amplification; SAF, scrapie-associated fibrils.

TABLE 6 Studies on Protein Content in Anorganic Bovine Bone Substitutes

Anorganic Bovine Bone Substitute	Reference	Detection Methods	Results
Tutoplast® (bovine)	Tadic et al. (2004) ²²	Infrared spectroscopy, thermogram	Tutoplast® (bovine): 26 wt% organic material including collagen
PepGen P-15®, Endobon®, Cerabone®	Tadic et al. (2004) ²²	Infrared spectroscopy, thermogram	PepGen P-15®, Endobon®, Cerabone®, Bio-Oss®: no organic material detected
Bio-Oss®	Tadic et al. (2004) ²²	Infrared spectroscopy, thermogram	No protein in both tests
	Schwartz et al. (2000) ⁴⁷	Western blot using TGF-β and rhBMP-2 antibodies	Proteins of molecular weight from 12 to 65 kDa were detected
Heat treated bovine tibia at 300°C, 500°C, 700°C, and 900°C for 18 hours	Murugan et al. (2003) ⁴⁸	FTIR spectroscopy	2 N-H bands indicating proteins were detected from bone heated at 300°C, but no proteins at ≥500°C
		Hydroxyproline quantification	52 mg/g of collagen was detected in bone samples treated at 300°C, but no collagen at ≥500°C

FTIR, Fourier transform infrared; TGF-β, transforming growth factor-beta; rhBMP-2, recombinant human bone morphogenetic protein-2.

sheep with particular genotypes, PrP^{Sc} was estimated up to 90% of PrP^{Sc}.²¹

Protein Detection in Anorganic Bovine Bone Substitutes

For the evaluation of protein content in anorganic bovine bone substitutes, three studies were identified (see Table 6).^{22,47,48} Organic compounds including collagen were detected in Tutoplast® (bovine), but not in PepGen P-15®, Endobon®, and Cerabone® 2.²² For Bio-Oss, Schwartz and colleagues⁴⁷ detected proteins with WB following matrix extraction, while Tadic and colleagues²² did not detect proteins using both infrared spectroscopy and thermogram. Protein bands and collagens were detected in tibia samples treated at 300°C for 18 hours, which is similar to the treatment condition for Bio-Oss deproteinization process, but no proteins after treatment at ≥500°C.⁴⁸

Wenz and colleagues⁴⁹ reported no protein content in Bio-Oss and Osteograft/N using Lowry protein assay, but the study was excluded from the current review because of their erroneous methodology; the Lowry protein assay is used to estimate the content of proteins already in a solution or easily soluble in dilute alkali,^{50,51} and bone matrix needs to be extracted and solubilized via defatting and decalcification procedures for the Lowry protein assay.⁵²

BSE Prion Inactivation by Anorganic Bovine Bone Substitute Manufacturing Process

No studies on the efficacy of BSE prion inactivation by the treatments used for anorganic bovine bone substitute manufacturing process were identified.

Wenz and colleagues reported the alkaline treatment used for Bio-Oss preparation inactivated BSE prions, determined by PrP^{Sc} disappearance in WB.⁴⁹ However, studies have suggested that the efficacy of prion inactivation should not be assessed by residual PrP^{Sc} levels in WB because of apparent discrepancies between residual PrP^{Sc} levels and prion inactivation levels measured by bioassay.^{24,26,27} For example, following 1% sodium dodecyl sulfate treatment in the presence of 0.5% acetic acid (pH 3.6) for 15 minutes at 37°C or room temperature, no PrP^{Sc} was detected in Sc237-infected brain homogenates by WB, but all bioassay hamsters developed the disease with prolonged incubation time.²⁷

Brain tissue from scrapie-infected hamsters was reported to have transmitted the disease after exposure to dry heat at 600°C for 15 minutes but no transmission at 1,000°C for 5 minutes,^{53,54} although whether BSE prion has similar resistance to inactivation as scrapie prion at the same treatment condition is unknown.

CONCLUSION

A systematic review revealed that:

1. Wide distribution of BSE infectivity and PrP^{Sc} was detected in BSE-infected bovine tissues including BSE-infected bovine bone marrow and blood serum. Discrepancies in tissue PrP^{Sc} distribution when different diagnostic test were used and lack of concurrence between PrP^{Sc} detection and BSE infectivity in BSE-infected bovine tissues raise questions about the validity of current BSE diagnostic tests.
2. Proteins including collagens were detected in some anorganic bovine bone substitutes including Tutoplast® (bovine), Bio-Oss®, and tibia samples treated at the similar condition for Bio-Oss deproteinization process.
3. The efficacy of BSE prion inactivation by the treatments used for anorganic bovine bone manufacturing has not been proven based on current literature review.

In conclusion, our systematic review indicates that bovine-derived graft biomaterials may carry a risk of BSE prion transmission to patients although the risk cannot be quantified by the information or research currently available.

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