Soil and the Environmental Transmission of Prion Disease

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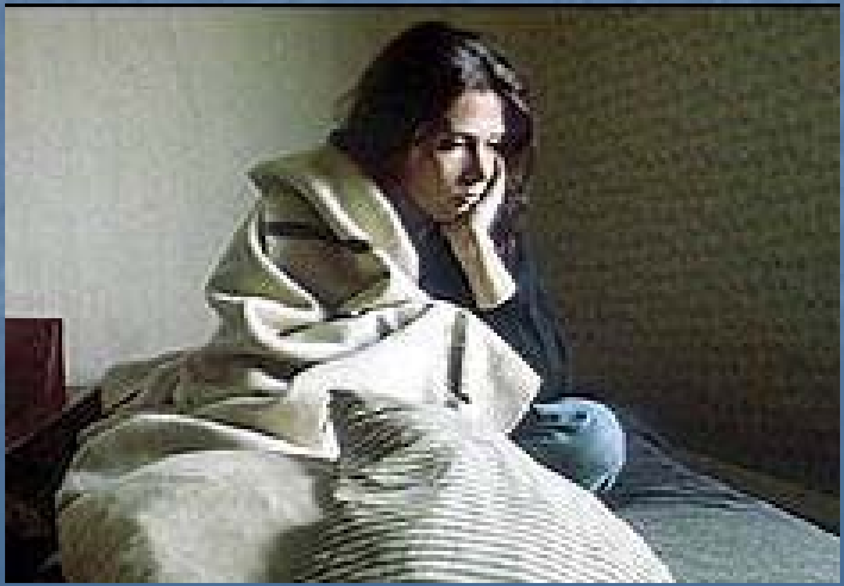
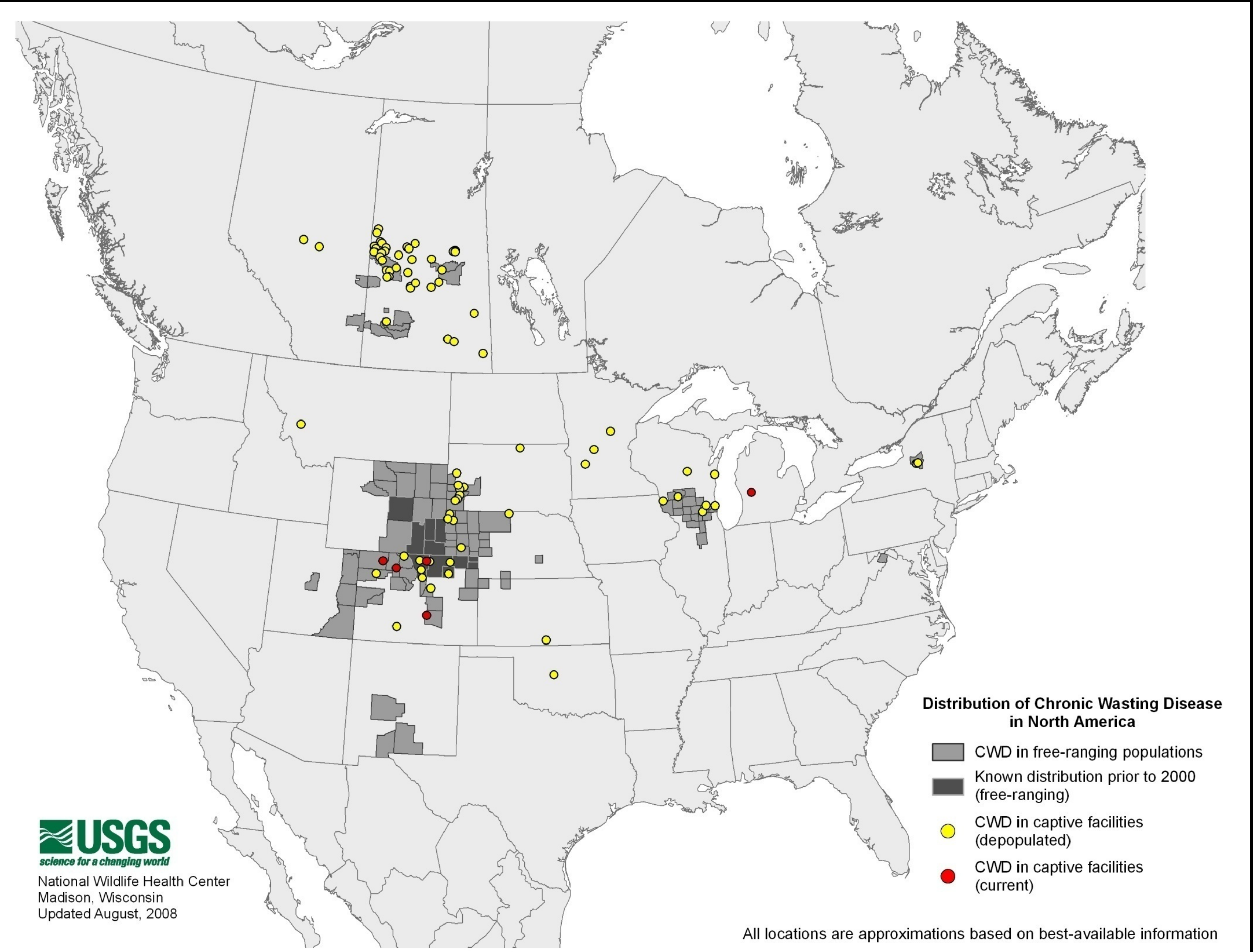
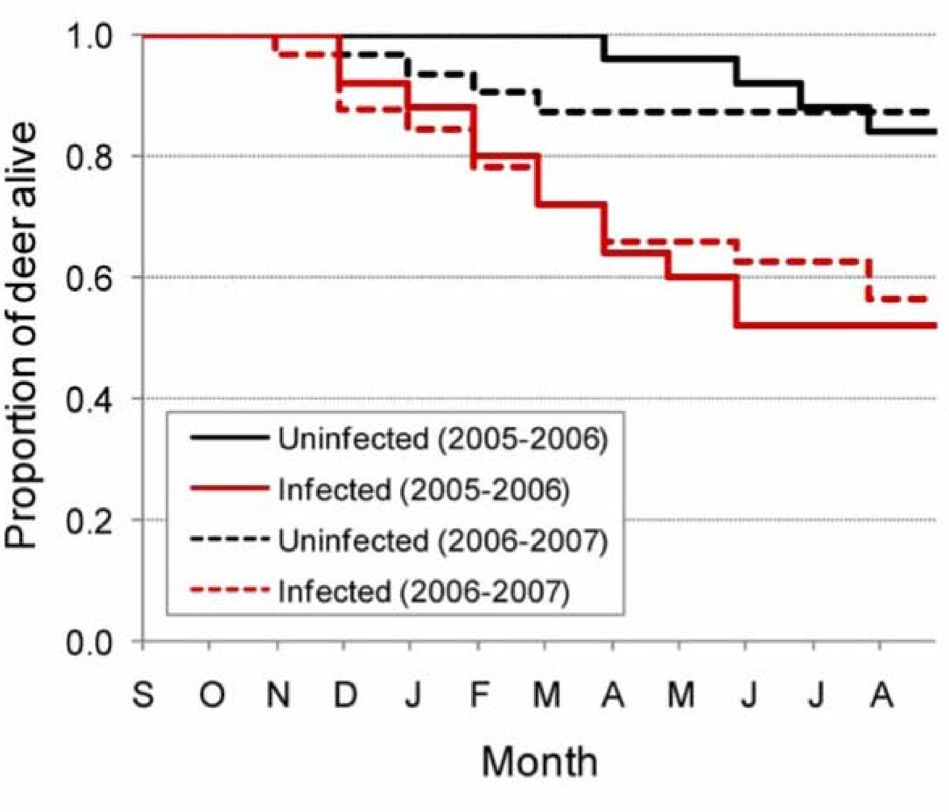
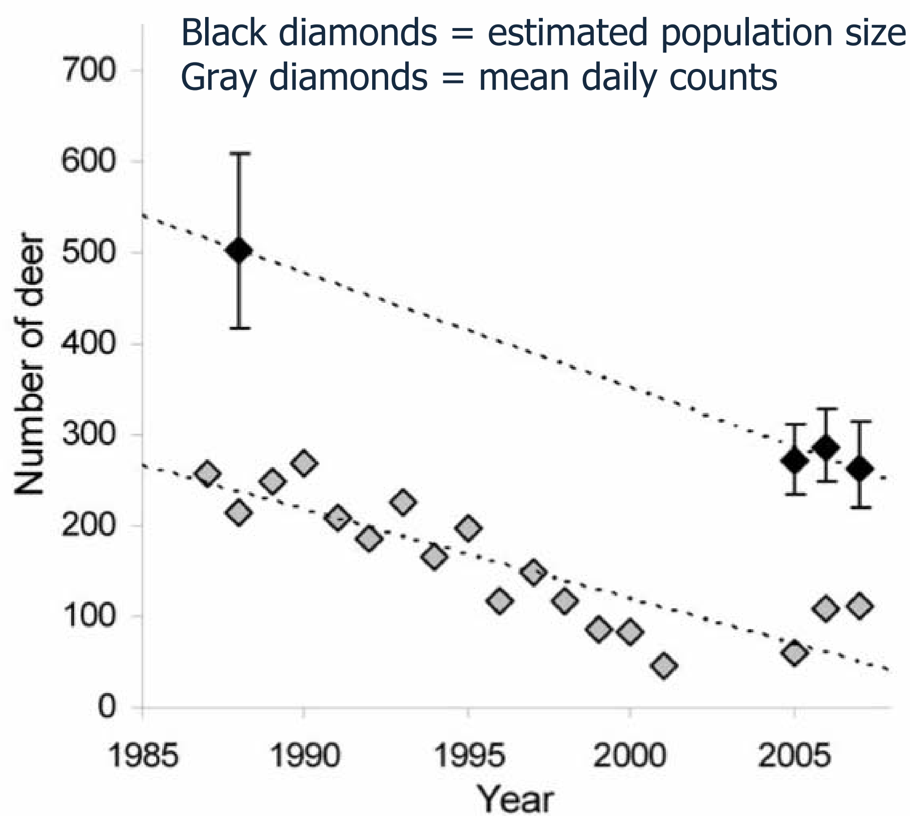
Transmissible Spongiform Encephalopathies


Distribution of Chronic Wasting Disease in North America
CWD in free-ranging populations
Known distribution prior to 2000 (free-ranging)
CWD in captive facilities (depopulated)
CWD in captive facilities (current)
USGS
science for a changing world
National Wildlife Health Center
Madison, Wisconsin
Updated August, 2008
All locations are approximations based on best-available information

Mule Deer Survival and Populations Trends at Table Mesa, CO

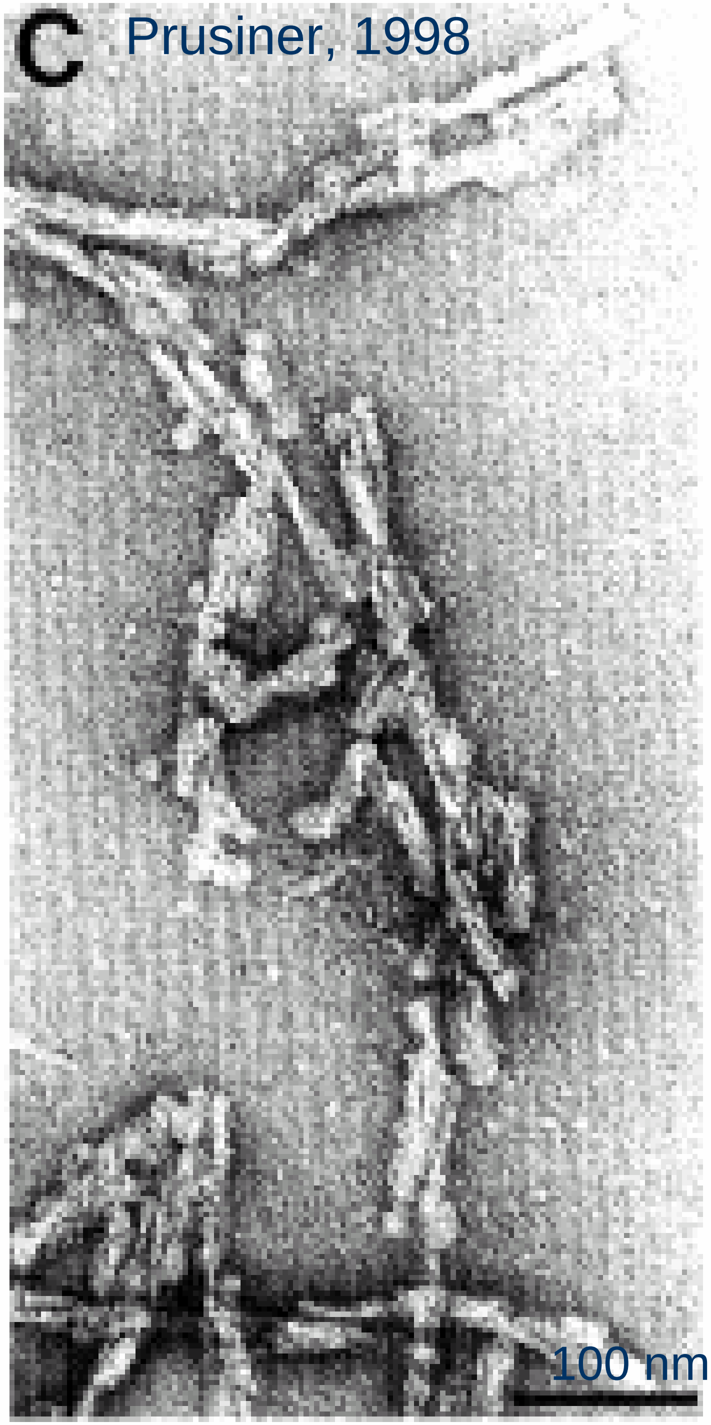




Estimated average life expectancy
Uninfected deer: additional 5.2 y
Infected deer: additional 1.6 y

Survival rates consistent with 45% decline in mule deer population since occurrence of CWD.

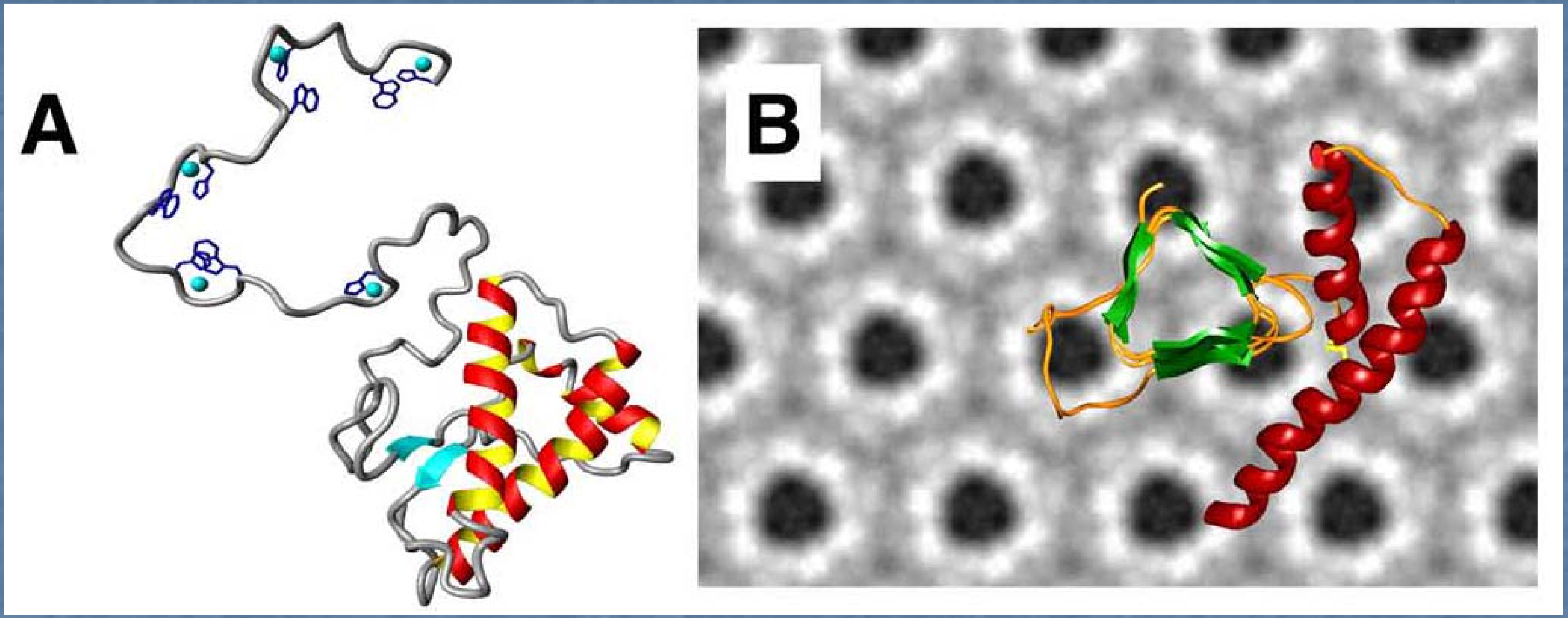
Miller et al. *PLoS ONE* **2008**, *3*, e4019



Common Features

- Spongiform degeneration
- Transmissible
- Accumulation of disease-associated prion protein
- Long incubation period/ extended preclinical stage
- No host immune response
- No cure
- Inevitably fatal
- Extreme resistance to inactivation

TSE Agent – Pathogenic Prion Protein



Watts et al. (2006) *PLoS Pathogens*

- PrP^{C} – normal, benign, cell-surface protein
  - 30-33 kDa sialoglycoprotein
  - Function unclear
- PrP^{TSE} (PrP^{Sc}) – misfolded isoform of prion protein
  - Co-purifies with infectivity
  - Major, if not sole, component of TSE agents

Persistence of PrP^{TSE}

- Resistant to UV, ionizing and microwave radiation
- Resistant to most chemicals that inactivate other pathogens
  - Alcohol, ammonia, benzene, β-propiolactone, ethylene oxide, formaldehyde, formalin, gluteradehyde, HCl, H_2O_2, peroxyacetic acid
- Resistant to limited protease digestion
- Heat treatment
  - Boiling has little effect
  - Autoclaving at 121-138°C not always sterilizing
  - 1-h exposure at 360°C or 15-min exposure at 600°C may leave residual infectivity

Taylor. *Vet. J.* **2000**, *159*, 10-17

Horizontal and Environmental Transmission

- Unlike most prion diseases, scrapie and CWD are communicable within a population
- Animals can contract scrapie or CWD following habitation in areas once holding infected animals
- An environmental reservoir of infectivity affects contact rate, duration of infectivity and efficiency of transmission
- Arthropod vectors (flesh flies, hay mites) have been postulated, but appear unlikely

Environmental Transmission Experiments

mule deer

exposure	CWD cases
infected deer	2/10
infected carcass decomposed *in situ* 1.8 y prior	3/12
residual excreta infected deer 2.2 y earlier	1/9

Unexposed controls: 0/4

Miller et al. *Emerg. Infect. Dis.* **2004**, *10*, 1003-1006

Soil as an Environmental Reservoir of Prion Infectivity

- Prions introduced into soils via decomposition of carcasses, alimentary shedding and urinary excretion [Mathiason etal. (2006) *Science*; Gregori et al. (2008) *EID*].
- Animals ingest soil
  - Incidental – feeding, grooming
  - Deliberate – mineral licks, scrapes [Weeks and Kirkpatrick (1976) *J. Wildl. Manage.*]
- Prion infectivity persists for years in soil when buried → impacts duration of infectivity [Brown and Gajdusek (1991) *Lancet* ; Seidel et al. (2007) *PLoS ONE*]

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Prions Adhere to Soil Minerals and Remain Infectious

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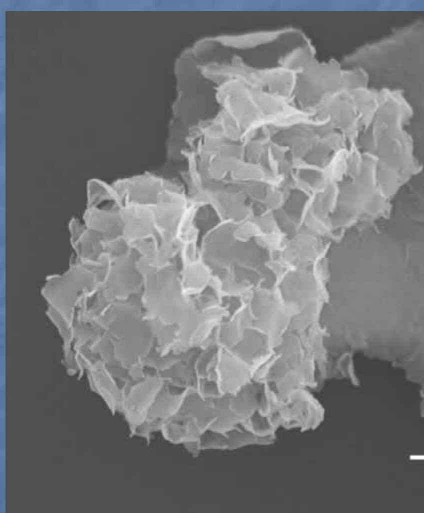
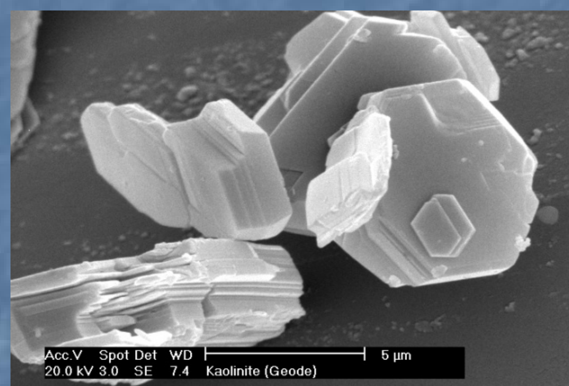
Montmorillonite (Mte)

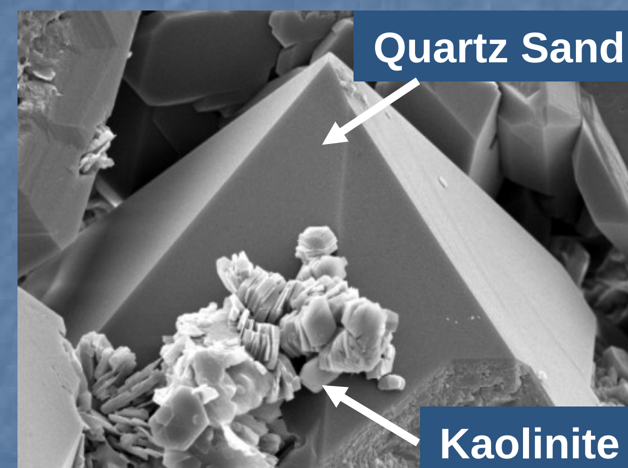


$(Na,Ca)_{0,3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot n(H_2O)$

Kaolinite (Kte)



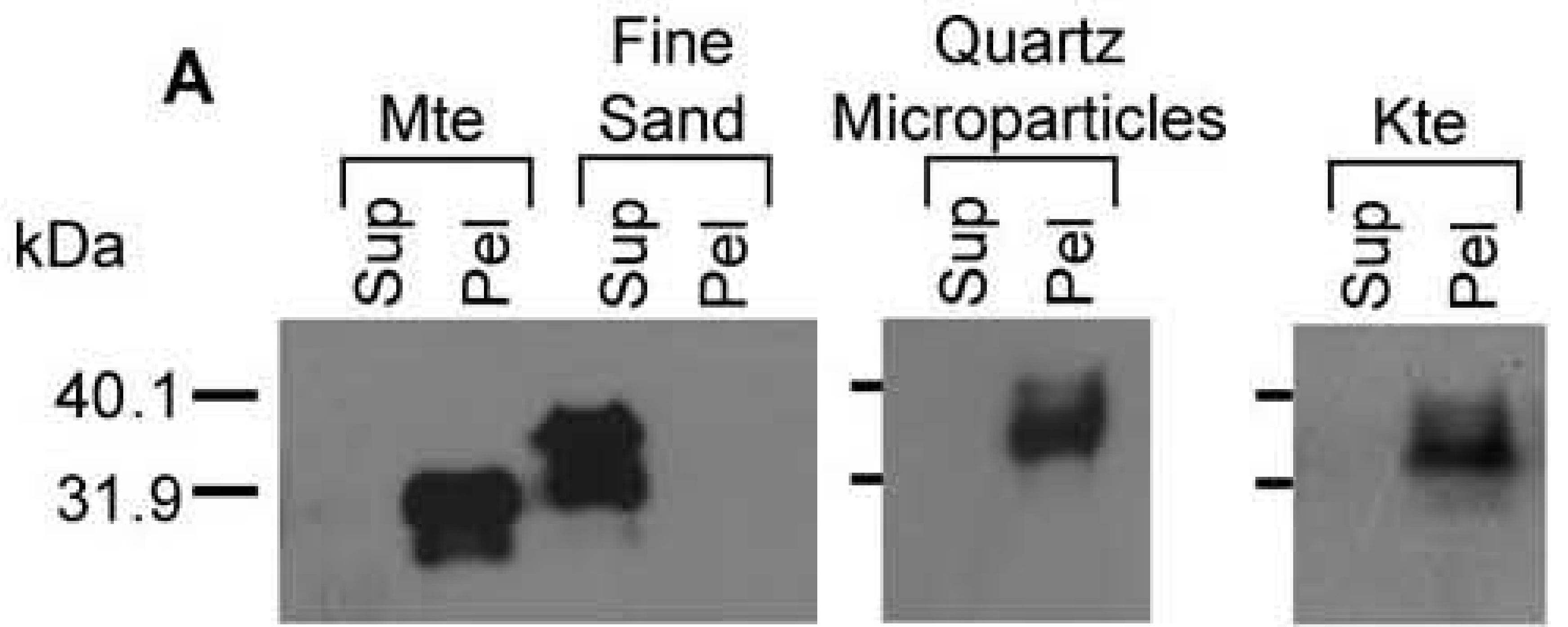
$Al_2Si_2O_5(OH)_4$



Images courtesy of Mineralogical Society

Johnson et al. *PLoS Pathogens* **2006**, *2*(4), 296-302.

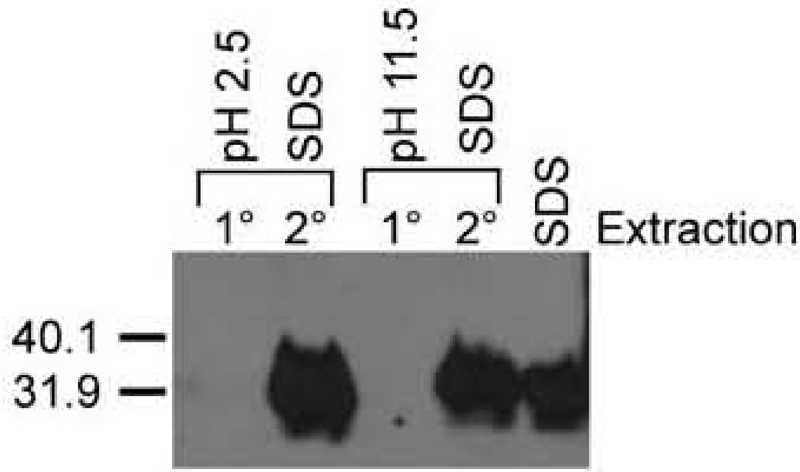
PrP^{TSE} Attachment to Mineral Particles

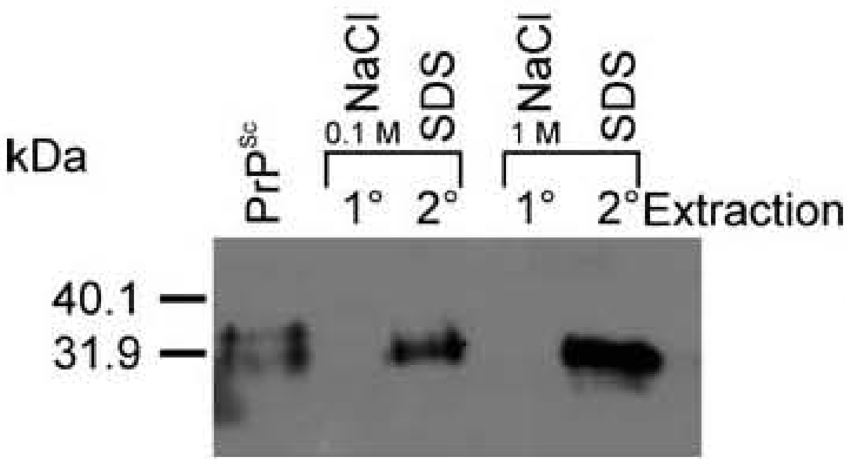


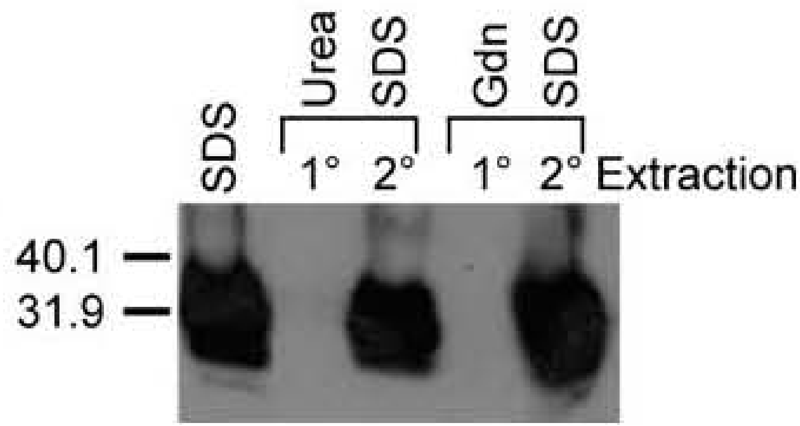
Sup = supernatant (free PrP^{TSE})
Pel = pellet (mineral particle-bound PrP^{TSE})

Johnson et al. *PLoS Pathogens* **2006**, *2*(4), 296-302.

Strength of PrP^{TSE} Attachment to Mte







1° = primary extractant

2° = secondary extractant (SDS)

Johnson et al. *PLoS Pathogens* **2006**, *2*(4), 296-302.

Table 2. Prions Adsorbed to Montmorillonite Clay Retain Infectivity

Inoculum	Positive Animals/ Total Animals	Onset of Clinical Symptoms (dpi)[a]
None	0/8	>200[b]
Mte (no PrP^{Sc})	0/8	>200[b]
Mte-PrP^{Sc} complex	10/10[c]	93 ± 4[d]
Mock supernatant[e] (no Mte)	8/8	103 ± 0[d]
Mock pellet[e] (no Mte)	8/8	178 ± 21[d]

[a]Mean dpi ± SD to the onset of clinical symptoms of TSE infection.

Johnson et al. *PLoS Pathogens* **2006**, *2*(4), 296-302.

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Oral Transmissibility of Prion Disease Is Enhanced by Binding to Soil Particles

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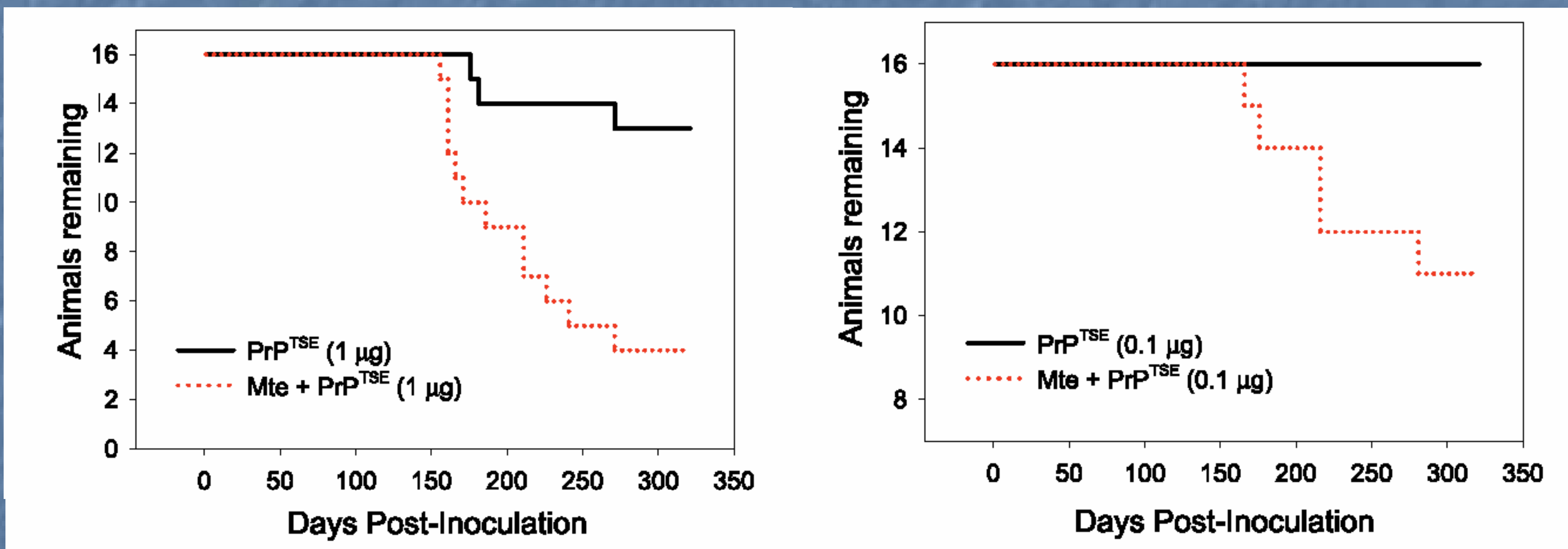


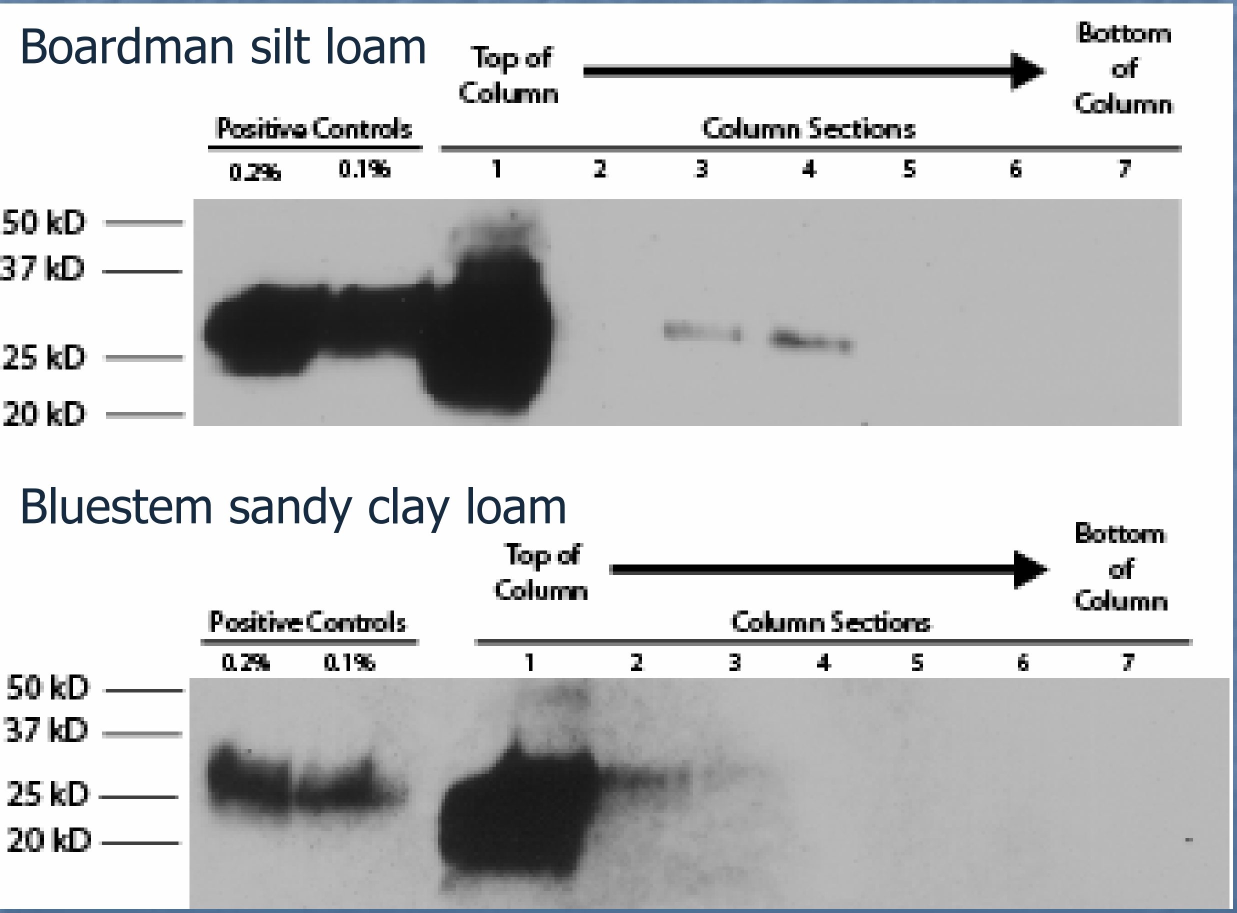
Equivalence factor:

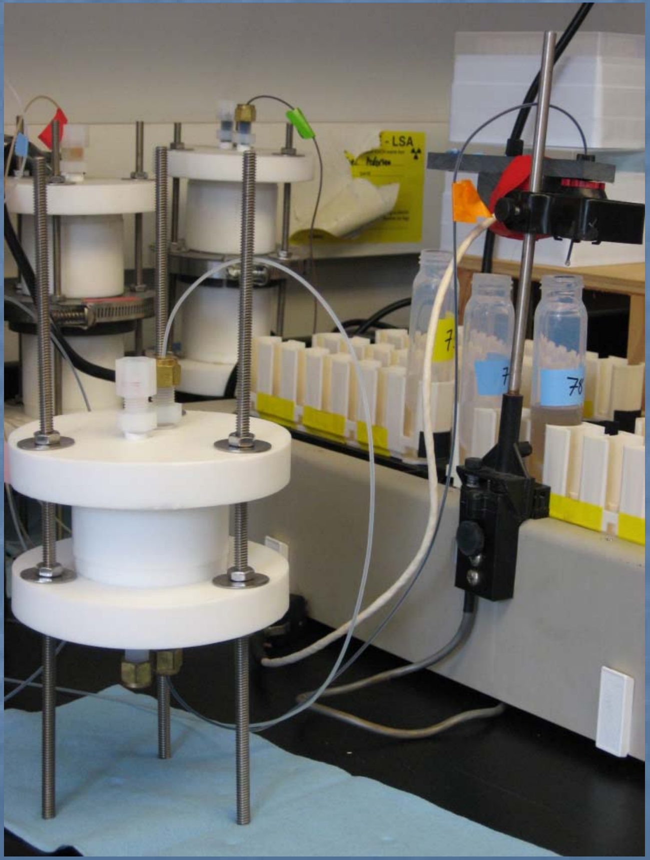
Mte increases transmission by a factor of 680

(95% CI: 16, ∞)

Johnson et al. *PLoS Pathogens* **2007**, *3*(7), 874-881.

PrP^{TSE} Transport through Soils

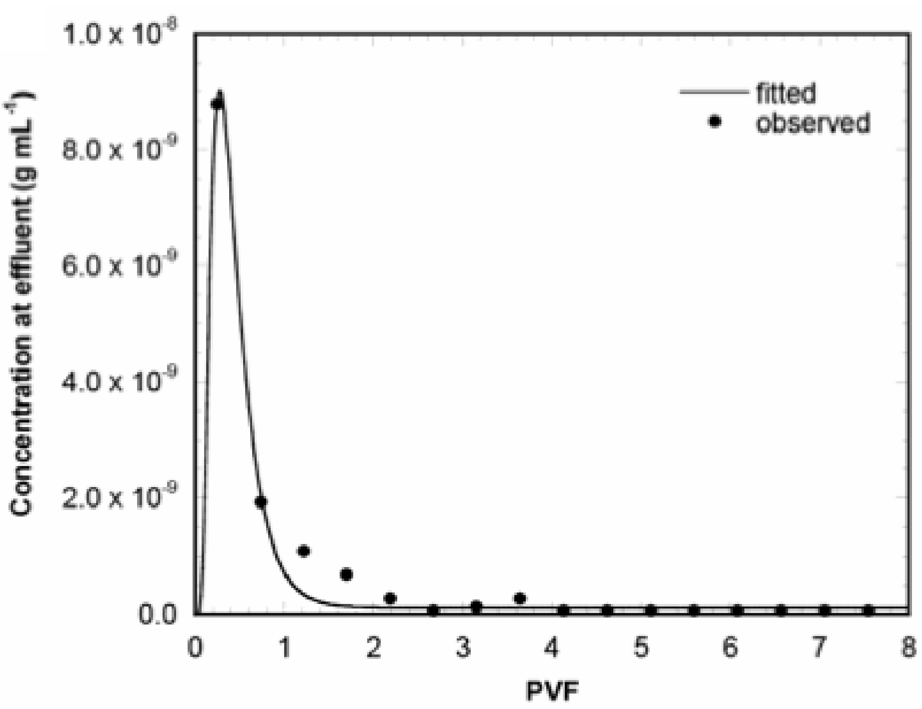




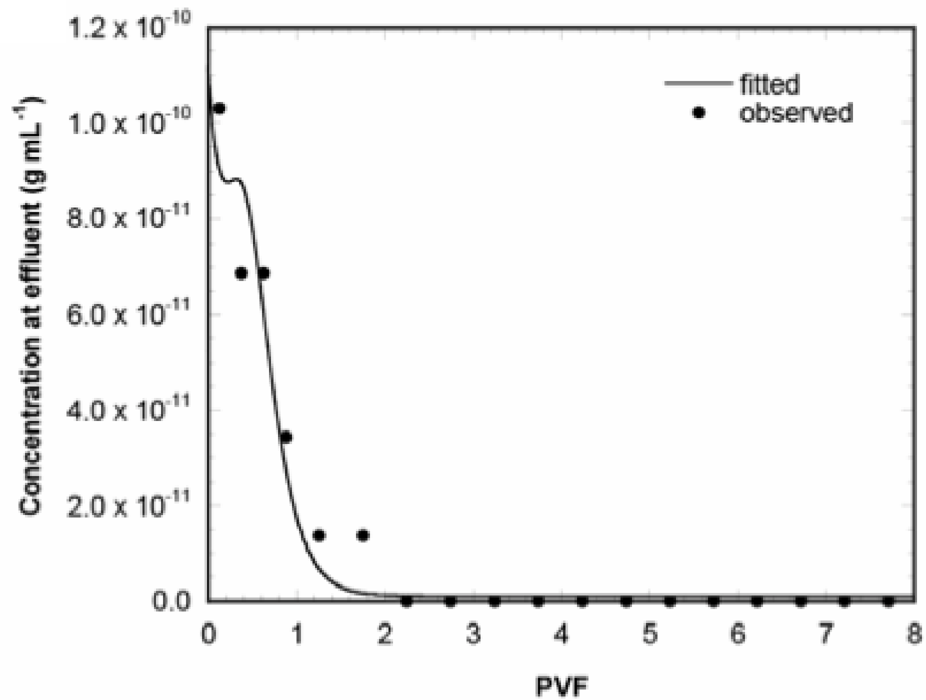
Each section ~ 3 mm

Jacobson et al. *Environ. Sci. Technol.* **2009**, *43*, (accepted)

PrP^{TSE} Transport through Municipal Solid Waste



Fresh MSW



Aged MSW

Jacobson et al. *Environ. Sci. Technol.* **2009**, *43*, (accepted)

Conclusions

- PrP^{TSE} interaction with montmorillonite is remarkably avid
- PrP^{TSE}-Mte complexes remain infectious and enhance disease transmission
- Prion transport appears limited in fine grained mineral soils, but mobility in MSW higher
- While recovery of PrP^{TSE} declines with time in aerobic soils, infectivity remains

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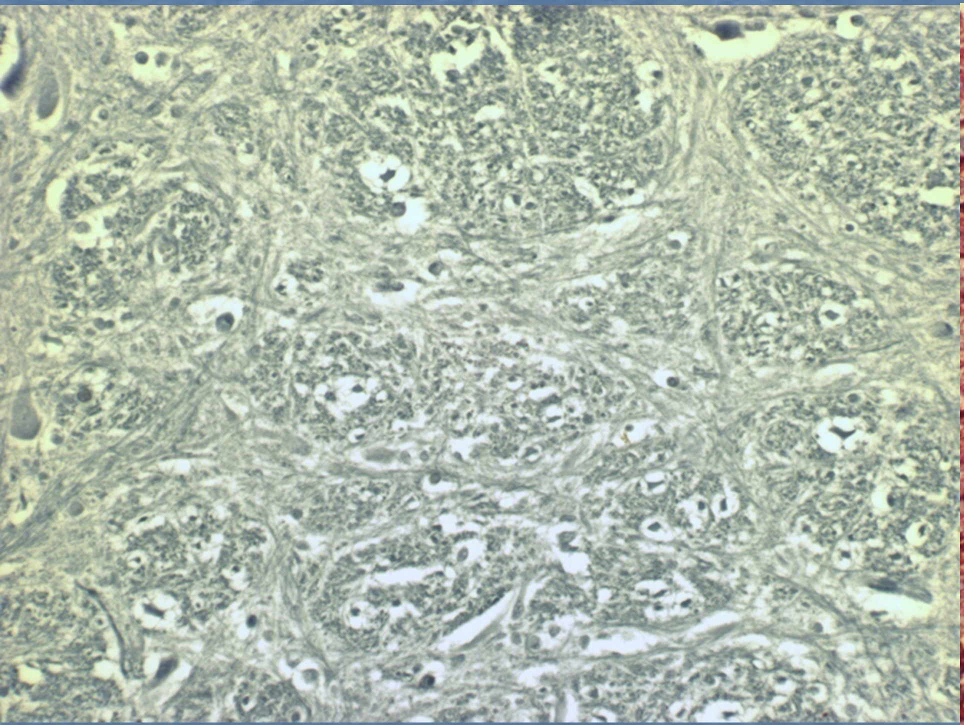
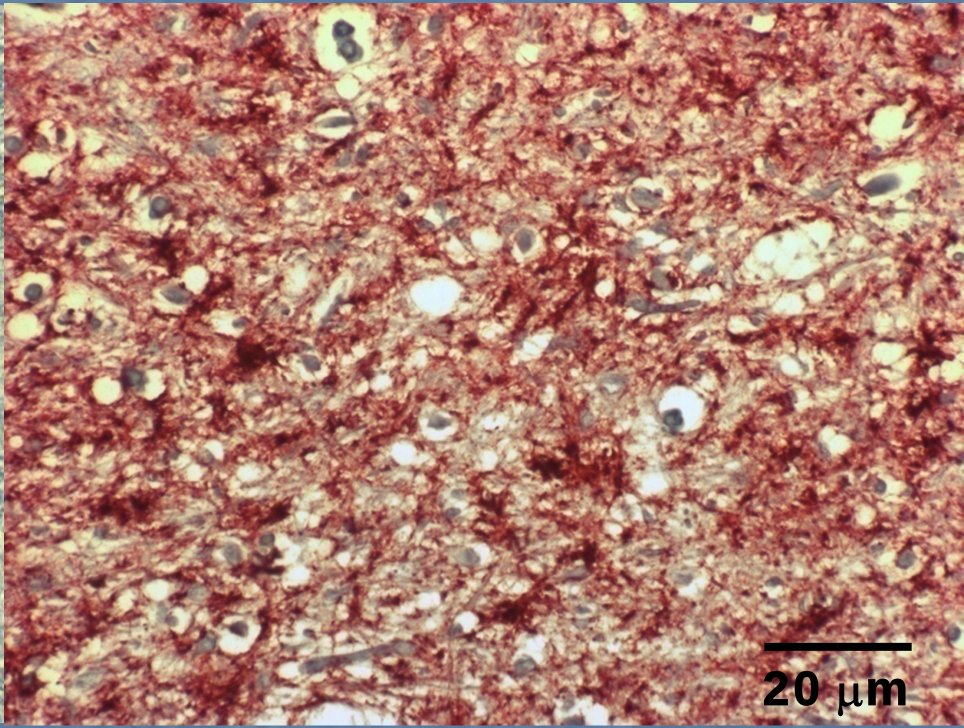
  - Wisconsin Department of Natural Resources




Spongiform Degeneration (obex region of brainstem)



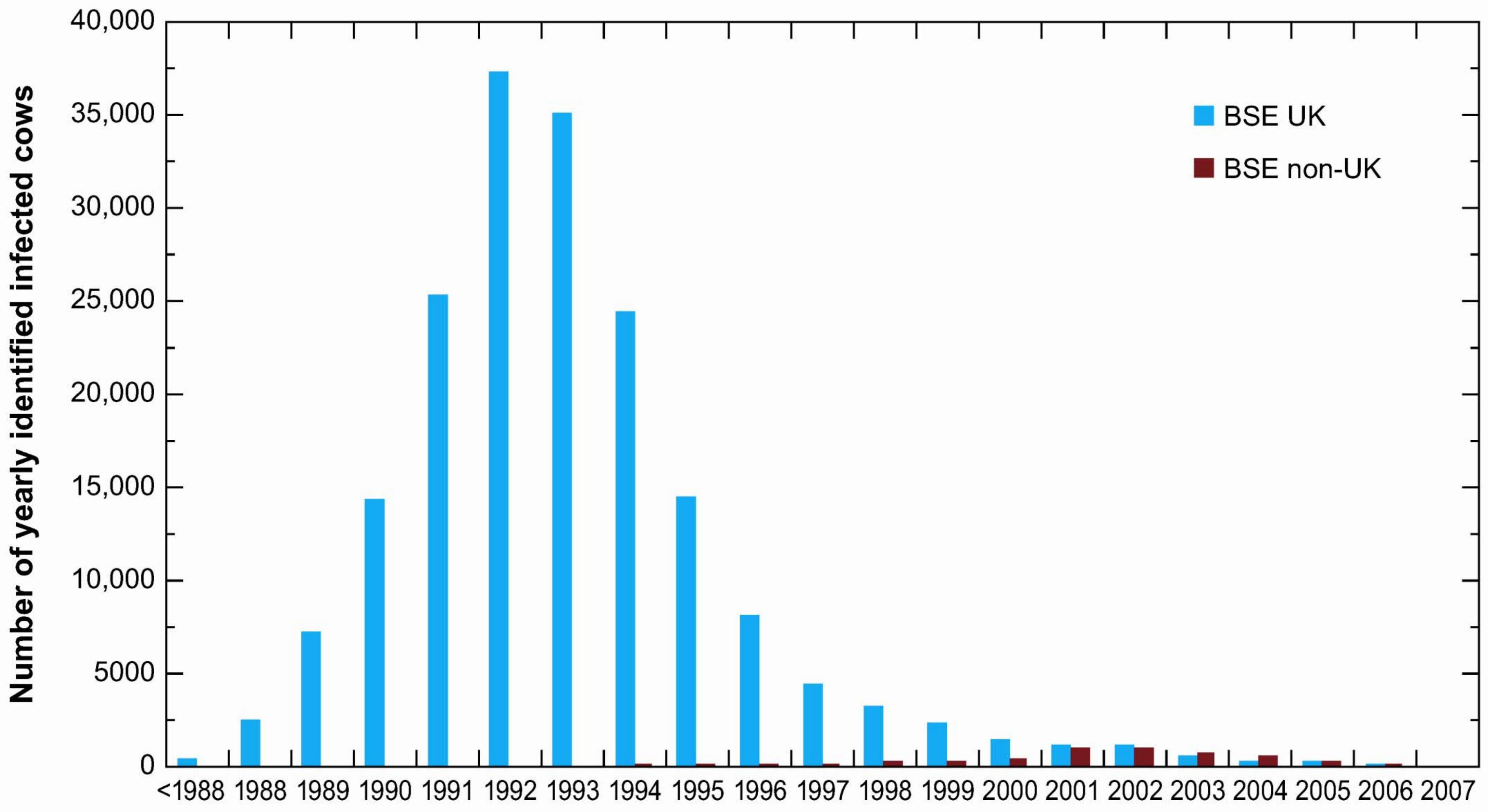
healthy tissue



CWD-infected tissue

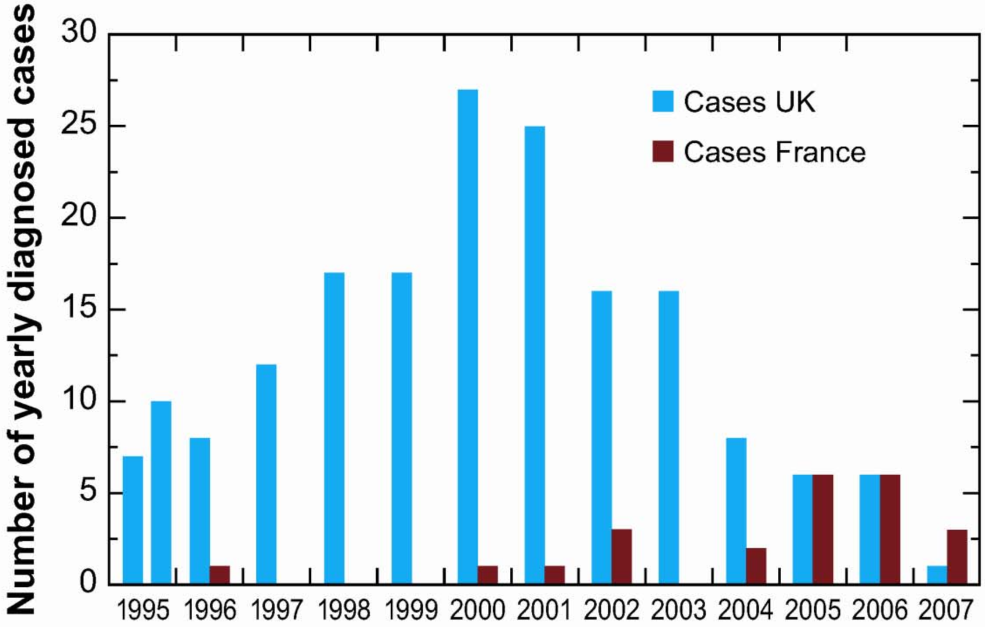
PrP^{TSE} immunostained red, counterstained blue
Image courtesy of Chad Johnson

Reported Cases of BSE



Aguzzi et al. (2008) *Annu. Rev. Neurosci.*

Reported Cases of vCJD



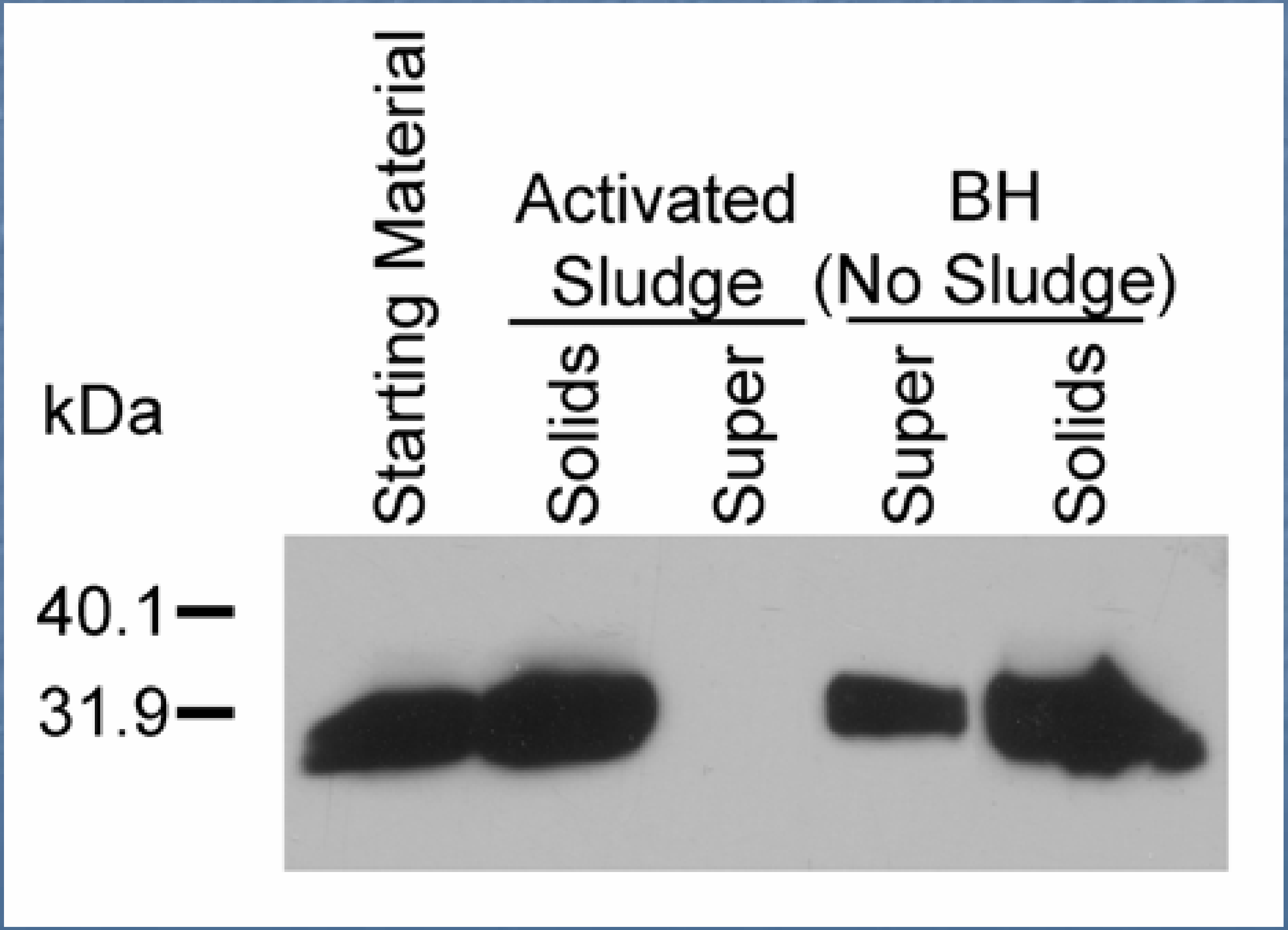
	vCJD cases (dead)	vCJD cases (still alive)	Secondary vCJD (blood transfusion)
UK	160	3	4
France	21	2	–
Republic of Ireland	4	0	–
Italy	1	–	–
USA	3	–	–
Canada	1	–	–
Saudi Arabia	–	1	–
Japan	1	–	–
Netherlands	2	–	–
Portugal	1	1	–
Spain	2	–	–

Aguzzi et al. (2008) *Annu. Rev. Neurosci.*

Potential Routes of Prion Introduction into WW Treatment Systems

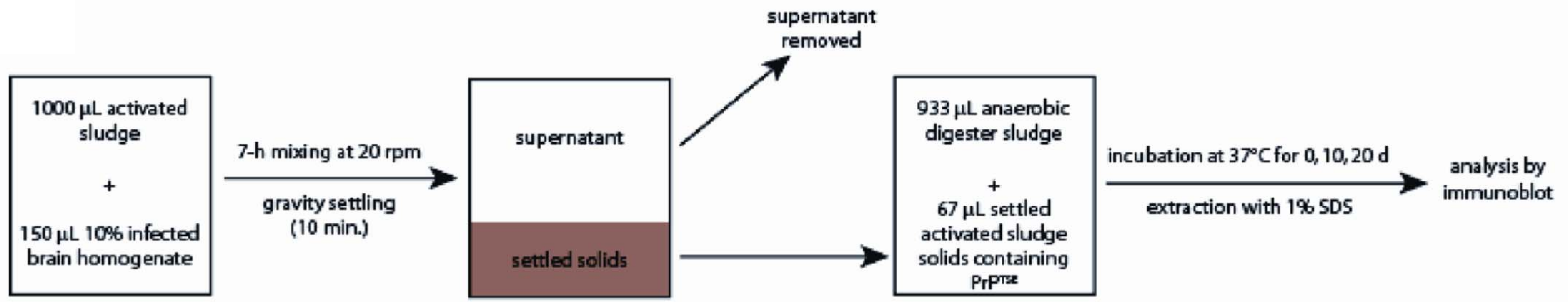
- Leachate from landfills accepting prion-infected materials
  - Unlikely if encased in fine-textured soil
- Improper discharge into WW collection systems
  - Necropsy labs, game processors, game dressing
- Shedding by CJD patients
  - Unlikely to be significant
- Disposal of downer animals in agricultural digesters
- Prions have not been detected in WWTP influent, effluent or biosolids

PrP^{TSE} Partitions to Activated Sludge Solids



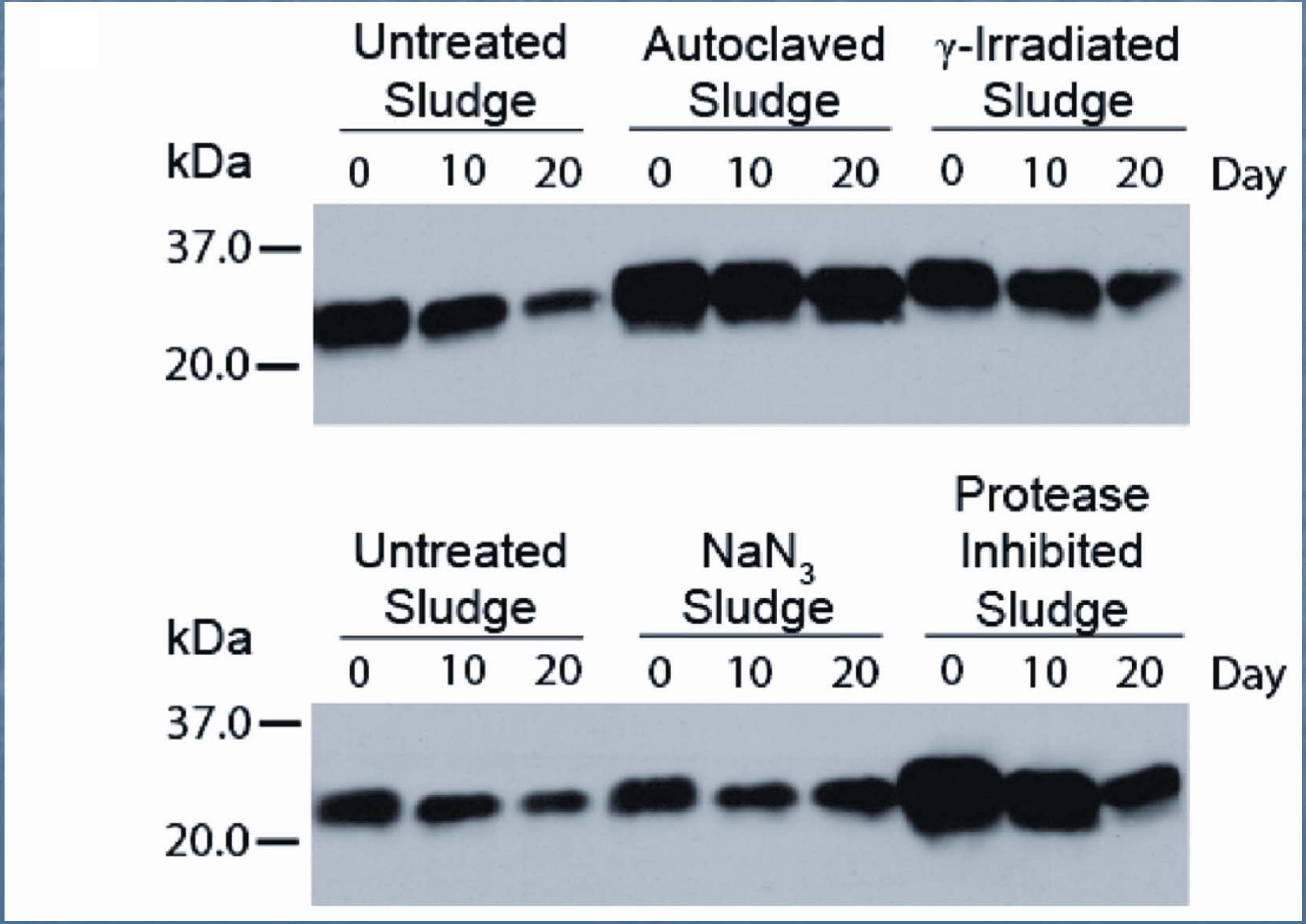
Hinckley et al. *Environ. Sci. Technol.* **2008**, *42*, 5254-5259

Mesophilic (37°C) Anaerobic Digestion of PrP^{TSE}



Hinckley et al. *Environ. Sci. Technol.* **2008**, *42,* 5254-5259

PrP^{TSE} Survives Simulated Mesophilic (37°C) Anaerobic Sludge Digestion



Hinckley et al. *Environ. Sci. Technol.* **2008**, *42*, 5254-5259

Transformation of PrP^{TSE} in Soil

- Microorganisms
  - Enzymes – proteases, phenoloxidases
  - Biogenic oxidants
- Soil macrobiota
- Solid-phase oxidants
  - Manganese oxide minerals

In vitro Biomimetic ·OH Production

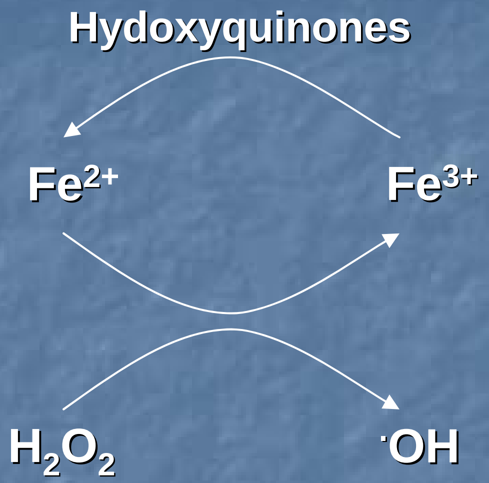
Brown Rot Fungus ***Gloeophyllum trabeum*** (courtesy of Ken Hammel)

Lignolytic Fungi

pH 3-5

produce H_2O_2 & hydroxyquinones

$[H_2O_2]$ = 50-300 μM

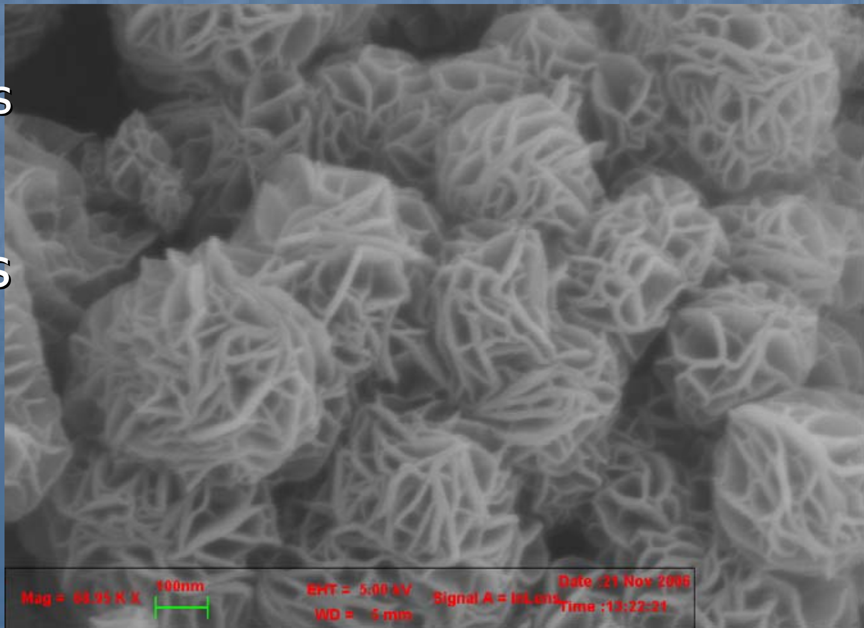


- Mimics extracellular chemistry of lignolytic fungi to produce environmentally relevant ·OH concentrations (Metz et al., in review)
  - 200 μM H_2O_2, 20 μM Fe^{2+}, 20 μM methoxyquinone, pH 4
  - anoxic conditions, dark, 16-18 h reaction time
- Environmentally relevant ·OH levels induced no change in PrP^{TSE} levels

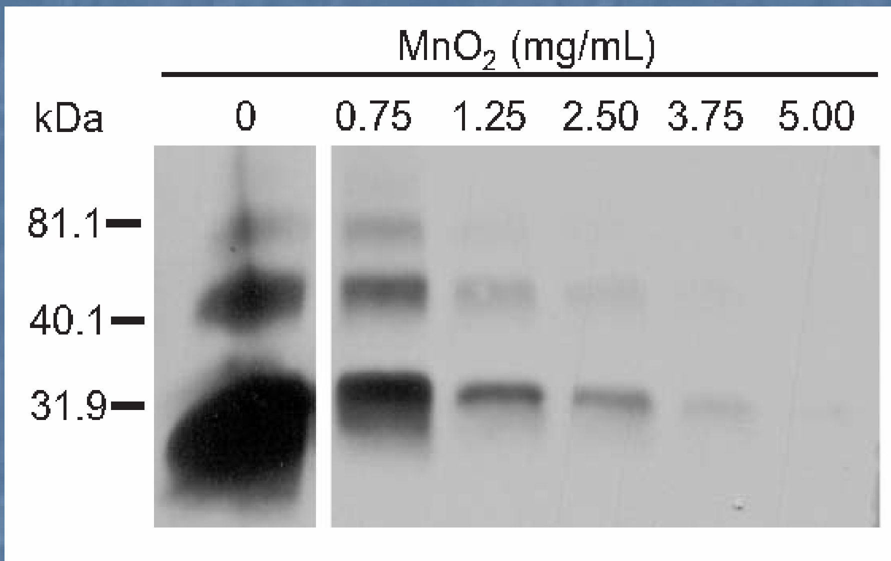
Metz et al. *Environ. Sci. Technol.* (in review)

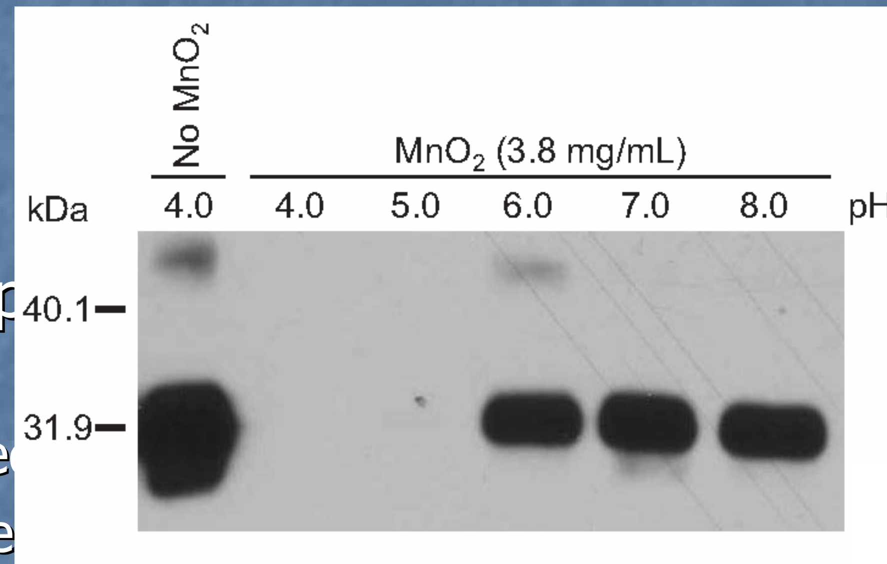
Manganese Oxides

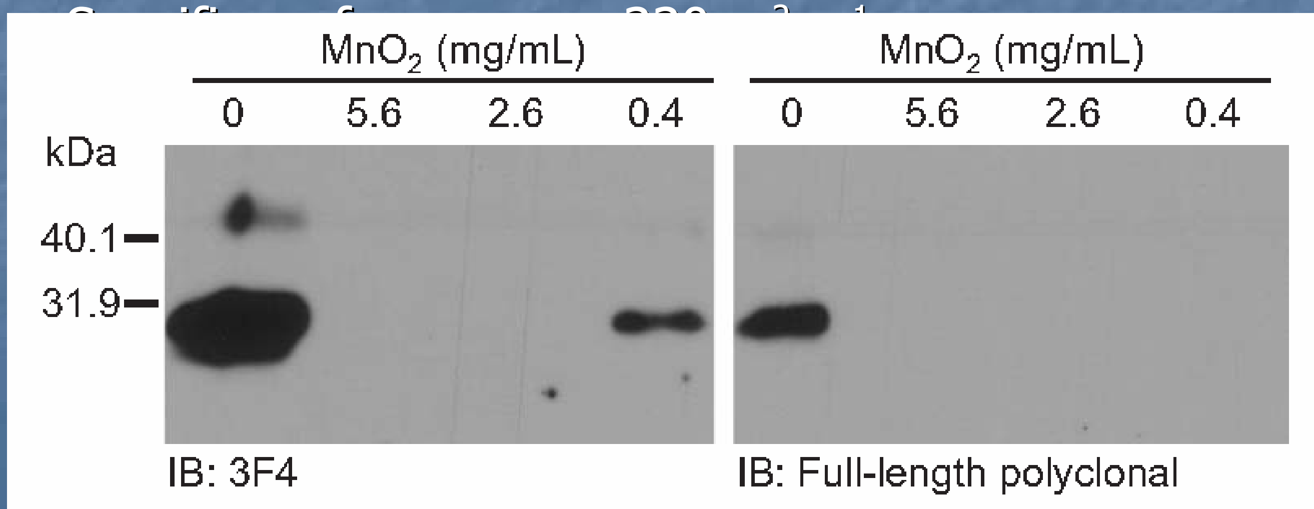
- Coatings and fine-grained aggregates in soils and sediments
- Birnessite among most common Mn oxides in soils
  - Layer type $Mn^{IV,III}$ oxides
  - Formed from bacterial oxidation of Mn^{II}
  - Important in soils with alternating reducing and oxidizing conditions
    - Seasonally waterlogged areas, areas with reduced drainage
    - However, many well-drained soils contain MnO_2 due to previous wet conditions
  - Among strongest natural oxidants in soils ($E_H^0 = +1.29$ V)
  - Mediates transformation of a variety of organic contaminants



Transformation of PrP^{TSE} by Acid Birnessite

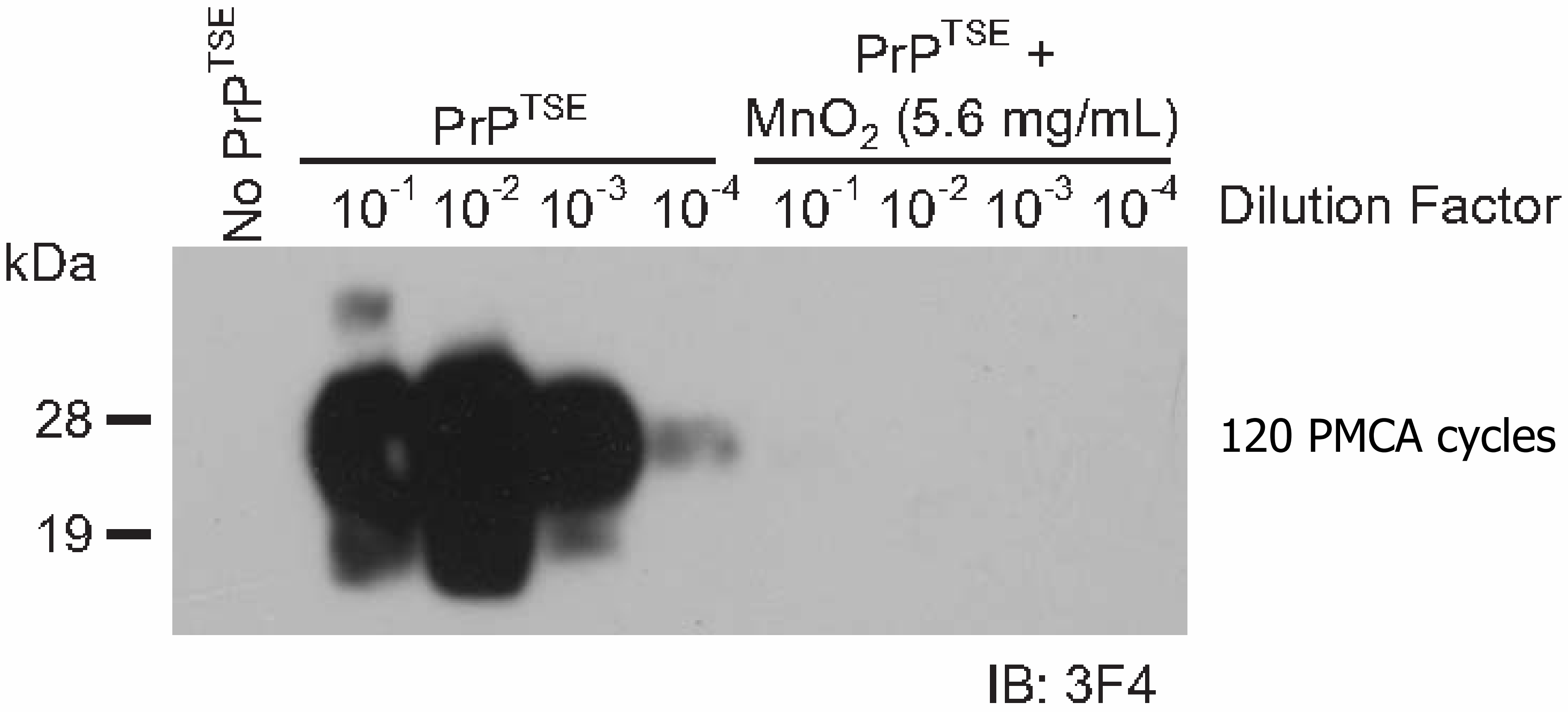






Russo et al. (2008) *J. Gen. Virol.*

PMCA Quantification of PrP^{TSE} Degradation



MnO_2 reduced prion converting levels by more than a factor of 10^4

Russo et al. (2008) *J. Gen. Virol.*