Dear Editor,

We are writing this letter with regard to an article recently published in *Clinical Implant Dentistry and Related Research* by Yeoungsug Kim and colleagues, entitled “Risk of Prion Disease Transmission through Bovine-Derived Bone Substitutes: A Systematic Review” (2013; 15:645–653). The aim of the article was “to assess the risk of BSE transmission through anorganic bovine bone substitutes.” This review concluded that “bovine-derived graft biomaterials may carry a risk of prion transmission to patients.” There are many shortcomings with the published review paper, which fails to substantiate the conclusions reached by the authors.

1. This publication was labeled as a systematic review; however, the methodology of this publication has significant scientific flaws. The study was limited to one database and included only an electronic search. As a “systematic review,” it does not follow established guidelines for evidence-based medicine (EBM). EBM guidelines such as the PICO process require more than one database and should also include hand searches of relevant journals.

2. Epidemiological data have failed to demonstrate evidence of an association between the application of bovine derived bone substitutes and prion-related diseases. Given the large number of bovine-derived bone substitutes used worldwide, prion-related diseases would have been observed at higher levels. On the contrary, there has been a decline in BSE cases. At the peak of the epidemic in 1992, there were over 37,000 reported cases of BSE worldwide, with 97% reported in the UK. In 2011, 15 cases of BSE were reported worldwide, and in 2013, there have been only four cases reported (two in the UK, one in Ireland, and one in Poland). This decline has been attributed to the implementation of stringent measures such as a ban on cattle consuming ruminant-derived animal protein, such as meat and bone meal.

3. Prions have never been detected in bovine bone tissue. The only reference cited by the authors refers to a study where prions were detected in bovine sternal bone marrow, which is not relevant as a precursor to bovine-derived biomaterial.

4. The distribution of infectivity and PrPTSE (prion protein) in cattle with BSE has been documented in scientific studies. In view of the risk assessment provided by scientific data, the World Health Organization has developed a classification of the risk associated with different tissues and has classified bovine bone in Category IC, that is, “tissues with no detectable infectivity.” This implies that bone tissues are regarded as low-risk materials for potential BSE infectivity.

5. The review author’s claim that “the inactivation of prions by the manufacturing process of bovine anorganic bone has not been proven” is not accurate. All current manufacturers of bovine anorganic bone keep their specific techniques for preparation of these materials confidential. As with all medical devices, these are information of proprietary nature, which are not disclosed by medical device manufacturers. Therefore, the authors of the review paper could not have judged the effectiveness of the methodology utilized by these device manufacturers. However, this information is disclosed to regulatory bodies such as the Food and Drug Administration. Nevertheless, the general methodology for generation of bovine anorganic bone includes sourcing of animals from low-risk regions as identified by the World Organisation for Animal Health (OIE). The process involves utilization of young, healthy bovine material; physical separation of bone tissue; treatment with harsh alkaline solution and organic solvents; and prolonged heat treatment at temperatures exceeding 300ºC (personal communication with Geistlich Pharma AB; NIBEC Co., Ltd.; Collagen Matrix, Inc.; Dentsply Implants; and Biomet 3i). The claim that manufacturing of bovine anorganic bone does not completely remove proteins is also not substantiated. It is well established that the etiologic agents for transmissible spongiform encephalopathy are resistant...
to many physical and chemical treatments for inactivating pathogens. The methods used to inactivate prions within biological tissue include physical treatment, such as dry or steam heat by autoclaving, and chemical treatment, such as exposure to acids, bases, alcohol, or other organic solvents, as well as various combinations of these methods. Some of these strategies have been ineffective, whereas most lead to significant reductions in infectivity. Studies have demonstrated that a high heat treatment in conjunction with strong alkaline treatment are effective methods for BSE prion inactivation/elimination, thus reducing the risk of infectivity.6

6. The validity of BSE diagnostic tests was questioned. However, the Prionics-Check is the most commonly utilized test and is considered the “gold standard.”

This review paper presents a completely biased point of view without scientific merit. One example is the discussion of a case report where the clinician had used anorganic bovine bone in conjunction with autogenous bone, platelet-rich plasma and cross-linked collagen membrane.15 The case report also stated that the patient developed an allergic reaction to penicillin. The case report found multinucleated giant cells near the grafted area. The outcome of the report was interpreted by the authors of the systematic review as implying that bovine anorganic bone induced the giant cells. The reality is that it is much more likely that these giant cells were generated from autogenous tissue, because the progenitors of giant cells are bone marrow mononuclear cells. An alternative possibility is that an allergic reaction to penicillin was responsible for this histologic manifestation. In the case report, the authors stated that they retreated the site with bovine anorganic bone again with a favorable outcome. The fact that this report is cited as an example of an adverse reaction to bovine anorganic bone clearly reveals the biased view of the authors of the review paper.

The safety of biomaterials utilized for patient care is an important consideration, and as clinicians we all strive to protect the safety of our patients. However, the publications of unsubstantiated claims that report positive or negative results are equally unjustified. The fact is that bovine anorganic bone is the most well-studied bone graft material for bone reconstruction in the craniofacial region. False claims not only do not serve to protect the public, they can actually have the opposite intended effect on patient care, because they can deprive patients of viable therapeutic options.

In summary, this review paper never presented direct evidence for the presence of prions within bovine anorganic bone, nor for the transmission of BSE to a single patient from bovine anorganic bone. Therefore, the conclusion of the review by Kim and colleagues that bovine-derived graft biomaterials may carry a risk of prion transmission to patients was not substantiated by scientific evidence as the authors claim in their review paper.

Sincerely,

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REFERENCES

With regard to the letter to the Editor from Dr. M.S. Block and colleagues, we would like to respond as follows.

Most importantly, we would like to emphasize that our review of the literature was conceived with the safety of patients in mind. Four questions were put forth for investigation by examining available literature. The questions asked are 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. The aim of the study was “to assess the risk of BSE transmission through bovine bone substitutes.” The fair and reasonable questions we asked were the following:

1. Does BSE prion infectivity exist in raw bovine bone?
2. If present, will the infectivity be inactivated by the treatment used for the anorganic bovine bone substitute 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 reference to the literature and summarized our search with the caution appropriate to the scientific method. Our final statement was “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.” We stand by our conclusion that risk of BSE prion transmission from bovine-derived graft biomaterials is a possibility, albeit unquantifiable.

1. With regard to Block and colleagues’ first point, we searched the database of the National Library of Medicine (PubMed/MEDLINE). We can only reiterate that our search was conducted through a highly respected database and yielded 1,704 studies related to our search terms. Examining this literature and the associated references consumed countless hours of meticulous work to screen for duplicates and relevance. Consequently, we confined the study to the one database as stated by Block and colleagues.

2. Point by Block and colleagues: “Epidemiological data have failed to demonstrate evidence of an association between the application of derived bone substitutes and prion-related diseases.” We agree with this statement, and that is why we conducted the study.

3. With regard to Block and colleagues’ third point: It is true that the World Health Organization has developed a classification of the risk associated with different tissues and has classified bovine bone in Category IC, that is, “tissues with no detectable infectivity”; however, this does not mean that the classification cannot be questioned, and furthermore, “low risk” does not mean “no risk.”

4. Point by Block and colleagues: “The review authors’ claim that ‘the inactivation of prions by the manufacturing process of bovine anorganic bone has not been proven’ is not accurate.” We acknowledge that manufacturers make efforts to prepare materials and assure safety. However, it is puzzling as to why these techniques should be kept “confidential” and shared only with such bodies as the FDA. This does not explain under what circumstances a body like the FDA would be receiving the information. We thank the manufacturers for sharing their “general” methodology of the preparation for the purposes of this debate; however, we feel that a general description of the process is inadequate for proving to the public that the process has successfully inactivated all possibility of prion presence.

5. Point by Block and colleagues: “The claim that manufacturing of bovine anorganic bone does
not completely remove proteins is also not substantiated.” Our paper stated, “Manufacturers of anorganic bovine bone products claim that their xenografts are completely devoid of organic materials. However, several studies have reported protein presence in bovine bone graft materials.” We cited four studies to support this statement that were not acknowledged by Block and colleagues. Furthermore, our study statement with regard to reference iv, “plastic surgeons detected proteins including collagens in Bio-Oss® blocks following uneventful patient recovery after orthognathic surgery,” was not mentioned by Block and colleagues. The four references are the following:


6. Point by Block and colleagues: “The validity of BSE diagnostic tests was questioned. However, the Prionics-Check is the most commonly utilized test and is considered the ‘gold standard.’ ” We do not doubt that manufacturers are complying with the “gold standard.” However, research and science are about revisiting accepted knowledge, expressing doubt, and positing new questions for consideration.

The responders have stated that “anorganic bone is the most well-studied bone graft material for bone reconstruction in the craniofacial region.” It should be noted that bovine bone itself is not a well-studied bone graft material for craniofacial bone reconstruction. 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.

An especially important recommendation is that 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.

Finally, we take issue with Block and colleagues’ claim that our paper presented a “completely biased point of view without scientific merit.” We are academicians and practitioners who conducted a literature review. We have no reason to protect anything or anyone except the patients in this matter.

Thank you for this opportunity to reply to Dr. Block and his colleagues, who brought these questions to the forefront.