Chapter 6
Prions in the Environment

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Abstract Scrapie and chronic wasting disease (CWD) are two prion diseases of particular environmental concern as they are horizontally transmissible. Prions are shed from diseased hosts in a diverse set of biologic matrices and are present throughout the diseased host. There is strong experimental evidence that properties of soil and water can significantly affect prion sorption, resistance to degradation, persistence, replication efficiency when bound to soil, and ultimately prion infectivity. Highly sensitive and accurate detection of prion infectivity in the environment is not currently possible, severely hampering informed management of disease. A more thorough understanding of the interaction of prions with the environment in combination with robust detection methods may lead to means to reduce or eliminating prion disease in free-range and captive animal populations as well as mitigating the risk of zoonotic prion transmission.

Keywords Chronic wasting disease • Environmental prion contamination • Prion diseases • Prion shedding • Scrapie

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6.1 Introduction

Scrapie and chronic wasting disease (CWD) are two prion diseases of particular environmental concern as they are horizontally transmissible and remain infectious after years in the environment (Greig 1940; Hadlow et al. 1982; Miller and Williams 2003; Miller et al. 2004). Recent experimental and epidemiological work suggests that soil may play a role in natural prion transmission (Saunders et al. 2008a, 2012a, b; Smith et al. 2011). Indirect, environmental transmission has been implicated in multiple CWD and scrapie outbreaks (Georgsson et al. 2006; Miller et al. 2006) and environmental transmission has been demonstrated in a number of studies (Greig 1940; Miller et al. 2004; Dexter et al. 2009; Mathiason et al. 2009; Rhyan et al. 2011).

One factor influencing environmental transmission of prion diseases is the long-term survival of prions in the environment. Unbound and soil-bound scrapie and BSE PrP^{Sc} were detectable after 18 months of room temperature incubation in the laboratory (Maddison et al. 2010a), and soil-bound hamster prions remained capable of replication after similar year-long incubations in a separate study (Saunders et al. 2011a). In addition, hamster prions mixed with soil and buried in the field remained orally infectious after 2 years (Seidel et al. 2007). Thus, long-term survival of prions in soil is possible and could explain the long-term environmental persistence of prions (Saunders et al. 2008a). Epidemiological records indicate numerous instances of scrapie recurrence upon reintroduction of animals on farms previously exposed to scrapie. Scrapie recurrence was documented following fallow periods of 1–19 years (Siguardson 1991; Georgsson et al. 2006) and pastures can retain infectious CWD prions at least 2 years after exposure (Miller et al. 2004). In addition, the disposal of mortalities during BSE outbreaks, both in the past and potential future disposal events, serves as another environmental source of prions with the potential to infect humans. Therefore, it is clear that prions pose a significant environmental concern.

Prions are shed from diseased hosts in a diverse set of biologic matrices, including feces, urine, saliva, blood, skin, milk, placenta, and nasal mucus and a comprehensive review of prion shedding was recently performed by Gough and Maddison (2010). Prion shedding can occur many months prior to clinical manifestation of the disease (Gough and Maddison 2010; Tamgüney et al. 2009). Prions also enter the environment after decomposition of diseased animal carcasses (Miller et al. 2004), as prions are present near-ubiquitously throughout a diseased host (Saunders et al. 2012a). Uptake of prions to naïve hosts can occur via ingestion or inhalation of contaminated material (Hamir et al. 2005, 2008; Kincaid and Bartz 2007; Sigurdson et al. 1999), although the significant routes of natural exposure remain uncertain (Saunders et al. 2012a).

Prions shed into the environment will interact with soil (Fig. 6.1). Given the close contact that animals, especially ruminants, have with soil through many routine behaviors, including ingestion of soil via feeding and mineral supplementation, there is significant opportunity for transmission of prions via soil (Saunders et al. 2008a, 2012a, b; Smith et al. 2011). No experimental work to date has directly investigated soil-mediated CWD or scrapie transmission in the natural hosts of these diseases.

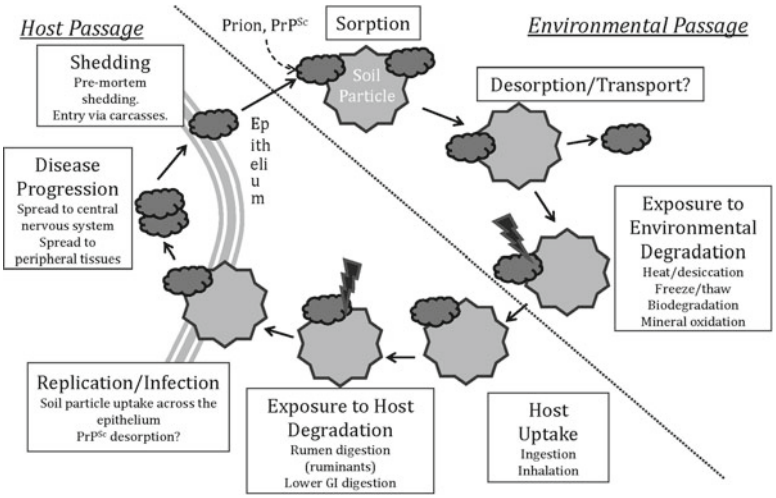

Fig. 6.1 Conceptual Model for soil-mediated prion transmission. From Saunders et al. (2012b)

Nevertheless, there is now ample evidence from studies in rodents that soil-mediated transmission is a viable and likely significant mechanism of natural prion transmission. Replication of soil-bound hamster and CWD prions (seeding conversion of PrP^{c} to PrP^{Sc}) has been demonstrated (Saunders et al. 2011b), and soil-bound hamster prions are infectious via oral (Johnson et al. 2007) and intracerebral routes (Saunders et al. 2012a).

6.2 Prion Sorption to Soil

There is strong experimental evidence that properties of soil and water can significantly affect prion sorption, resistance to degradation, persistence, replication efficiency when bound to soil, and ultimately prion infectivity (Table 6.1). Soil type, which we define broadly here as a soil's distinct texture (particle size distribution), mineralogy, and organic carbon content, is a strong determinant of prion sorption (Table 6.1). PrP^{Sc} has a higher affinity for clays and clay soils compared with sand and sandy soils. For instance, in one study, the sorption capacity of a silty clay loam soil was at least three times higher than a sandy loam soil (or 400 times higher at initial equilibrium) and 2,000 times higher than fine quartz sand (Saunders et al. 2009a). In another, sorption of purified PrP^{Sc} to montmorillonite clay was at least 100 times greater than fine quartz sand (Johnson et al. 2006). PrP adsorption kinetics are also significantly different between clay soil and sand or sandy soil. In one study, maximum adsorption for fine quartz sand and a sandy loam soil was observed after 7–30 days, while maximum adsorption for a silty clay loam soil took only 24 h (Saunders et al. 2009a). Thus, prions contacting clay soils could be rapidly

Table 6.1 Variance in prion–soil interactions with respect to soil type

Prion–soil property	Soil type/component			References
	Clay/clay soils	Sand/sandy soils	Organic content	
PrP^{Sc} sorption capacity	Higher	Lower	Unknown	Johnson et al. (2006), Saunders et al. (2009a)
PrP^{Sc} desorption with SDS (% recovery)	Low (<5–50%)	High (20–95%)	Low (5–20%)	Cooke et al. (2007), Jacobson et al. (2009), Maddison et al. (2010a), Saunders et al. (2010)
PrP^{Sc} sorption kinetics in tissue homogenate	Faster (<1 day)	Slower (>1–30 days)	Unknown	Saunders et al. (2009a)
Role of the PrP^{Sc} N-terminus in sorption	Enhances sorption	Inhibits sorption	Unknown	Cooke et al. (2007), Johnson et al. (2006), Maddison et al. (2010a), Saunders et al. (2009b, 2010)
Replication efficiency[a]	Reduced	Equal	Reduced	Saunders et al. (2011b)
Intracerebral infectivity[a]	Reduced	Unknown	Unknown	Saunders et al. (2011b)
Oral infectivity[a]	Increased	Unknown	Unknown	Johnson et al. (2007)

[a]Soil-bound prions compared with unbound prions
From Saunders et al. (2012b)

immobilized on the soil surface, forming potent reservoirs for efficient transmission. In contrast, prions contacting sandy soils may be more readily transported below the surface and diluted by surface or groundwater.

The role of N-terminal region of PrP^{Sc} in soil adsorption also varies with soil type. Although the N-terminus is not required for prion infectivity (Bessen and Marsh 1994) or for soil sorption (Saunders et al. 2009b), its presence enhances adsorption of PrP^{Sc} to clay but may hinder adsorption to sand surfaces (Saunders et al. 2009b). In addition, numerous studies have observed cleavage of the N-terminus following PrP desorption from clay surfaces using anionic detergents (Cooke et al. 2007; Johnson et al. 2006; Maddison et al. 2010a; Saunders et al. 2010). Cleavage is not observed following desorption from sand, sandy soils, or organic matter, suggesting the N-terminus is actively involved in PrP sorption to clay particles but not other soil components. Both truncated and full-length forms of PrP^{Sc} will enter the soil environment (Saunders et al. 2008b), and given that the N-terminus is not required for prion infectivity or soil sorption, there may be little effect of interactions between the PrP N-terminus and soil on prion transmission. However, it does strongly suggest mechanistic differences in prion sorption between clay surfaces and other soil surfaces.

Soil water chemistry can also influence prion adsorption. The chemistry of soil–water–prion mixtures will vary with soil components, soil moisture, and the source of infectious prions (e.g., excreta, saliva, and tissue). While solution ionic strength and ionic composition may not significantly affect PrP^{Sc} adsorption (Saunders et al. 2011a), the biologic matrix in which prions enter the environment (prion source) can significantly alter soil sorption kinetics and capacity (Saunders et al. 2009a). For example, the magnitude and kinetics of PrP adsorption from tissue homogenate are significantly reduced compared with adsorption of pure or purified PrP (Saunders et al. 2009a), most likely due to competitive sorption (Saunders et al. 2009b). Importantly, adsorption of PrP introduced in biologic matrices besides tissue homogenate has yet to be studied.

Desorption of PrP from soil has not been observed under mild, environmentally relevant conditions or in the presence of harsh chaotropic agents, nonionic detergents, or extreme pH (Cooke et al. 2007; Johnson et al. 2006; Seidel et al. 2007). Thus, desorption of prions once bound to soil may be rare in natural settings. However, it is interesting to note the ability to desorb PrP with anionic detergents varies with soil type, where extraction from sand and sandy soils is significantly higher than from clays, clay soils, and organic matter (Table 6.1) (Cooke et al. 2007; Maddison et al. 2010a; Saunders et al. 2010; Jacobson et al. 2009).

6.3 Prion Transport in the Environment

Due to their insolubility and high affinity for clays and silts, prions are unlikely to be transported long distances in surface water. Recent studies simulating prion fate in wastewater found that PrP strongly partitioned into the sludge solids (Hinckley et al. 2008; Kirchmayr et al. 2006). Three studies have evaluated the mobility of prions in soil. One found only slight recPrP migration in a soil column over a 9-month incubation (Cooke and Shaw 2007). Jacobson et al. (2009, 2010) observed minimal HY TME PrP^{Sc} migration in columns packed with five different soils. Purified PrP^{Sc} was more mobile in columns packed with municipal solid waste (Jacobson et al. 2009). The potential for prion transport facilitated by mobile soil colloids has not been investigated. Colloid-facilitated transport has been shown to be a significant transport process for many strongly sorbing contaminants (de Jonge et al. 2004). In addition, infectious prions can form aggregates of colloidal size (Silveira et al. 2005) and might be transported unassociated. Macropore colloid-facilitated transport could quickly move prions into groundwater or surface waters and therefore warrants further study.

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6.4 Degradation and Mitigation of Prions in the Environment

Prions are subject to degradation in the natural environment; however, prions are resistant to degradation and inactivation, especially when compared with bacterial or viral pathogens (Taylor 1999). Bacterial enzymes which effectively degrade prions have been identified, but they are most effective at high pH (10–12) and high temperature (50–60°C) (McLeod et al. 2004; Yoshioka et al. 2007) conditions which are atypical of most natural environments. Microbiological consortia taken from the rumen and colon of cattle could degrade PrP^{Sc} to undetectable levels within 20 h under anaerobic conditions at 37°C, although infectivity remained (Scherbel et al. 2006, 2007). Degradation of PrP^{Sc} by select lichen extracts has been shown (Johnson et al. 2011) and treatment with manganese oxide (naturally occurring in certain soils) under acidic conditions also leads to PrP^{Sc} degradation (Russo et al. 2009).

A limited number of studies have investigated degradation of soil-bound prions. Laboratory studies suggest prions bound to soil with high organic content may degrade more rapidly when compared to prions bound to clay and sand minerals (Maddison et al. 2010a; Saunders et al. 2011a). Soil-bound prions in highly dilute aqueous solutions may also exhibit lower persistence compared to prions in solutions of higher ionic strength (Saunders et al. 2011a). An additional study reported significantly higher survival of clay-bound PrP in the presence of manganese (Davies and Brown 2009). Enzymatic digestion of soil-bound prions under environmentally relevant conditions is effective across all soil types (Saunders et al. 2010, 2011c), although prions bound to soil organic matter may be more susceptible than prions bound to other surfaces (Saunders et al. 2011c). Preliminary data indicate that binding to soil decreases prion resistance to heat desiccation irrespective of soil type (authors' unpublished data), which suggests soils that retain moisture could favor prion persistence.

In addition to studies evaluating prion persistence in soil, there has been some work to determine the risk of prions in wastewater and biosolids (Epstein and Beecher 2005; Pedersen et al. 2006). Prions could enter wastewater through effluent from slaughterhouses unknowingly rendering prion mortalities or through contaminated effluent from hospital or research facilities. Hinckley and colleagues determined that most PrP^{Sc} and prion infectivity would associate with the activated sludge solids, survive mesophilic anaerobic digestion, and be present in the remaining biosolids (Hinckley et al. 2008). Likewise, Kirchmayr et al. (2006) found no significant decrease in PrP^{Sc} after 16-day incubation in mesophilic anaerobic sludge and observed PrP^{Sc} solids association. PrP^{Sc} degradation was observed in thermophilic anaerobic sludge, although maximum degradation occurred in sterilized samples (Kirchmayr et al. 2006). Others found a large decrease in PrP^{Sc} within 15 days after

incubating BSE brain homogenates in municipal sewage at 20°C (Maluquer de Motes et al. 2008). Sheep scrapie brain homogenates were somewhat more resistant to degradation. Based on these studies, it can be assumed that most prion infectivity will be conserved during normal wastewater treatment processes, and prions would thus enter the environment, highly diluted, via landfill disposal or landspreading of biosolids.

6.5 Do Environmental Factors Influence Prion Incidence?

Prion disease incidence exhibits significant geographic variance, including CJD in humans, CWD, and scrapie (Blanchong et al. 2008; Conner and Miller 2004; Holman et al. 2010; Joly et al. 2006; Stevens et al. 2009; Walter et al. 2011). There are a wide range of potential factors influencing spatial variance in these diseases, including population genetics (Blanchong et al. 2008; Hunter 2007), animal movement patterns and habitat prevalence (Conner and Miller 2004; Joly et al. 2006), predator prevalence (Wild et al. 2011), and human impacts (Krumm et al. 2005; Stevens et al. 2009). Environmental factors such as local climate, the presence of potential vectors, and vegetation, water, and soil characteristics may also influence prion disease incidence for a given area, either by altering the susceptibility of the host to infection or directly affecting the prion along its transmission pathway.

With respect to the former, a number of groups have investigated trace metal levels in forage, water, and soils of scrapie and CWD endemic areas, given that copper, manganese, or other metals may play key roles in prion pathogenesis (Davies and Brown 2009). No consistent correlations have been observed to date (Chihota et al. 2004; Imrie et al. 2009; McBride 2007), suggesting abnormal environmental exposure to trace metals may not be a significant factor in prion incidence. In contrast, a number of studies have observed significant soil factors that may directly affect prion transmission pathways. Although a study of scrapie in Great Britain did not find a significant correlation between soil texture (only roughly delineated as “sand”, “loam”, “peat”, or “clay”) and scrapie incidence, a soil drainage factor was significant, where soils classified as “naturally wet” had higher risks of scrapie than “freely draining” soils (Stevens et al. 2009). In addition, Imrie and colleagues found possible correlations between soil pH and organic content and scrapie incidence in Great Britain, but no correlation with soil clay content (Imrie et al. 2009). As the authors acknowledge, these studies must be considered preliminary as the spatial resolutions were very low and the datasets were limited.

Recently, a more robust study of CWD in northern Colorado has suggested a correlation between soil texture and CWD incidence in free-ranging cervids. Along with the previously known risk factors of age and sex, the soil clay content of a deer’s home range appeared to be positively correlated with risk of CWD infection (Walter et al. 2011). Results for the other deer habitat factors analyzed, which were distance to riparian habitat, location near wintering concentration areas, and landownership (private/public), were less conclusive. The results of this study are somewhat difficult

to interpret as clear goodness-of-fit parameters were not presented. Nevertheless, this study not only supports a link between soil and CWD transmission but also implicates a specific soil factor, clay content, with increased local prion incidence.

6.6 Detection of Prions in the Environment

One current limitation in our ability to evaluate environmental prions is that highly sensitive and accurate detection of prion infectivity in the environment is not currently possible. Standard methods such as western blotting fail to detect significant levels of infectivity (Barron et al. 2007; McLeod et al. 2004; Scherbel et al. 2006), and the most reliable method of prion detection, animal bioassay, would be impractical for use on large numbers of environmental samples. Protein misfolding cyclic amplification (PMCA) (Saa et al. 2006), developed by Soto and colleagues for detecting small amounts of PrP^{Sc}, has generated much interest for use as an environmental detection method. PMCA has been used successfully with CWD samples (Kurt et al. 2007) and with hamster PrP^{Sc} exposed to soil (Nagaoka et al. 2010; Seidel et al. 2007). A recent study reported detection of scrapie PrP^{Sc} on metal and wooden fencing from a scrapie endemic farm using PMCA, but infectivity was not determined (Maddison et al. 2010b). The QUIC (quake-induced conversion) method (Atarashi et al. 2007, 2008), which uses recPrP as a substrate instead of uninfected brain homogenate, might be a viable alternative to PMCA as an environmental diagnostic tool. Quantitative tandem mass spectrometric techniques (Onisko et al. 2007) may also be developed as a sensitive environmental detection and quantification method for PrP.

6.7 Conclusion

As prion diseases, and CWD in particular, continue to spread geographically and disease residence times in cervid populations and habitats increases, environmental factors may play an increasingly important role in sustaining or heightening disease prevalence (Almberg et al. 2011). The critical parameters of environmental prion transmission are the mean residence time of prions in environmental reservoirs and the efficiency of transmission via these reservoirs (Sharp and Pastor 2011). We predict these parameters could vary significantly based on environmental factors such as soil properties.

Influence of soil factors on disease incidence is certainly not without precedent. Numerous experimental studies have reported variance in the survival, transport, and transmission of enteric pathogens with respect to soil type and soil factors (Cilimburg et al. 2000). Biotic and abiotic soil factors have been linked to the prevalence of agriculturally relevant soil-borne diseases (Mazzola 2002). Recently, clay soils have been linked to an increased risk of the parasitic nematode *Baylisascaris procyonis* in Texas raccoons (Kresta et al. 2010), organic carbon and clay content was positively correlated with prevalence of ovine Johne's disease, caused by

Mycobacterium avium, in Australia (Dhand et al. 2009), and poorly drained clay soils with high organic content were associated with the abundance of *Culicoides imicola*, primary vector for the bluetongue virus (Acevedo et al. 2010).

The epidemiological data on prion–soil risk factors are as yet limited. Thus, robust spatial epidemiological studies of well-established CWD endemic areas should be conducted to build on the work of Walter et al. (2011). In addition, reliable methods for detecting and quantifying infectious prions in the soil environment are clearly required. Although detection of prions in natural soil samples has not yet been reported, use of protein misfolding cyclic amplification (PMCA) or similar methods appears to be the most promising avenue (Saunders et al. 2012a). PMCA has been used successfully with soil-bound prions (Saunders et al. 2011a, b, c).

If soil properties are indeed significant in local prion incidence, a number of important disease management implications arise. In captive settings, herd owners could favor pastures with low-risk soils, perhaps even amending soils to decrease prion transmission. In free-range populations, epidemiological modeling could use soil properties to predict temporal and spatial trends in prion incidence. Soil could be considered to prioritize disease surveillance efforts. High-risk soils, especially those with the potential for human exposure, could be targeted with treatments to reduce transmission (Saunders et al. 2010, 2011c). These measures offer hope for reducing or eliminating prion disease in free-range and captive animal populations as well as mitigating the risk of zoonotic prion transmission.

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