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MEAT AND BONE MEAL AND MINERAL FEED ADDITIVES MAY INCREASE THE RISK OF ORAL PRION DISEASE TRANSMISSION

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Abstract

Ingestion of prion-contaminated materials is postulated to be a primary route of prion disease transmission. Binding of prions to soil (micro)particles dramatically enhances peroral disease transmission relative to unbound prions, and it was hypothesized that micrometer–sized particles present in other consumed materials may affect prion disease transmission via the oral route of exposure. Small, insoluble particles are present in many substances, including soil, human foods, pharmaceuticals, and animal feeds. It is known that meat and bone meal (MBM), a feed additive believed responsible for the spread of bovine spongiform encephalopathy (BSE), contains particles smaller than 20 μm and that the pathogenic prion protein binds to MBM. The potentiation of disease transmission via the oral route by exposure to MBM or three micrometer-sized mineral feed additives was determined. Data showed that when the disease agent was bound to any of the tested materials, the penetrance of disease was increased compared to unbound prions. Our data suggest that in feed or other prion–contaminated substances consumed by animals or, potentially, humans, the addition of MBM or the presence of microparticles could heighten risks of prion disease acquisition.

Prion diseases (transmissible spongiform encephalopathies, TSE) are unique in that the etiological agent appears to be a misfolded isoform of the host prion protein (PrP^{TSE}) capable of resisting conditions and treatments sufficient to inactivate other pathogens (Prusiner, 1998; Taylor, 2000; Watts et al., 2006). Prion diseases are typically acquired via ingestion of infectious material, and disease manifests following an extended incubation period (Maignien et al., 1999). Compared to intracerebral or intraperitoneal exposure, peroral infection requires titers that are several orders of magnitude higher (Kimberlin & Walker, 1989). In animals challenged with low doses of agent, only a subset, and potentially no animals, manifest clinical disease (Baier et al., 2003).

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The stability of the prion agent leads to unexpected modes of disease transmission. For example, bovine spongiform encephalopathy (BSE) is thought to be spread to cattle and other animals through feeding BSE agent-tainted materials, such as meat and bone meal (MBM), and environments contaminated with sheep scrapie or cervid chronic wasting disease (CWD) agents spread disease to exposed animals (Miller et al., 2004; Morley et al., 2003; Palsson, 1979). A growing number of studies suggest that soil particles may play a role in the transmission of sheep scrapie and cervid CWD by binding and retaining the infectious agent in a bioaccessible form (Brown & Gajdusek, 1991; Johnson et al., 2006; Leita et al., 2006; Seidel et al., 2007). Johnson et al. (2007) previously demonstrated that prion binding to the soil mineral montmorillonite (Mte) or whole soils significantly enhance oral disease transmission relative to unbound prions. The mechanism responsible for enhanced prion disease transmission by soil-bound prions remains to be elucidated, but protection from digestive processes, increased residence time in the gastrointestinal tract, attachment-induced physicochemical changes to the agent, or enhanced prion uptake or exposure may all contribute to pathogenesis.

The presence of insoluble microparticles in a variety of substances prompted consideration that the effect could represent a more general mechanism of prion disease transmission with implications for other TSE. Animal feeds are often supplemented with a variety of substances including vitamins, enzymes, microorganisms, binders, fillers, and nutritional supplements; MBM and aluminosilicate minerals are two classes of such supplements (Olson & Hollis, 2007). The role of additives is varied, but feeds are typically amended with materials to increase concentration or bioavailability of nutrients. MBM is a common, low-cost additive that can be derived from many animal species or mixtures of species and is used to augment the protein content of feeds (Wang & Parsons, 1998). Most MBM are composed of >50% protein and 20–30% ash, which contain inorganic materials such as minerals. Bovine-derived protein, such as that in bovine MBM, is banned for consumption by ruminants in Canada, the United States, and most European Union countries, but is still consumed by monogastric livestock (e.g., swine, horses), pets, fish, and poultry in many countries. Ruminant consumption of MBM derived from nonruminant species remains permitted in some countries.

Aluminosilicate minerals are added to animal feeds to modulate water content, provide bioavailable nutrients adsorbed to the particles, and detoxify feed contaminants (Trckova et al., 2004). Further, aluminosilicates increase residence time of feed in the digestive system (Bringe & Schultz, 1969; Collings et al., 1980; Quisenberry, 1968), preserve consumed nitrogen in usable forms for rumen bacteria (Mumpton, 1999), and aid in drug delivery (Byrne & Deasy, 2005). Johnson et al. (2007) previously reported that the aluminosilicate mineral Mte increased the oral transmissibility of a low dose of prions by a factor of 680. It was postulated that insoluble particles, such as those added to feeds, or supplements that may be partially composed of insoluble materials, like MBM, may affect oral prion disease transmission. In this study, the effects of three minerals used in animal feeds and MBM were determined on the penetrance of a low dose of prion agent.

MATERIALS AND METHODS

Porcine MBM was acquired from two sources: MBM 1 was from Hormel Foods Agri-Nutrition (Austin, MN), and MBM 2 was the generous gift of Dr. Carl Parsons (University of Illinois, Urbana-Champaign). MBM 1 contained 55% protein and 27% ash, and MBM 2 had 50% protein and 24% ash. Particle size measurements of each MBM were conducted using the Bouyoucos method (Klute & Page, 1982), and results are presented in Table 1. Preparation of montmorillonite (Mte; d_h = 0.5– 2 μm), kaolinite (Kte; d_h = 0.5–2 μm), and quartz microparticles (d_h = 1–5 μm) were described previously (Johnson et al., 2006). The

HY strain of hamster-passaged transmissible mink encephalopathy agent was used for all experiments (Bessen & Marsh, 1992). Purified PrP^{TSE} was prepared as described previously (Bolton et al., 1987; Johnson et al., 2006).

Incubation of purified PrP^{TSE} with particles was performed to (1) assess whether agent would associate with these particles and (2) generate inocula for animal bioassays. These methods are previously described and were performed here with only minor variations (Johnson et al., 2006, 2007). For attachment studies, PrP^{TSE} was clarified by two sequential centrifugation steps at 800 × g to remove large aggregates (Johnson et al., 2006), and nonclarified (total PrP^{TSE}) was used for bioassays (Johnson et al., 2007). Attachment studies and bioassays used 0.2 μg clarified PrP^{TSE} and 1 μg total PrP^{TSE}, respectively. Purified PrP^{TSE} was added to MBM (1 or 10 mg), Mte (500 μg), Kte (1.5 mg), quartz microparticles (3.2 mg), or samples lacking particles in 500 μl of 10 m*M* NaCl. The surfaces areas available for binding to the three minerals were equivalent (Johnson et al., 2007). Samples were incubated with agitation for 1 h prior to analysis of attachment or inoculation into hamsters for bioassay.

For analysis of attachment to MBM particles, control samples lacking MBM and suspensions of PrP^{TSE} with MBM were placed above a 750-m*M* sucrose cushion and centrifuged for 3 min at 3000 × g, generating pellets containing bound PrP^{TSE}. Pellets were extracted with 10 × SDS-PAGE sample buffer at 95°C for 10 min. Samples were separated on 4–20% poly-acrylamide gels, then transferred to polyvinyl difluoride membranes and immuoblotted with monoclonal antibody 3F4.

For animal bioassay, 52 hamsters were orally challenged with microparticle or MBM-associated PrP^{TSE} (40 animals, inocula prepared as described earlier) or an equal amount of unbound PrP^{TSE} (12 animals). Hamsters receiving each class of microparticle or 10 mg of MBM 1 without PrP^{TSE} (4 animals per treatment; 16 animals total) served as negative controls. Animals were dosed via voluntary consumption of the entire sample (solution and particles) as previously described (Johnson et al., 2007). Animals were scored twice weekly for onset of clinical symptoms; diagnoses were confirmed by analyzing brains from clinically positive animals for proteinase K-resistant PrP. All procedures requiring animals were performed in accordance with all institutional protocols.

RESULTS AND DISCUSSION

Each of two preparations of MBM contained insoluble particles, and the distributions of particle sizes are presented in Table 1. Both preparations had roughly similar particle size distributions, and both MBM samples contained approximately 8% microparticles <2 μm and approximately 10% particles of −20 μm size. Previously, Johnson et al. (2006) reported binding of PrP^{TSE} to Mte, Kte, and quartz and found that the oral transmissibility of a given dose of TSE agent is increased when bound to <2-μm Mte particles (Johnson et al., 2007). Studies examined whether PrP^{TSE} would bind to insoluble particles in MBM samples to determine whether PrP^{TSE} present in MBM could be particle bound when dosed into animals (Figure 1). Following incubation of the protein with each MBM sample, it was found that PrP associated with sedimentable particles. Little PrP^{TSE} was detected in pellets of mock samples lacking MBM but treated identically (i.e., subjected to sedimentation). These data indicate that PrP^{TSE} spiked into MBM, or potentially present in contaminated feed, is likely to be associated with insoluble particles.

Animals were challenged with PrP^{TSE} that had been incubated with 1 or 10 mg of MBM 1 to allow association of the agent with MBM particles, or bound to Mte, Kte, or quartz microparticles. Control animals received an equal amount of identical PrP^{TSE} in the absence

of any particles. For these transmission experiments, a dose of PrP^{TSE} was chosen with a low level of peroral infectivity to facilitate detection of microparticle-associated enhancement of disease penetrance. The feed additive MBM and all tested microparticles, when complexed with prions, increased the oral transmission of disease in experimental animals (Figure 1). Increasing the dose of MBM from 1 mg to 10 mg raised the number of infected animals from 37 to 62%. One potential explanation is that more PrP^{TSE} was particle bound in the presence of more MBM. The aluminosilicates Mte and Kte enhanced prion disease transmission to a similar extent, whereas quartz was less effective. Compared to an equal amount of unbound prions, a dose not resulting in clinical disease in any of the animals, MBM and all tested microparticles substantially increased disease penetrance.

CONCLUSIONS

Should prions be present in animal feed, their association with MBM or insoluble particles such as those tested (viz. Mte, Kte, and SiO_2) could substantially increase the risk of disease acquisition compared to consumption of prions alone. While care needs to be exercised when extrapolating results from laboratory rodent models to ruminants, these data may explain how MBM produced BSE transmission despite the presumably low titers of infectious agent present in cattle feed (Taylor et al., 1995). In addition, inorganic particles in commercial feeds or mineral licks may pose a previously unrecognized risk of enhancing TSE transmission to domestic and wildlife species from agents contaminating these materials or infectivity shed onto feed or licks.

Following consumption, (sub)micrometer-sized particles can be absorbed by the gut. Goblet cells (Doyle-McCullough et al., 2007), M cells in Peyer's patches (Florence, 1997), and persorptive mechanisms at the tips of broken intestinal villi (Hillyer & Albrecht, 2001; Volkheimer, 2001) all contribute to intestinal particle uptake. Increased uptake of particle-bound prions might explain enhanced oral transmissibility. Alternatively, aluminosilicates may increase residence time of agent in the digestive system (Bringe & Schultz, 1969; Collings et al., 1980; Quisenberry, 1968) and elevate exposure time at sites of conversion. Further investigation into these mechanisms, the effect of digestive processes on bound prions, and alterations of agent physicochemical properties upon binding is warranted.

It is noteworthy that microparticles find widespread use as human food and pharmaceutical additives in Western diets (Lomer et al., 2000, 2004). The average daily consumption dietary microparticles from food, pharmaceuticals, and dentifrices is approximately 40 mg (approximately 10^{12} particles) per person (Powell et al., 2007). The extent to which microparticles in the human diet influence prion disease acquisition is currently unknown, but these data suggest that microparticle consumption needs to be investigated as a potential risk factor in human TSE acquisition.

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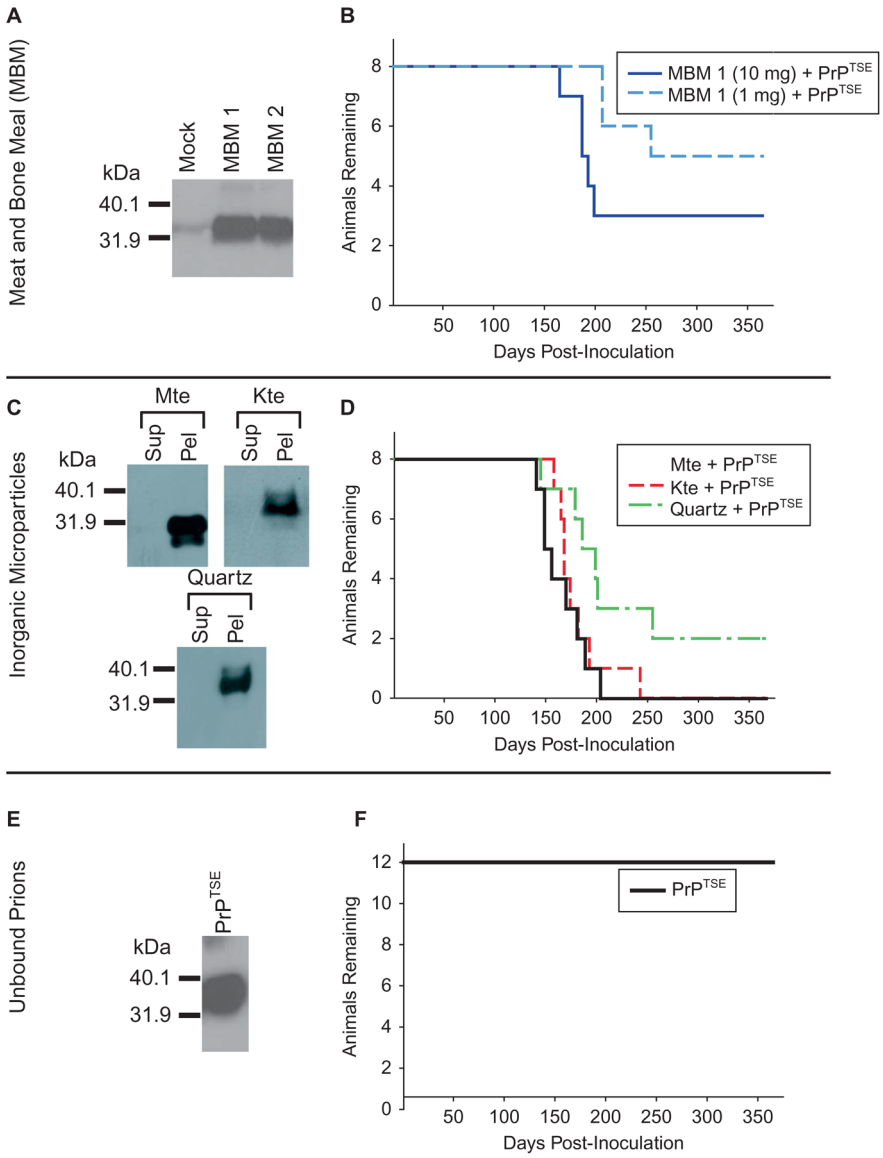

FIGURE 1.

Dietary microparticles and MBM bind PrP^{TSE} increasing prion disease transmission via the oral route of exposure. (A) Meat and bone meal (MBM, two sources MBM 1 and MBM 2; 1 mg) was incubated with abnormal prion protein (~0.2 μg, PrP^{TSE}), and attachment was analyzed by centrifugation and immunoblotting. Mock samples lacking microparticles demonstrated limited PrP^{TSE} sedimentation in the absence of MBM. (B) Either 1 or 10 mg of MBM 1 was incubated with purified agent (1 μg PrP^{TSE}) and fed to hamsters. Survival curves of the number of hamsters developing clinical disease within 1 yr of oral inoculation are shown. Tenfold increase in the amount of MBM 1 used in oral bioassay produced a corresponding increase in the number of animals presenting clinical disease. (C) The inorganic microparticles montmorillonite (Mte), kaolinite (Kte), and quartz all bind PrP^{TSE} (supernatant [Sup] and pellet [Pel] fractions representing unbound and particle-bound PrP^{TSE}, respectively). (D) Survival curves of hamsters perorally challenged with 1 μg PrP^{TSE} bound to these particles are shown. Hamsters dosed with an equal amount of unbound prions (immunoblot, panel E; survival curve panel F) remained healthy throughout

the course of the experiment. Likewise, negative-control treatment groups (four animals per group) challenged with microparticles (Mte, Kte, and quartz alone) or MBM 1 lacking prions did not acquire disease (not shown). Immunoblot data for Mte, Kte, and quartz are from (Johnson et al., 2006).

TABLE 1

Particle Size Distributions in Two Preparations of Meat and Bone Meal

	Percentage of particles in fraction		
Preparation	**2000–20 μm**	**20-2 μm**	**<2 μm**
MBM 1	85	8	7
MBM 2	79	12	9