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Treatment of periodontal disease for glycaemic control in people with diabetes

Transport of the Pathogenic Prion Protein through Soils

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Abstract

Transmissible spongiform encephalopathies (TSEs) are progressive neurodegenerative diseases and include bovine spongiform encephalopathy of cattle, chronic wasting disease (CWD) of deer and elk, scrapie in sheep and goats, and Creutzfeldt-Jakob disease in humans. An abnormally folded form of the prion protein (designated PrP^{TSE}) is typically associated with TSE infectivity and may constitute the major, if not sole, component of the infectious agent. Transmission of CWD and scrapie is mediated in part by an environmental reservoir of infectivity. Soil appears to be a plausible candidate for this reservoir. TSE agent transport through soil is expected to influence the accessibility of the pathogen to animals after deposition and must be understood to assess the risks associated with burial of infected carcasses. We report results of saturated column experiments designed to evaluate PrP^{TSE} transport through five soils with relatively high sand or silt contents. Protease-treated TSE-infected brain homogenate was used as a model for PrP^{TSE} present in decomposing infected tissue. Synthetic rainwater was used as the eluent. PrP^{TSE} was retained by all five soils; no detectable PrP^{TSE} was eluted over more than 40 pore volumes of flow. Lower bound apparent attachment coefficients were estimated for each soil. Our results suggest that TSE agent released from decomposing tissues would remain near the site of initial deposition. In the case of infected carcasses deposited on the land surface, this may result in local sources of infectivity to other animals.

Transmissible spongiform encephalopathies (TSEs) are a family of fatal neurodegenerative disorders affecting a variety of mammalian species. Examples include Creutzfeldt-Jakob disease (CJD) and kuru in humans, bovine spongiform encephalopathy (BSE), scrapie in

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goats and sheep, and chronic wasting disease in deer, moose, and elk (cervids). Although TSE agents have not been fully characterized at the molecular level, a major component of the infectious agent is the prion protein, PrP. In infected tissues, the normal cellular PrP (PrP^C) has assumed abnormal conformations (referred to here as PrP^{TSE}) that are often found in protease-resistant sedimentable aggregates (Prusiner 1998). After infection, TSE infectivity accumulates slowly, mainly in central nervous system and lymphoid tissues. Due to the extreme resistance of TSEs to common physical, chemical and enzymatic methods of inactivation (Taylor 2000), the likelihood is high that TSE infectivity entering the environment through disposal of infected tissues, *in situ* decay of infected carcasses, and excreta from infected animals will persist for long periods of time (years) serving as a pool of infectivity (Schramm et al., 2006). Environmental transmission of CWD to captive cervids has been demonstrated (Miller et al., 2004; Mathiason et al., 2009).

Among the potential environmental reservoirs of TSE infectivity, soil appears highly plausible due to the deposition of TSE agent to this medium, the persistence of TSE agent in soils (Brown and Gajdusek, 1991; Seidel et al., 2007), maintenance of infectivity by soil-particle bound prions (Johnson et al., 2006; Johnson et al., 2007a; Seidel et al., 2007), potentially enhanced transmission of soil-bound TSE agent (Johnson et al., 2007a), and the consumption of soil by herbivores (Beyer et al., 1994; Atwood and Weeks, 2003).

Several prior studies examined TSE agent survival in soil and the interaction of PrP^{TSE} with soil components. Brown and Gadjusek (1991) recovered infectivity from TSE-infected hamster brain material buried in garden soil for 3 years. The experiment was not designed to assess migration of infectivity in soils; however, the authors noted little vertical movement of the infectious agent. More recent studies have investigated attachment and persistence in the environment in more detail. Johnson et al. (2006) found that TSE infectivity adhered to common soil minerals and that soil mineral particle-prion complexes were infectious by the intracerebral route of exposure. Subsequently, enhanced oral TSE transmission was demonstrated for prions bound to montmorillonite and to some whole soils (Johnson et al., 2007a). Ma et al. (2007) measured PrP^{TSE} aggregate attachment to quartz sand and found attachment to be maximal near the average isoelectric point (pH_{IEP}) of the PrP^{TSE} aggregates (i.e., the pH at which the ensemble of PrP^{TSE} aggregates exhibited zero average electrophoretic mobility; see the Supplemental Information for further discussion) and at high solution ionic strength (I). Infectivity was shown to persist in the soil for at least 29 months when brain tissue from hamsters infected with the 263K strain of hamster-adapted scrapie was buried outdoors in soil lysimeters (Seidel et al., 2007). Although these burial experiments were not specifically designed to investigate transport of the pathogenic prion protein, movement of PrP^{TSE} into the underlying soil was noted to be minimal. Neither this study nor the earlier work by Brown and Gajdusek characterized the saturation state of the soils or the flux of liquid moving through the area containing infectious material.

We recently evaluated the potential for PrP^{TSE} to migrate through different landfill materials (Jacobson et al., 2009). All landfill materials examined retained PrP^{TSE} near the point of initial loading. A fraction of the loaded PrP^{TSE} migrated through columns packed with shredded municipal solid waste and green waste residual material (a potential burial material derived from composted yard waste); detectable PrP^{TSE} did not break through columns packed with natural soils used for daily cover (viz. a silty loam and a sandy clay loam) over 40 pore volumes of flow. The study focused on landfill disposal of infected carcasses and, therefore, employed landfill leachate as the eluent. Jacobson et al. (2009) used pathogenic prion protein enriched from the brain tissue of infected animals as the PrP^{TSE} source (Bolton et al., 1987; McKenzie et al., 1998). The enrichment procedure concentrates PrP^{TSE}, but promotes formation of protein aggregates that are likely larger than those formed *in vivo* (released from decomposing carcasses or present in excreta or secretions from infected

organisms). The enrichment procedure also eliminates many of the biomacromolecules present with PrP^{TSE} in disposed tissue. The need exists to investigate the mobility of PrP^{TSE} in a form more closely corresponding to that released from decomposing carcasses. Studies examining PrP^{TSE} transport resulting from on-farm carcass burial in soil and deposition to surface soils (e.g., from infected carcasses, hunter-deposited "gut piles", urine, feces) have not been reported.

We hypothesized that PrP^{TSE} in partially decomposed infected brain homogenate would exhibit limited migration in natural soils. The disposal scenario we chose to examine was on-farm burial in soil, and the experiments were designed to parallel an on-going field study in the UK. The soils chosen were those used in the UK study. The field study used disturbed soils; we, therefore, employed packed columns. Protease-treated, infectious brain homogenate was used as the PrP^{TSE} source. This matrix includes cellular debris (e.g., membrane fragments) that would be found in infectious material released from decaying tissue, and protease treatment was used to simulate the breakdown of PrP^{TSE} (Saunders et al., 2008) and its surrounding tissue matrix as infected carcasses decay. We chose synthetic northern European rainwater as the eluent to represent the liquid phase in contact with the PrP^{TSE} and soil. Boardman silt loam was included in the current study to provide a link with previous work (Johnson et al., 2009).

MATERIALS AND METHODS

Soils

Transport of PrP^{TSE} was investigated in five soils: Site S Topsoil (silt loam), Site S Subsoil (sandy loam), Site C Topsoil (sandy clay loam), Site C Subsoil (sandy clay loam), and Boardman (silt loam). The first four soils originated in central Scotland and represent those used in ongoing field-scale study of survival and migration of TSE infectivity from buried cattle heads. These soils were selected for the field study to represent areas of the UK where BSE occurred at high incidence and to incorporate properties assumed to influence movement or retention of infectivity. Cooke et al. (2006) detail the origins of these soils. Boardman silt loam, originating from eolian deposits in eastern Oregon, USA, is used as a municipal solid waste landfill cover material and was employed by Jacobson et al. (2009) to study transport of purified PrP^{TSE}. Properties of the soils are summarized in Table 1.

Source of PrP^{TSE}

Brain homogenate (BH, 10% (w/v)) was prepared in 10 mM 3-(N-morpholino)propanesulfonic acid (MOPS) (pH 7) from the brain tissue of Syrian golden hamsters (*Mesocricetus auratus*) clinically affected with the HY strain of hamster-adapted transmissible mink encephalopathy. To simulate the effect of endogenous proteases during the decay of tissues and to eliminate the non-infectious, cellular form of the prion protein (PrP^{C}), BH was treated for 2 h with 200 μg·mL^{-1} (final concentration) proteinase K (PK) at 37°C. The protease was then inhibited by addition of 5 mM phenylmethylsulphonyl fluoride (PMSF). To control for potential PrP^{TSE} degradation by residual PK activity in the PK-treated brain homogenate, infected BH was treated with PK and inhibited with PMSF as above, and then maintained on the benchtop for a time period corresponding to the duration of the column experiments. Immunoblot analysis (*vide infra*) of this control indicated no further loss of PrP^{TSE} (data not shown). The size distribution of particles in PK-digested infected BH was determined by dynamic light scattering (ZetaSizer Nano ZS, Malvern Instruments, Worcestershire, UK). These analyses were preformed on aliquots of the same PK-digested infected BH used in transport experiments.

PrP^{TSE} Transport Experiments

All column experiments were conducted in custom fabricated, 10-mm internal diameter, 24-mm high, poly(tetrafluoroethylene) (PTFE) columns containing a 1-mm-thick perforated PTFE frit at the bottom (Jacobson et al., 2009). All other components (ferrules, fittings, seals and tubing) were constructed from PTFE, PTFE-coated Viton, or fluorinated ethylene polypropylene, materials shown to minimize PrP^{TSE} binding (data not shown).

The eluent used in all transport experiments was a solution simulating northern European rainwater (Boyd, 2000) composed of 89.2 μM (2.05 mg·L^{-1}) Na$^+$, 9.0 μM (0.35 mg·L^{-1}) K$^+$, 35.4 μM (1.42 mg·L^{-1}) Ca^{2+}, 16 μM (0.39 mg·L^{-1}) Mg^{2+}, 174 μM (6.16 mg·L^{-1}) Cl$^-$, 34.2 μM (2.19 mg·L^{-1}) SO$_4^{2-}$, and 4.4 μM (0.27 mg·L^{-1}) NO$_3^-$ in distilled deionized water (ddH$_2$0; 0.18MΩ-m resistivity). The pH of the synthetic rainwater was 5.7, and I was 0.3 mM.

Prior to packing columns, soil water contents were adjusted to field conditions (*cf.* Table 1) by addition of ddH$_2$O and overnight equilibration. Following equilibration, soils were packed into columns by gentle tamping to dry densities representative of collection site conditions (*cf.* Table 1). The soil was then saturated by pumping >20 pore volumes (PV) of simulated rainwater through the soil using a syringe pump (KD Scientific, Holliston, MA). Hydrodynamic properties of the soils were determined by pumping simulated rainwater spiked with 1.2 mM KBr through the columns. Bromide concentrations in the effluent were determined using a microplate-based colormetric method (Lepore et al., 2009). The advection-dispersion-reaction equation (ADRE) was fit to the Br$^-$ data by adjusting the effective porosity and dispersion coefficient following the procedure in Lee and Benson (2004). Tailing in the Br$^-$ data was not observed, and excellent fits ($R^2 > 0.9999$) were obtained with the ADRE, suggesting that the dominant transport mechanisms in the columns were well modeled and represented by advection-dispersion theory.

Following the Br$^-$ tracer, the columns were flushed with >20 PV of simulated rainwater. The top plate of the column was then removed, and 50 μL of PK-digested 10% BH was pipetted directly onto the top of the soil column. The column was resealed, and simulated rainwater was pumped through the column at a seepage velocity of ~0.2 m·d^{-1}. Effluent samples were collected from the columns in ~0.5 PV increments in Protein Lo-Bind microcentrifuge tubes (Eppendorf AG, Hamburg, Germany) using a Retriever II autosampler (Teledyne Isco, Lincoln, NE) and stored at −80°C until analysis.

Prior to analysis, PrP^{TSE} in the effluent samples was concentrated by methanol precipitation. Samples were mixed with 4× their volume of ice-cold methanol and 10 μL of 10 mg·mL^{-1} solution of porcine thyroglobulin (to facilitate formation of a visible pellet). Samples were stored overnight at −20°C and centrifuged for 30 min at 24,400 *g* at 0°C. After the supernatant was aspirated and discarded, the pellet was dried in a vacuum centrifuge (Speed Vac SC110, Thermo Savant, Waltham, MA). Dry pellets were re-suspended in a 1:1 mixture of 5× SDS-PAGE sample buffer (100 mM Tris, 7.5 mM EDTA, 100 mM DTT, 350 mM SDS, pH 8.0) and ddH$_2$O.

After collecting 40 PV of flow, columns were frozen for 1 h at −80°C and sectioned into 3-mm slices using a razor blade (except for the Site C subsoil which was frozen and sectioned after 20 PV of flow due to the large pressure drop encountered with this material). PrP^{TSE} was extracted from the sectioned column portions for 10 min in 100°C 5× SDS-PAGE sample buffer (Johnson et al., 2006).

SDS-PAGE and Immunoblot Analysis

Samples in SDS-PAGE buffer were fractionated by SDS-PAGE, electrotransferred to a polyvinylidene fluoride membrane, and immunoblotted with a 1:40,000 dilution of monoclonal antibody (mAb) 3F4 (Signet, Dedham, MA) that targets residues 109-112 of PrP (hamster numbering). The secondary antibody was a 1:10,000 dilution of goat anti-mouse IgG (Bio-Rad, Hercules, CA) conjugated to horseradish peroxidase. Detection was achieved by exposing the membrane to West Pico peroxidase detection substrate (Pierce, Rockford, IL). As in our previous study (Jacobson et al., 2009), samples were run on the same gel as serial dilutions of the starting material to facilitate semiquantitation of PrP (data not shown). The amount of PrP in samples was determined by interpolation of immunoblot band intensities to those from the standard curve generated from serial dilutions of PK-treated BH.

Data Analysis

We previously modeled PrP^{TSE} transport in saturated porous media successfully using the ADRE equation with instantaneous linear adsorption and first-order attachment and detachment (Eq. 1) (Jacobson et al., 2009):

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial z}\left(D\frac{\partial C}{\partial z}\right) - \frac{\partial qC}{\partial z} - \rho_d K_d \frac{\partial C}{\partial t} - k_{att}C + \rho_d k_{det}S$$

(1)

where C is the PrP^{TSE} concentration in the aqueous phase, t is time, z is distance in vertical direction, q is the Darcy velocity, D is the dispersion coefficient, ρ_d is the dry density of media in column, K_d is distribution coefficient describing linear, instantaneous and reversible sorption, k_{att} is first-order attachment coefficient, k_{det} is first-order detachment coefficient, and S is the solid-phase (attached) PrP^{TSE} concentration.

PrP was not detected in any effluent samples in the current study. In such cases, we have shown that retention of PrP^{TSE} during steady, saturated flow can be described by first-order attachment alone (Jacobson et al. 2009). For this case, Eq. 1 becomes:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - v\frac{\partial C}{\partial z} - k_{att}C$$

(2)

Solving Eq 2 for an initial slug input of mass at $z = 0$ (the top of the column) to yields:

$$C(z,t) = \left\{ \frac{M/A}{\sqrt{4\pi Dt}}\exp\left[-\frac{(z-vt)^2}{4Dt}\right]\right\} e^{-k_{att}t}$$

(3)

where A is the cross sectional area of the column. A lower bound estimate of k_{att} can be obtained by solving Eq. 3 for k_{att} assuming the concentration of PrP^{TSE} in the first pore volume of effluent is at the detection limit of the immunoblotting assay. In this formulation, the attachment coefficient must be considered an apparent value (k_{att}^{app}) because a single value is used to describe PrP^{TSE} attachment to soil particle surfaces with a distribution of properties. The resolution of the experimental data does not justify use of a probability density function for the attachment coefficient distribution (Bradford et al., 2006). Straining was not explicitly included in our analysis (*vide infra*). To the extent that straining occurred in any of the porous media investigated, it would be captured in the k_{att}^{app} .

RESULTS AND DISCUSSION

PrP^TSE Transport Experiments

Effluent samples from columns analyzed up to pore volume 40 (pore volume 20 for the Site C subsoil column) did not contain PrP detectable by immunoblot analysis. Detection limits for immunoblotting were 0.1 μL of 10% BH or 0.2% of the total BH spiked into the columns (approximately 0.1 ng PrP^{TSE}). Extraction of PrP^{TSE} from the column sections demonstrated that detectable levels of PrP^{TSE} were confined to the upper 3 to 6 mm of the soil column (Fig. 1). Penetration of detectable PrP^{TSE} beyond 3 mm occurred only in the Site S Topsoil (detectable PrP^{TSE} in upper 6 mm, Figure 1). Compared to the other soils used, the Site S Topsoil had the largest porosity and organic carbon fraction (Table 1), two properties that may increase the propensity for PrP^{TSE} transport (Jacobson et al., 2009). The similarity in migration in the other columns prevented an assessment of the effect of soil properties on transport (e.g., textural class, CEC, mineralogy). The minimal migration of detectable PrP^{TSE} in PK-digested BH suggests, however, that transport of PrP^{TSE} from decomposing tissue will be limited in many soils, especially those that having finer texture than the soils used here. We note, however, that the organic carbon contents of the soils employed were low (f_{oc} = 0.0024 – 0.0049). Previous work suggests that attachment of PrP^{TSE} to soil particles diminishes as f_{oc} increases (Jacobson et al., 2009), but the interaction of the pathogenic prion protein with natural organic matter in soils warrants more thorough investigation.

We previously examined transport of purified PrP^{TSE} through Boardman silt loam under saturated conditions and reported penetration of detectable PrP^{TSE} to a depth of 12 mm when landfill leachate was used as the eluent (Jacobson et al., 2009). In the present study, we used N-terminally truncated PrP^{TSE} in a less aggregated state and in the presence of cellular debris, and used synthetic rainwater as the eluent. The more limited mobility of PrP^{TSE} in the present study may have been due to differences in the form of PrP^{TSE}, solution chemistry, or both. The PrP^{TSE} aggregates in the BH, although presumably smaller than those in purified preparations, were likely associated with larger cellular fragments that may have been retained in the porous medium by straining, thus limiting their movement (*vide infra*). PK digestion of PrP^{TSE} results in the removal of the N-terminal 67 amino acids in the HY strain of TME (Bessen and Marsh, 1994), six of which are basic residues. This N-terminal truncation is expected to lower the pH_{IEP} of the protein. As of the time of writing, the pH_{IEP} of N-terminally truncated PrP^{TSE} has not been reported. The reduction in pH_{IEP} would be expected to favor migration. Eluent chemistry differed between the two sets of experiments: the present study used synthetic rainwater with pH 5.7, I = 0.3 mM, and a dissolved organic carbon concentration ([DOC]) of 0 mg·L^{-1}; the landfill leachate was pH 7.7, I = 37 mM, and [DOC] = 175 ± 2 mg·L^{-1} (Jacobson et al., 2009). PrP^{TSE} aggregate size is maximal near the average isoelectric point of aggregates of full-length PrP^{TSE} ($pH_{IEP} \approx$ 4.6) and tends to increase with increasing I (Ma et al., 2007). The differences in eluent chemistry in the two sets of experiments, the lack of electrophoretic mobility measurements for N-terminally truncated PrP^{TSE}, and the association of PrP^{TSE} with membrane fragments and other cellular debris in brain tissue homogenate complicates comparison of expected PrP^{TSE} aggregate size based on the results of Ma et al. (2007). The relatively high DOC concentration in the landfill leachate may have facilitated PrP^{TSE} transport in the Boardman silt loam.

The only other study explicitly designed to examine movement of PrP in soil used recombinant ovine (sheep) prion protein (α-recPrP) expressed in *Escherichia coli* rather than the disease-associated prion protein (Cooke and Shaw, 2007). Penetration of α-recPrP was, at most, 10 mm in loamy sand and sandy clay loam (i.e., the Site C Topsoil used in our study) under unsaturated conditions. The biophysical properties of this surrogate differ

substantially from those of the purified and BH PrP^{TSE} used in our studies. In contrast to PrP^{TSE}, α-recPrP has low β-sheet content, lacks glycosylation, and has a much higher isoelectric point (pH$_{IEP}$ = 9.2 for α-recPrP [Rao et al., 2007]). Although both PrP^{TSE} and α-recPrP exhibited limited mobility in the soils examined, the pronounced biophysical differences between these proteins limits the degree to which results from experiments using α-recPrP results can be extrapolated.

PrP^{TSE} Recovery in Transport Experiments

Recovery of PrP^{TSE} loaded onto the columns by extraction of soils with 100°C SDS-PAGE sample buffer was incomplete. Recoveries ranged from 23% for the Boardman silt loam column to 76% from the Site C Subsoil column and averaged near 50% (Table 2). If PrP was present at the detection limit of immunoblotting for sections containing no observable PrP, recoveries would increase by ~3% (Table 2).

The PrP^{TSE} recoveries observed in the present study, while incomplete, are consistent with those reported previously (Johnson et al., 2006; Leita et al., 2006; Cooke et al., 2007; Seidel et al., 2007; Jacobson et al., 2009), and higher than those reported for α-recPrP after 1 month in the column (Cooke and Shaw, 2007). Possible sources of loss in the present study that were considered and discounted or controlled for include (1) sorption to experimental equipment, (2) degradation, and (3) interference of the leachate matrix and soil extracts with detection. First, poly(tetrafluoroethylene) (PTFE, Teflon®) or comparable fluoropolymers were used for column components in contact with PrP^{TSE} during transport experiments (*vide supra*) based on trials that indicated minimal PrP^{TSE} binding to PTFE relative to other potential column materials (viz. glass, polyvinyl chloride, polymethyl methacrylate). Furthermore, the surface area of the soil particles (23-28 m^2) greatly exceeded that available for attachment to the column (~8 × 10^{-4} m^2). All collection tubes used were manufactured to minimize protein binding. Second, degradation of PrP^{TSE} by residual, active PK in the BH could not account for the incomplete recoveries. The BH used was digested with PK prior to use. This treatment was expected to eliminate the majority of labile PrP in the brain homogenate (PrP^C and protease-sensitive PrP^{TSE}), leaving protease-resistant PrP^{TSE} (resPrP^{TSE}) (Pastrana et al., 2006). Significant degradation of resPrP^{TSE} by soil microorganisms over the time course of experiments would not be anticipated; PrP^{TSE} is resistant to most proteases tested (McLeod et al., 2004). Prior to addition to the column, PK was inhibited with PMSF. Control experiments demonstrated that further degradation of PrP^{TSE} did not occur after PMSF addition. Third, all standards used in immunoblotting experiments were treated in the same manner as the experimental samples to eliminate matrix effects on the detection of PrP. The standards were exposed to and loaded onto the polyacrylamide gel in effluent liquid from the relevant column collected immediately prior to introducing PrP^{TSE} to the column.

A possible explanation for the incomplete recovery is a time-dependent decline in extractability caused by a strengthening of the attachment between PrP^{TSE} and components of the porous material. Time-dependent declines in extractability from soil have been well-documented of small organic molecules (Hatzinger and Alexander, 1995; Alexander, 2000), noted in previous laboratory-scale experiments analyzing PrP^{TSE} in soils conducted in our laboratory, and observed or suspected in experiments carried out by others (Cooke et al., 2007; Seidel et al., 2007). Mechanisms governing declines in PrP^{TSE} extractability warrant further investigation.

Estimated Attachment Coefficients

PrP was not detected in any effluent samples. Thus, Eq. 3 was used to estimate a lower-bound k_{att}^{app} that would yield PrP^{TSE} at the detection limit of the immunoblot assay in the

first pore volume of effluent. The actual k_{att}^{app} of PrP in these soils is expected to be higher. In this analysis, all possible retention mechanisms (e.g., attachment, straining) are collapsed into a single first-order kinetic parameter (see discussion of straining below). This simplification of the factors controlling PrP-media interactions is consistent with the level of detail in our dataset. Of the five soils tested in this study, the Site C Subsoil had the lowest k_{att}^{app}, 1.8 h^{-1}, while the Boardman silt loam had the highest k_{att}^{app}, 2.8 h^{-1}. The Site S Topsoil, the Site S Subsoil, and the Site C Topsoil had intermediate lower-bound k_{att}^{app} values (2.2, 2.1, and 2.0 h^{-1}, respectively).

These k_{att}^{app} are lower than the lower-bound k_{att}^{app} values (2.6 to 3.3 h^{-1}) we previously reported (Jacobson et al., 2009). This difference is, however, due at least in part to differences in the detection limits of the immunoblot for PK-treated infected BH versus enriched PrP^{TSE}, and does not imply an actual difference in attachment. The k_{att}^{app} for prion proteins are at least one order of magnitude larger than k_{att}^{app} reported for virus particles in porous media (Jin et al., 2000; Schijven et al., 1999), the most comparable colloidal entities.

Straining and wedging were not explicitly included in the analysis; to the extent that straining and wedging occurred in the porous media investigated, they would be captured in the apparent attachment coefficients. Straining can occur regardless of the presence or absence of an energy barrier to attachment (Johnson et al., 2007b). The transport experiments were conducted under solution conditions favoring attachment (Johnson et al., 2006; Cooke et al., 2007). However, we have not identified whether the initial contact between PrP^{TSE} and soil particles resulted from direct attatchment or straining.

If physical straining was the sole mechanism for retention of PrP^{TSE}, we would expect a substantial proportion of PrP^{TSE} to be removed by a simple water extraction. We tested this possibility using a procedure outlined by Bradford et al. (2006). At the conclusion of a transport experiment, rather than freeze the column, we excavated the soil from the column and added it to microcentrifuge tubes containing an excess of eluent. After gentle mixing and centrifugation, all detectable PrP^{TSE} remained associated with the soil particles (data not shown). When PK-digested BH was subjected to the same centrifugation conditions, the majority of PrP^{TSE} was in the supernatant fraction. These results indicate that PrP^{TSE} in BH was attached to the soil particles, regardless of the mechanism responsible for initial contact. Detachment of PrP^{TSE} from soil particles requires extraction more aggressive than resuspension in simulated rainwater. The most effective extractants for PrP^{TSE} to date are solutions of anionic surfactants, and even these are not fully effective (Johnson et al., 2006; Cooke et al., 2007). The work on PrP^{TSE} extraction suggests that hydrophobic and electrostatic interactions must be disrupted to release PrP^{TSE} from soil particles.

In well-sorted, uniform porous media, straining can be an important colloid retention mechanism for colloid-to-median grain (or "collector") diameter ratios (d_c/d_{50}) $\geq$ 0.005 (Johnson et al., 2007).. In the present study, d_c/d_{50} ratios were 0.0027 to 0.0031 for the Scottish soils and 0.006 for the Boardman silt loam using d_c set equal to the Z-average hydrodynamic diameter of particles in PK-treated BH suspended in synthetic rainwater (240 nm, determined by dynamic light scattering). These d_c/d_{50} ratios suggest that the experiments conducted with one soil (Boardman silt loam) may have been at the very edge of the straining regime. However, the applicability of such d_c/d_{50} thresholds for the onset of straining is unclear for natural soils containing a broad range of particle sizes and surface chemistries, and for colloids exhibiting substantial polydispersity. Straining of colloids exhibiting substantial polydispersity in natural soils with a broader range of pore sizes may occur at d_c/d_{50} ratios less than 0.005.

Environmental Implications

The present study examined migration of N-terminally truncated PrP[TSE] in several soils with relatively high sand and silt contents; retention of full-length PrP[TSE] is expected to be similar or more limited. The PrP[TSE] in BH decomposed in a laboratory setting was reported to be primarily N-terminally truncated (Saunders et al., 2008), and this is likely true of PrP[TSE] released from decomposing carcasses. We therefore digested infected BH with PK to N-terminally cleave PrP[TSE]. Proteinase K treatment had the added benefits of eliminating PrP[C] and partially degrading the BH matrix. Since the N-terminus is implicated in the strong binding of PrP[TSE] to clay particles (Johnson et al., 2006; Cooke et al., 2007), and it is expected that full-length PrP[TSE] would be retained more strongly than N-terminally truncated PrP[TSE], at least by clay minerals.

The persistence of TSE infectivity in the environment (Brown and Gajdusek, 1991; Miller et al., 2004; Georgsson et al., 2006; Seidel et al., 2007) may lead to release and migration of PrP[TSE] over time scales considerably longer than those simulated. Furthermore, the slow decay of the tissue of an infected carcass, but not the associated PrP[TSE], might also slow release of the infectious agent. Other factors that warrant investigation include the influence of natural organic matter (dissolved and particle-associated), the effects of pore size and distribution, and transport through preferential flow paths formed by biota and physical pedogenic processes (Kung et al., 2000; Cey et al., 2009). The present study was not designed to investigate the potential for macropore transport of PrP[TSE]. Transport via preferential flow paths may allow more extensive migration of PrP[TSE] in soils than observed here.

Although the present study was designed to provide insight into the potential for PrP[TSE] to migrate soil in the context of on-farm burial of infected carcasses, implications for environmental transmission of CWD and scrapie are apparent. TSE agent in infected tissues deposited at the soil surface (e.g., carcasses of deceased animals, gut piles, placenta) may remain near the soil surface in a bioavailable form (Johnson et al., 2007a, Seidel et al., 2007) and contribute to the spread of CWD and scrapie through relatively small, contaminated patches of soils serving as "hotspots" of infectivity. The strong binding of PrP[TSE] to some soil components and maintenance of the agent near the soil surface also raises the possibility that disease-associated prion protein could be delivered to surface waters attached to particles during runoff-producing rainfall and snow melt events (Nichols et al., 2009).

CONCLUSIONS

Our results suggest that migration of PrP[TSE] released from decomposing infected tissues will be minimal in natural soils with textures similar to silt loam, loamy sand, sandy loam, and sandy clay loam. The majority of PrP[TSE] released into soil will remain at or near the location of deposition. The experiments in the present study were conducted under saturated conditions; transport under unsaturated conditions is expected to be equally if not more limited (Powelson et al., 1990). Migration of PrP[TSE] (and most likely infectivity) deeper into the soil column is expected to be restricted in finely textured soils with relatively low organic carbon contents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

ADRE	advection-dispersion-reaction equation
BH	brain homogenate
BSE	bovine spongiform encephalopathy
CEC	cation exchange capacity
CJD	Creutzfeldt-Jakob disease
CWD	chronic wasting disease
DOC	dissolved organic carbon
DTT	dithiothreitol
EDTA	ethylenedinitrilotetraacetic acid
HY	Hyper strain of hamster-adapted transmissible mink encephalopathy
I	ionic strength
IgG	immunoglobulin G
mAb	monoclonal antibody
MOPS	3-(N-morpholino)propanesulfonic acid
PAGE	polyacrylamide gel electrophoresis
PFTE	poly(tetrafluoroethylene)
pH$_{IEP}$	isoelectric point
PK	proteinase K
PMSF	phenylmethylsulphonyl fluoride
PrP	prion protein
PrP^C	cellular prion protein
PrP^{TSE}	disease-associated prion protein
PV	pore volume
recPrP	recombinant prion protein
resPrP^{TSE}	proteinase K-resistant PrP^{TSE}
SDS	sodium dodecyl sulfate
TME	transmissible mink encephalopathy
Tris	Tris-(hydroxymethyl) aminomethane HCl
TSE	transmissible spongiform encephalopathy

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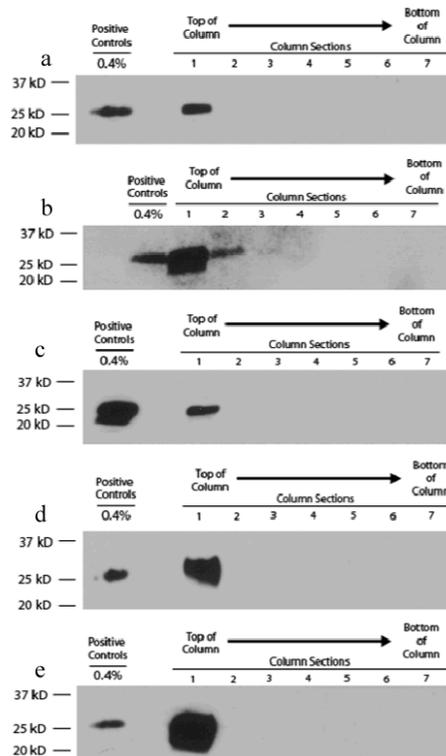
FIGURE 1.
PrP[TSE] extracted from (a) Boardman silt loam, (b) Site S Topsoil (c) Site S Subsoil (d) Site C Topsoil and (e) Site C Subsoil. Each section represents ~3 mm or 12.5% of the column height. Section 1 is the topmost section of each column. 50 μL of 10% brain homogenate (BH, ~50 ng PrP[TSE]) was applied to the top of the column. Positive controls are given as a percentage of the total BH volume initially applied to the column. PrP[TSE] is detected using the monoclonal antibody, 3F4. Protein molecular mass is indicated at the left as determined by use of pre-stained protein standards (Bio-Rad Precision Plus, Hercules, CA).

Table 1

Soil Properties

Parameter	Site S Topsoil	Site S Subsoil	Site C Topsoil	Site C Subsoil	Boardman silt
specific surface area (m²·g⁻¹)[†]	9.4	14.6	10.6	12.7	13.0
fraction organic carbon (f_{oc})[‡]	0.0049	0.0024	0.0024	0.0032	0.0047
dry density (Mg·m⁻³)	1.31	1.39	1.61	1.54	1.47
gravimetric water content	0.14	0.16	0.12	0.18	0.14
effective porosity	0.40	0.34	0.39	0.28	0.32
pore volume (mm³)	754	641	735	528	603
dispersivity (mm)	1.21	1.62	1.15	1.13	12.5
CEC (cmol⁺·kg⁻¹)	16.7	15.0	16.3	19.0	17.0
Mineralogy (%) [§]					
Quartz	56	44	67	53	34
K-Feldspar	4	11	4	4	6
Plagioclase	26	26	5	8	35
Pyrite	0	0	1	1	0
Hematite	1	2	0	0	1
Total Phyllosilicates[¶]	13	17	23	34	24
Particle Size Distribution [††]					
% sand	82	65	63	64	37
% silt	12	19	14	15	55
% clay	6	16	23	21	8

[†] Specific surface area determined by N2 adsorption

[‡] f_{oc}, fraction of organic carbon, determined by the organic carbon dry combustion method using a CNS-2000 instrument (Leco, St. Joseph, MO)

[§] Mineralogy determined by X-ray diffraction (K/T Geosciences, Argyle, TX)

[¶] Full phyllosilicate data can be found in the Supplemental Information

[††] Particle size distribution determined by wet sedimentation (Université Montpellier).

Table 2

Lower-bound attachment coefficients and recoveries

Soil	Apparent attachment coefficient, k_{att}^{app} (h^{-1})	% PrP^{TSE} recovered [†]
Site S Topsoil	> 2.2	40 (42)
Site S Subsoil	> 2.1	54 (57)
Site C Topsoil	> 2.0	68 (71)
Site C Subsoil	> 1.8	76 (78)
Boardman Silt	> 2.8	23 (26)

[†] Recoveries of PrP^{TSE} determined by comparison of immunoblots of dilutions of soil column extracts to known amounts of PrP^{TSE}. The numbers in parentheses indicate PrP^{TSE} recovery if samples with no detectable PrP^{TSE} are assumed to have an amount of PrP^{TSE} equivalent to the detection limit.