Can Composting Solve Specified Risk Material Issues?

Shanwei Xu, Tim McAllister

Agriculture and Agri-Food Canada, Lethbridge Research Centre, Canada

Tim Reuter, Kim Stanford

Alberta Agriculture and Rural Development, Government of Alberta, Canada

What Farmer Educators Need to Know about Mortality Composting – Beyond the basis

Langston University OK April 25 2014

Agriculture et Agroalimentaire Canada

Agriculture and Agri-Food Canada

Government of Alberta

Alberta

Freedom To Create. Spirit To Achieve.

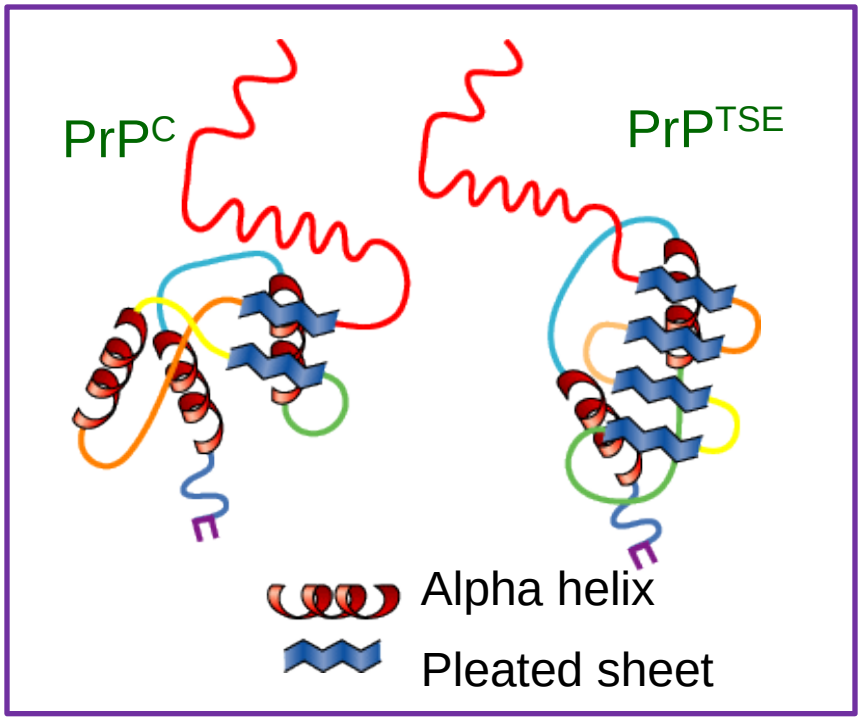
American Institute for Goat Research

Langston University

E (Kika) de la Garza

TSE Disease

- Scrapie – Sheep and goats
- CWD – Deer and elk
- BSE – Cattle
- CJD – Humans



TSE in Canada and USA

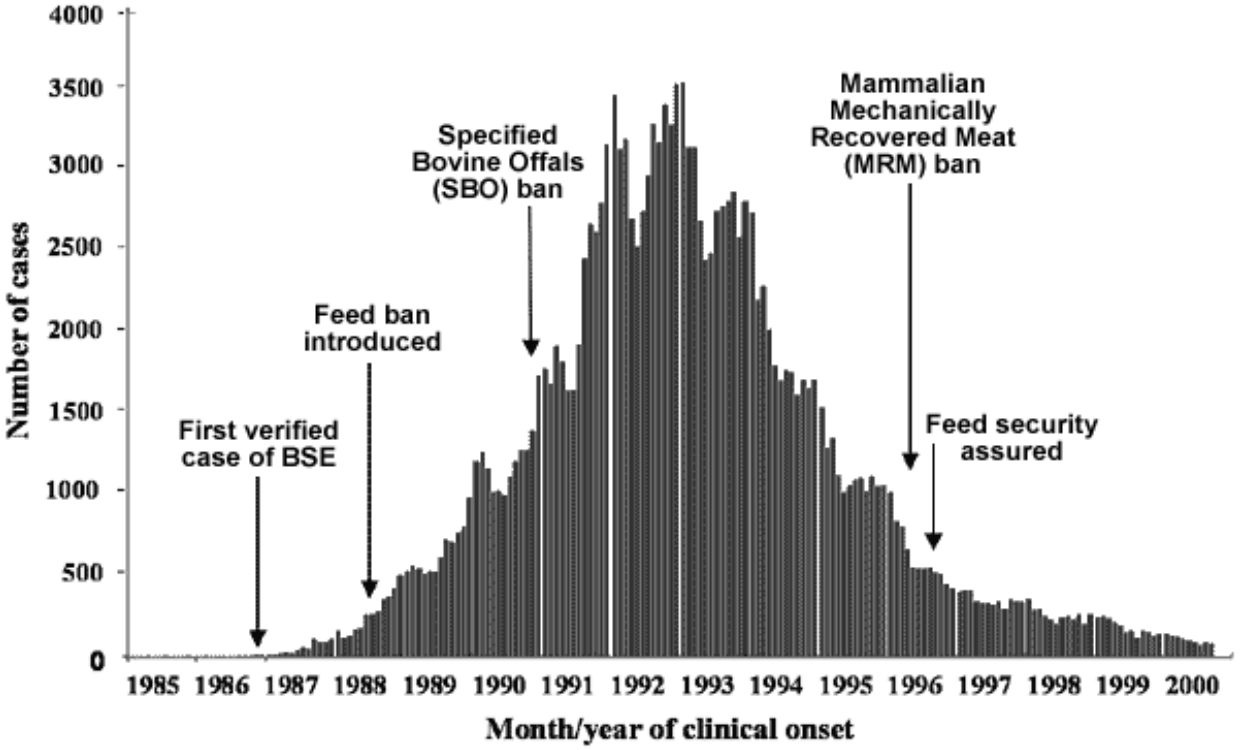
- 19 BSE cases in Canada and 4 cases in USA
- 35 Scrapie cases in Canada
- 173 CWD cases in Alberta Canada
- More CWD prevalence in 19 states of USA
- Economic losses

1. Cattle industry – $11 billion in Canada and USA
2. Cervid industry – $43 million in Canada

BSE Control

- BSE in Canada

1. 17 cases – typical BSE (Feed contamination)
2. 2 cases – atypical BSE (Spontaneous mutation)

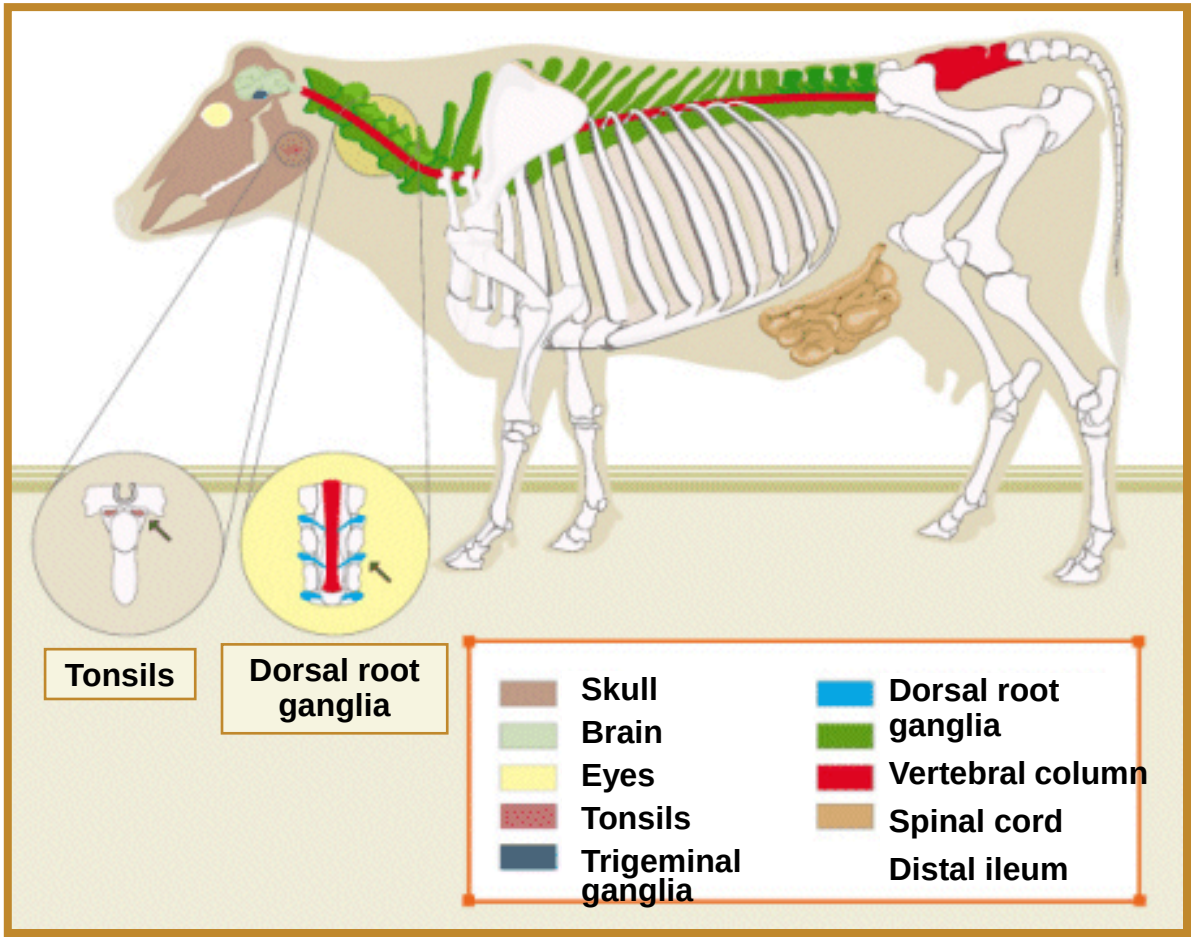


Source: Centers for Disease Control and Prevention of the United States (CDC)

Specified Risk Materials

Enhanced Feed Ban

- 2007 in Canada
- 2008 in USA
- SRM banned from
  - All animal feeds
  - All pet foods
  - All fertilizers



Specified Risk Materials

- ✓ 250,000 tonnes SRM in Canada
- ✓ 74,000 tonnes SRM in Alberta
- ✓ 50,000 cervid carcasses from farms and road kills
- ✓ SRM are rendered, dehydrated and disposed of in landfills

Methods for Prion Inactivation

Prion Inactivation

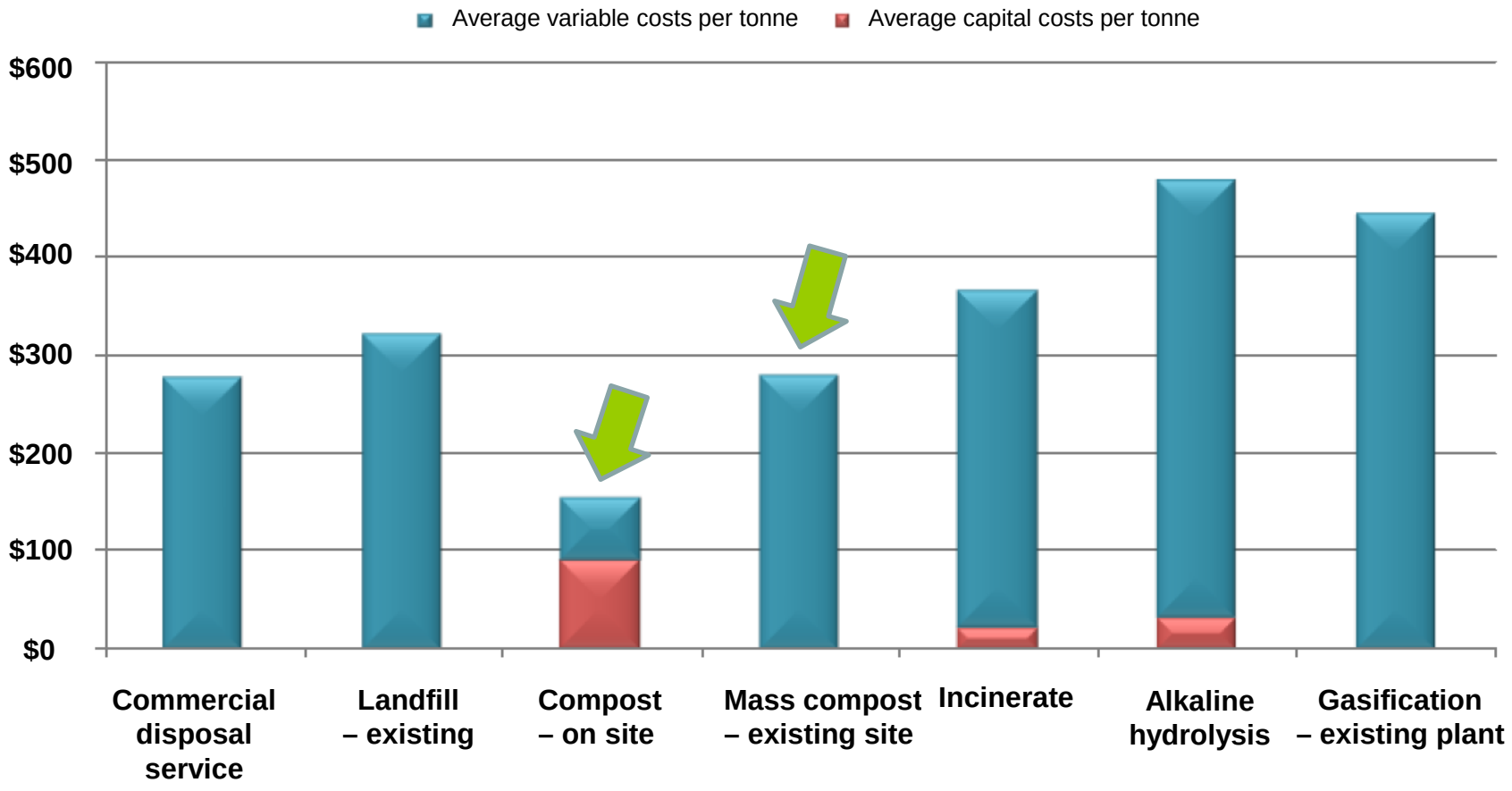
Alkaline or thermal hydrolysis
pH 14 and 150-180°C at 4-12 atmospheric pressure for 1-3 h

Incineration
Two stages at 850°C and 1000°C for 16 h

Sodium hydroxide
2 N for 1 h

Autoclaving
134°C for 1 h in saturated steam

Costs for SRM Disposal



Source: Ministry of Agriculture and Lands, British Columbia, Canada

Enzymes Effective Against Prion

Composting?

Enzymes	Source microbes	Enzyme reaction	
		Temp. (°C)	pH
Keratinase	*Bacillus licheniformis*	50	8
Alcalase	*Bacillus licheniformis*	35	7.2
Proteinase K	*Tritirachium album*	50	7.2
Properase, protease M	*Bacillus* spp.	60	12
Alkaline proteinase	*Streptomyces* sp.	60	11
Protease E	*Thermus* sp.	80	7
Keratinolytic protease	*Nocardiopsis* sp.	60	10

Susuki et al. (2006)

Outline

- **Lab-Scale Composting**
- Biocontainment laboratory level 3
- Approx. 40 kg
- Composted for 28 days; Two 14 day cycles
- SRM; Scrapie 263K, CWD, BSE (WB & PMCA)

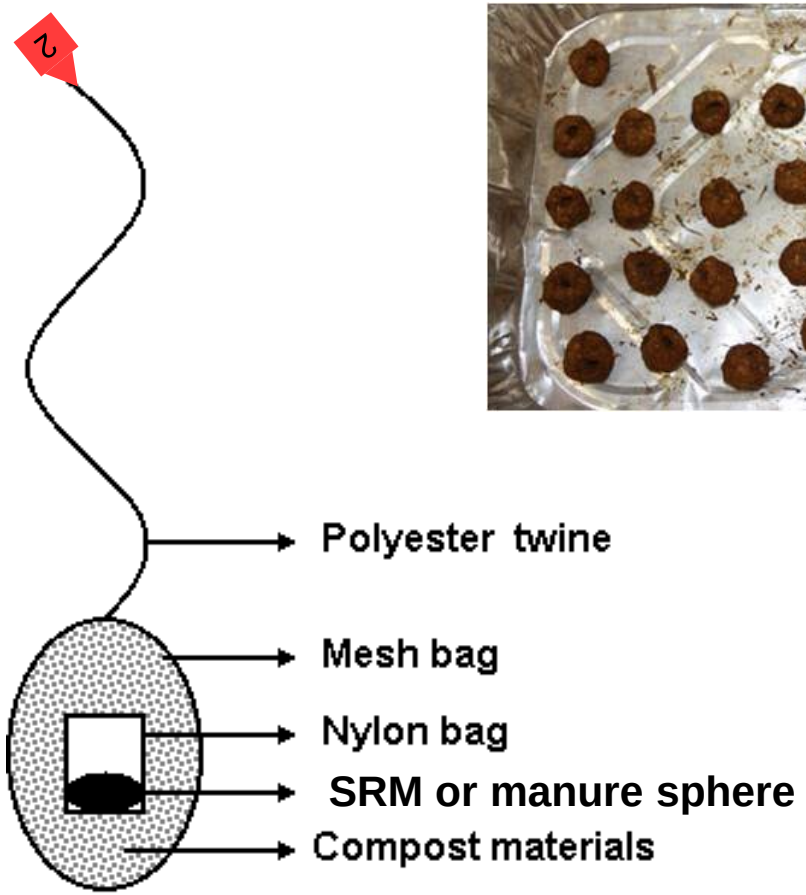
- **Field-Scale Composting**
- Approx. 85,000 kg;
- Composted for 230 days
- 16 cattle mortalities; SRM; Scrapie 263K (Bioassay)

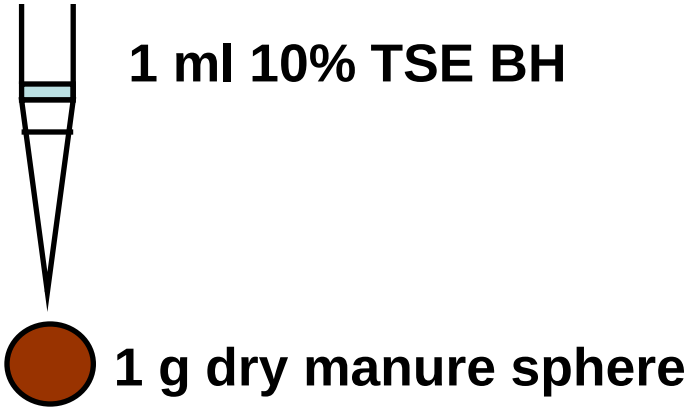
Laboratory Compost

Lethbridge Research Centre
(AAFC)

Level 3 laboratory
(CFIA)

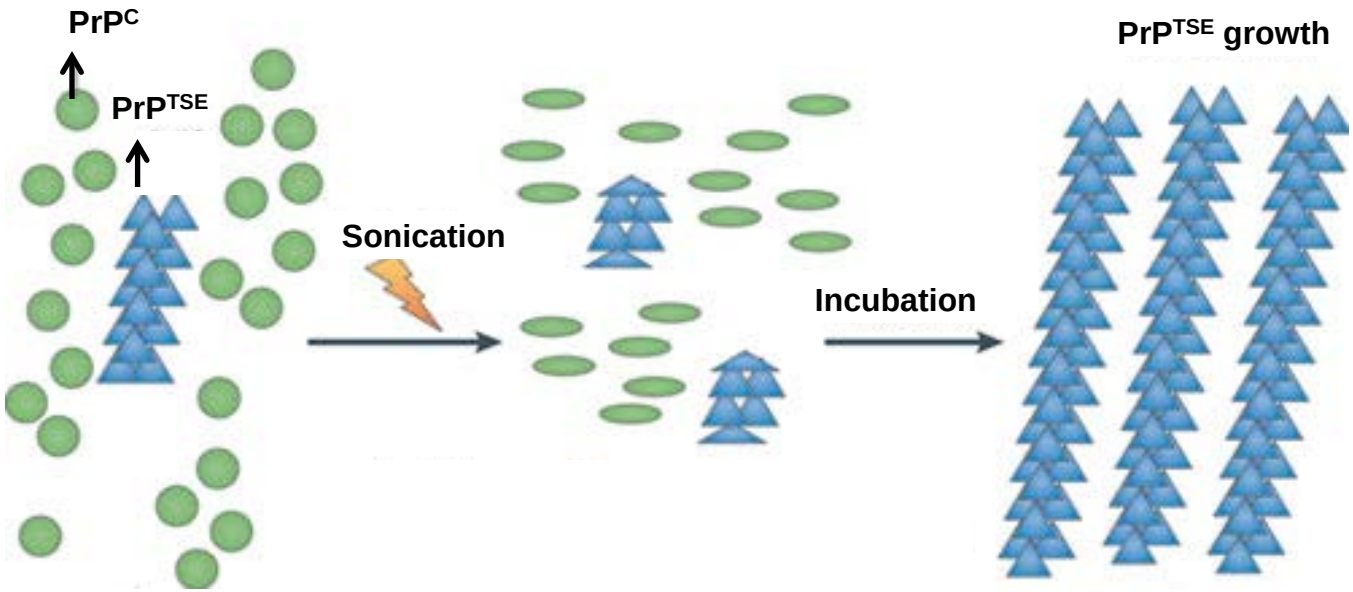
Laboratory Compost - Sampling





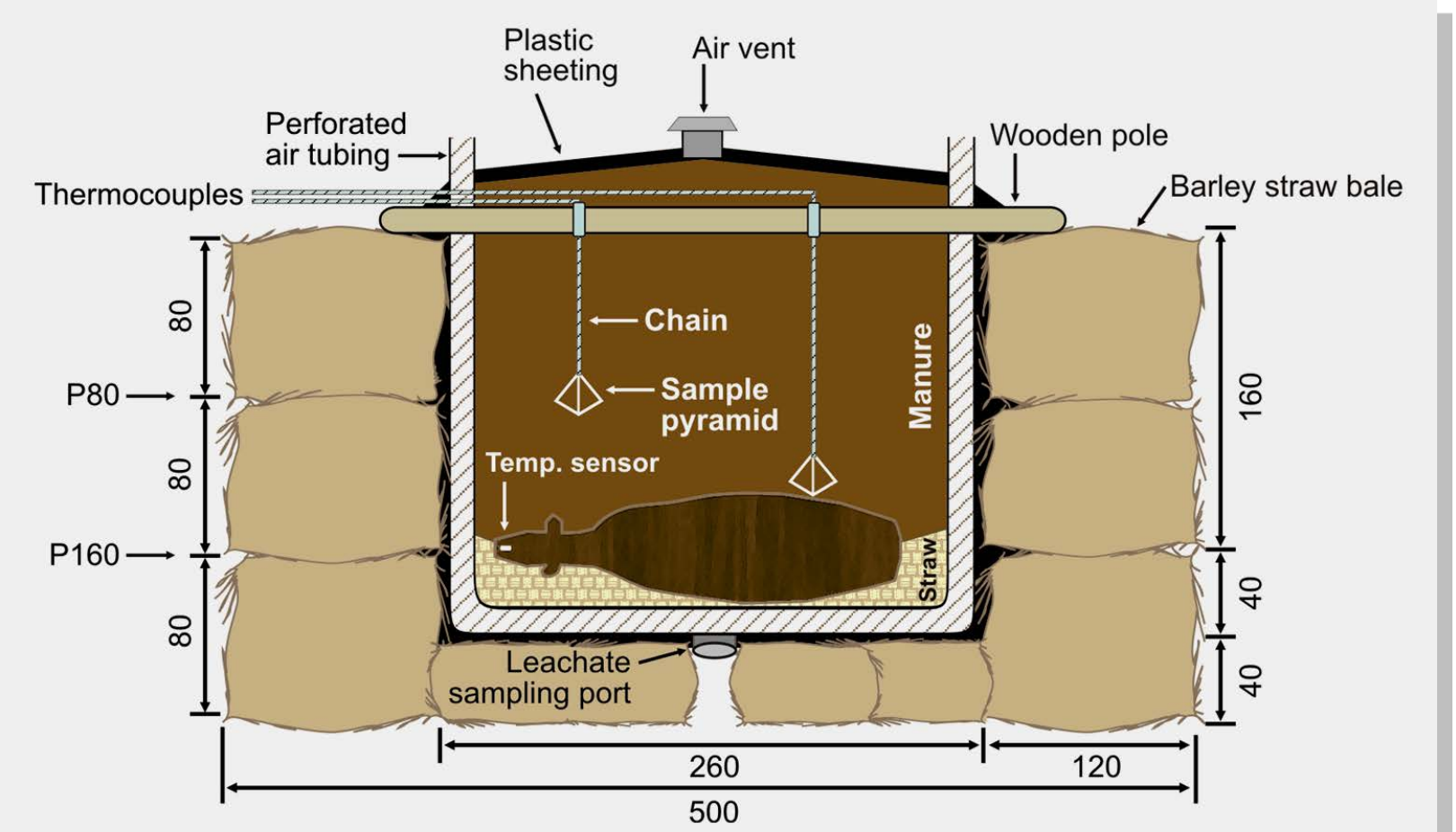
Methods for Prion Detection

- Western blot (WB)
- Protein misfolding cyclic amplification (PMCA)



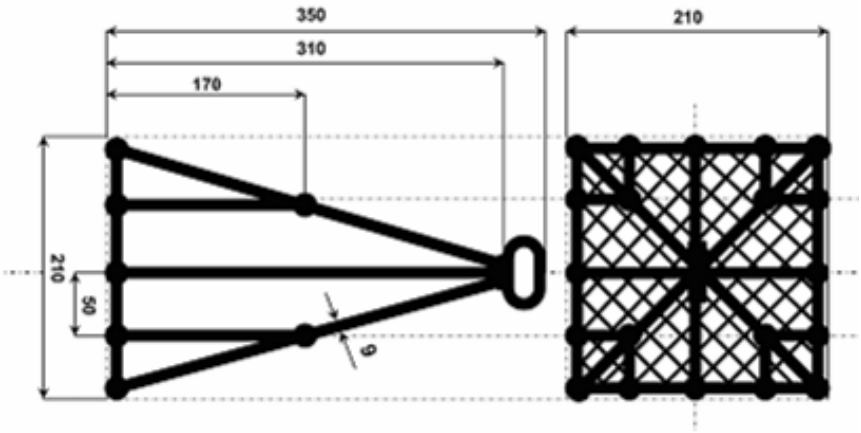
Colby and Prusiner (2011)

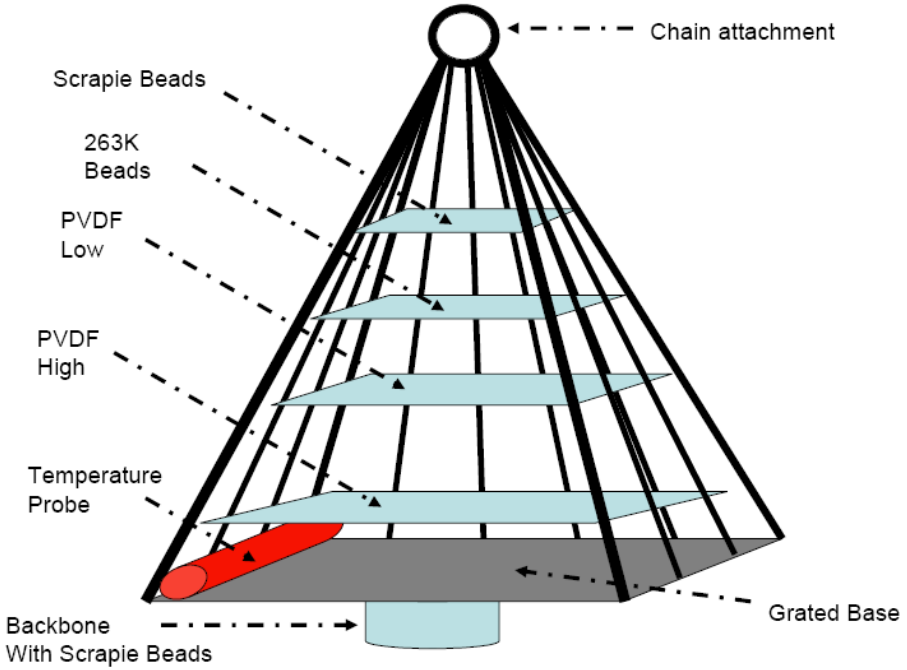
Field Compost Layout



- All dimensions in cm

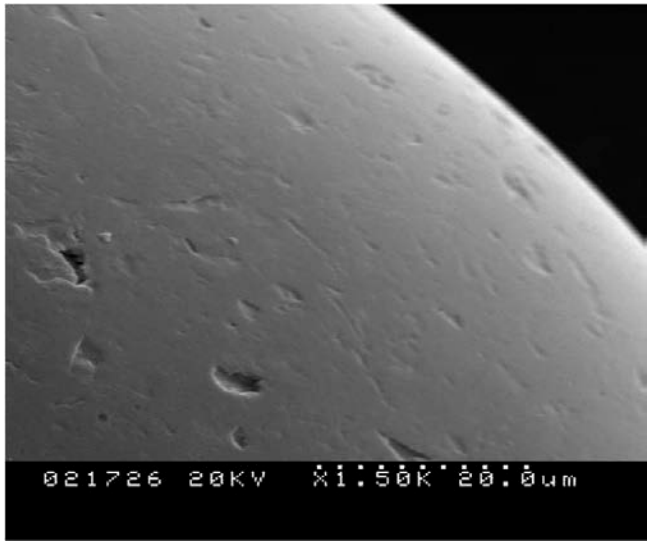
Baker Retrieval Pyramid



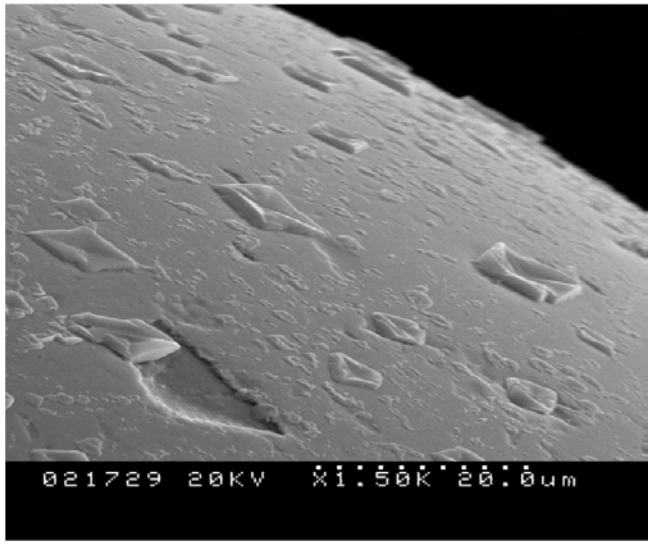


Binding to Stainless Steel Beads

- 440C Stainless Steel; Hamster 263K
- Mass: ~0.5 mg; Diameter: 0.5 mm
- Surface area: 0.8 mm^2; Density: 7.68 g cm^{-3}
- Syrian hamster model – IC implant



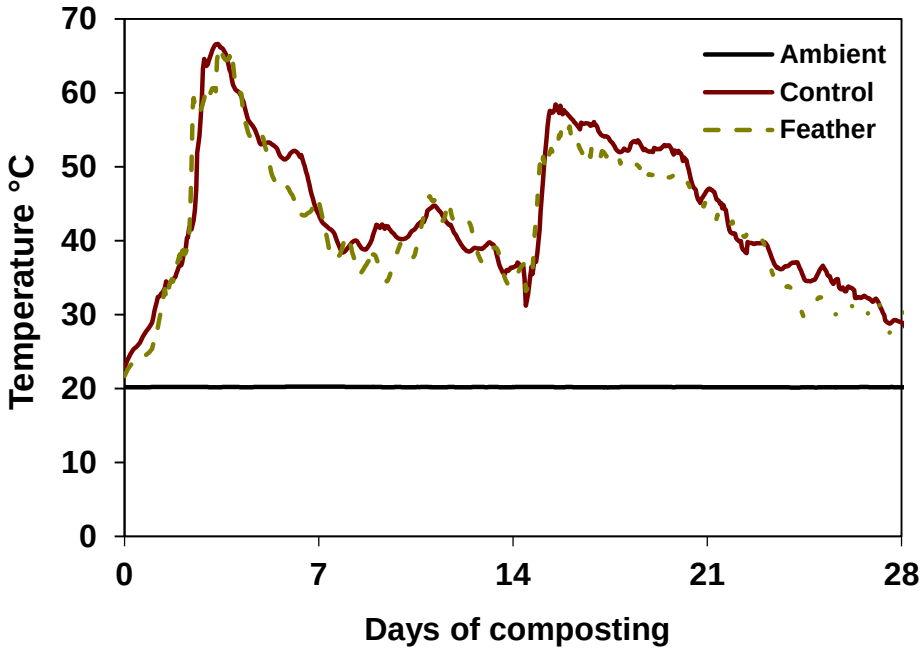
Clean beads



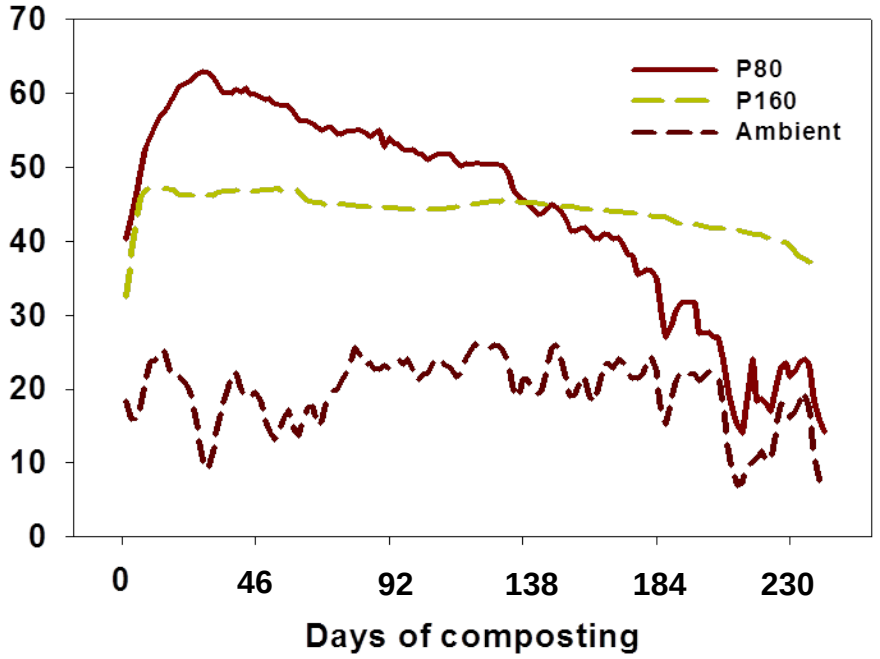
Beads coated with brain homogenate

Temperature

- Lab-scale compost



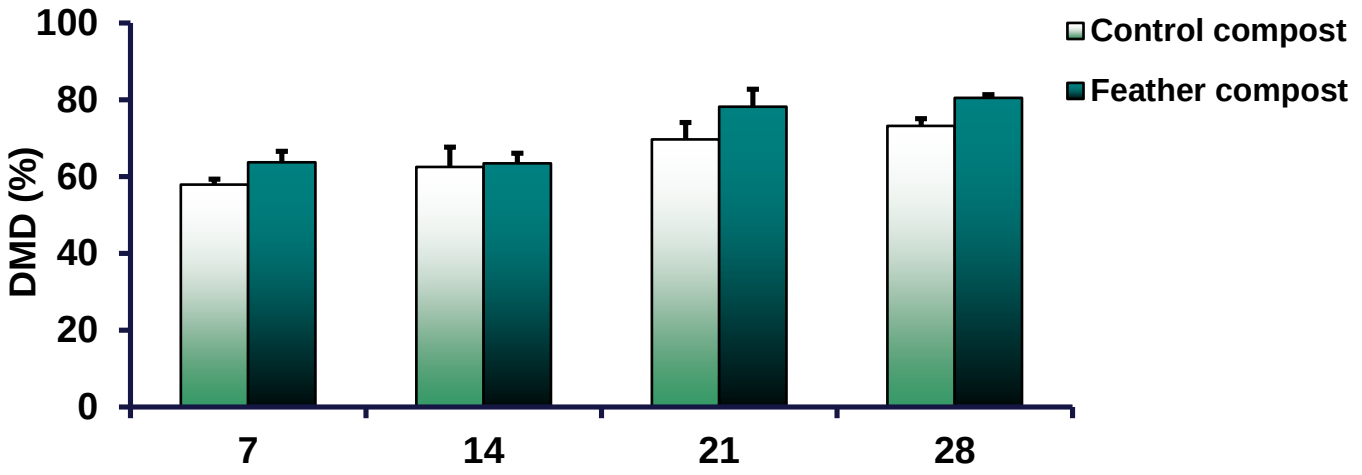
- Field-scale compost



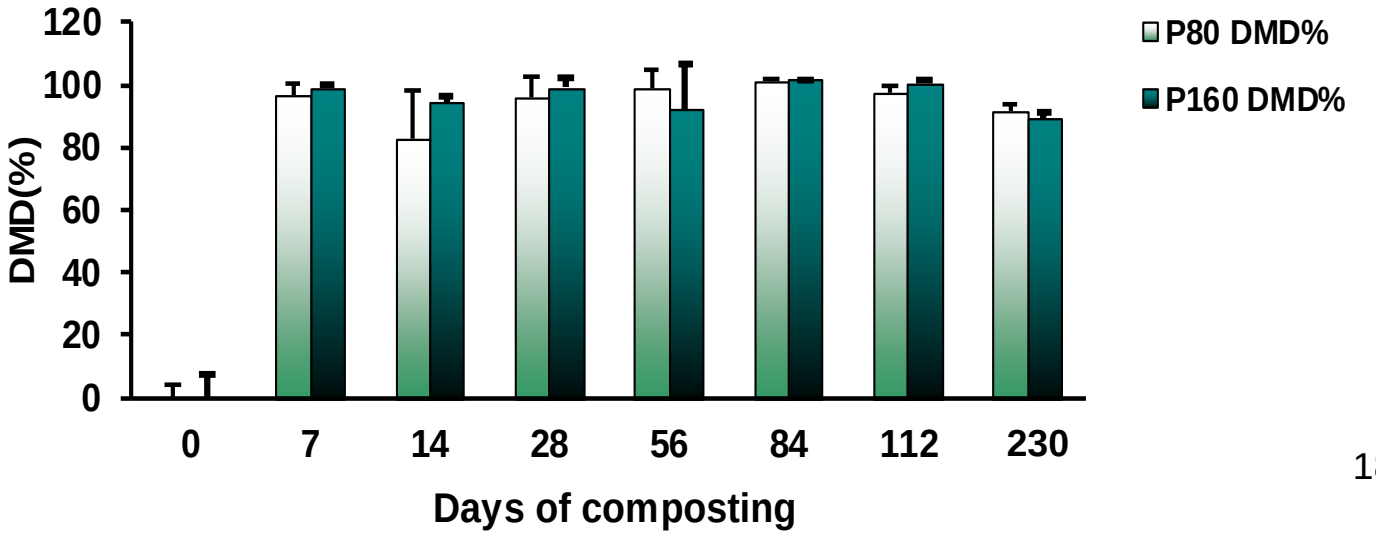
SRM Degradation

Dry Matter Degradation

- Lab-scale

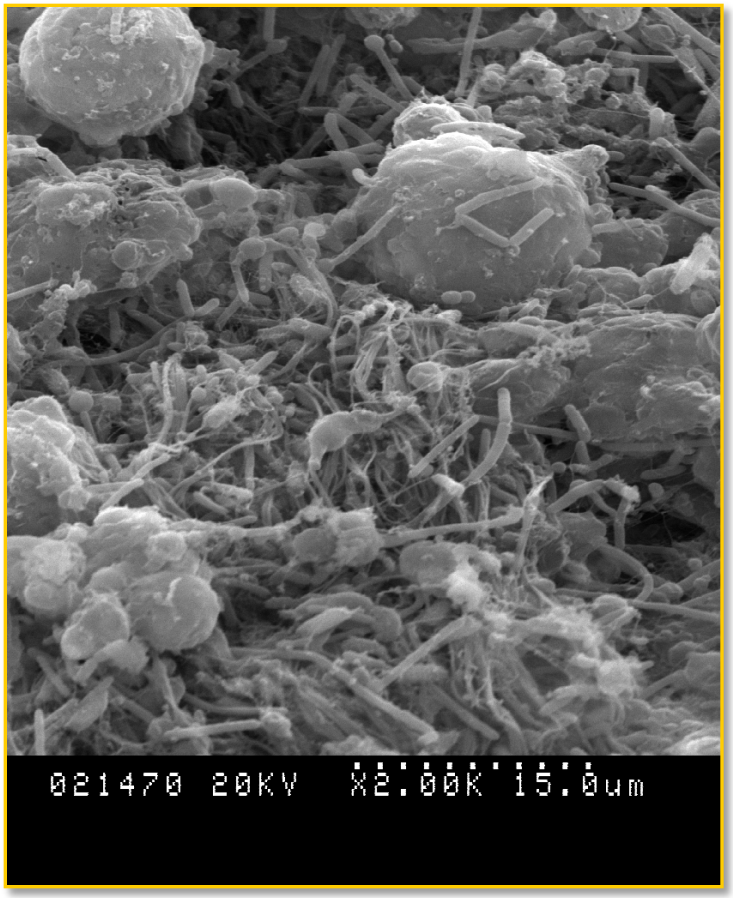


- Field-scale

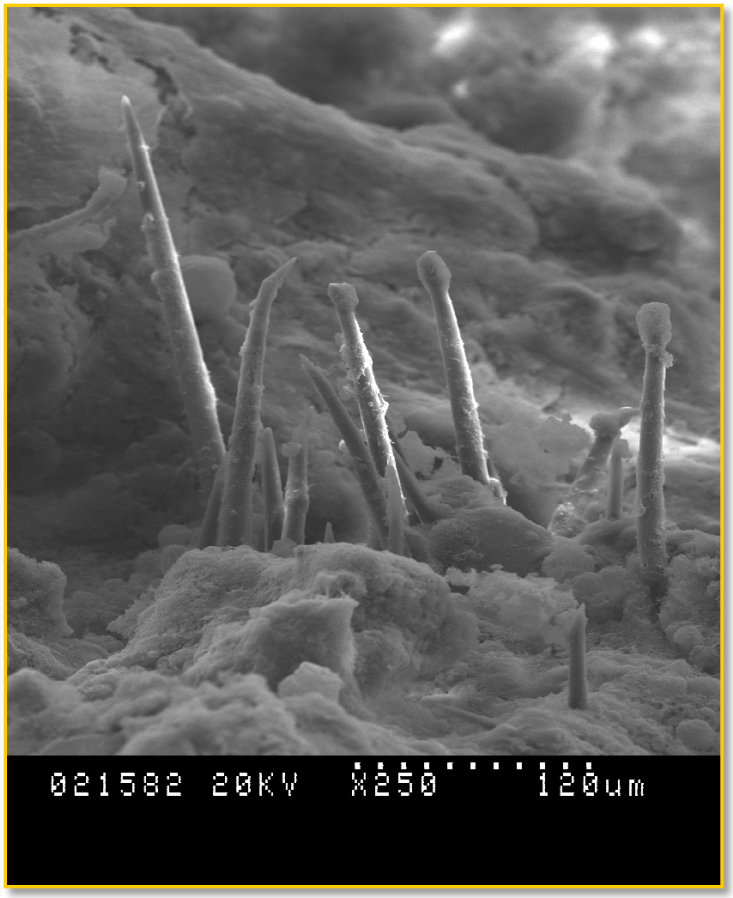


Microbial Invasion Brain

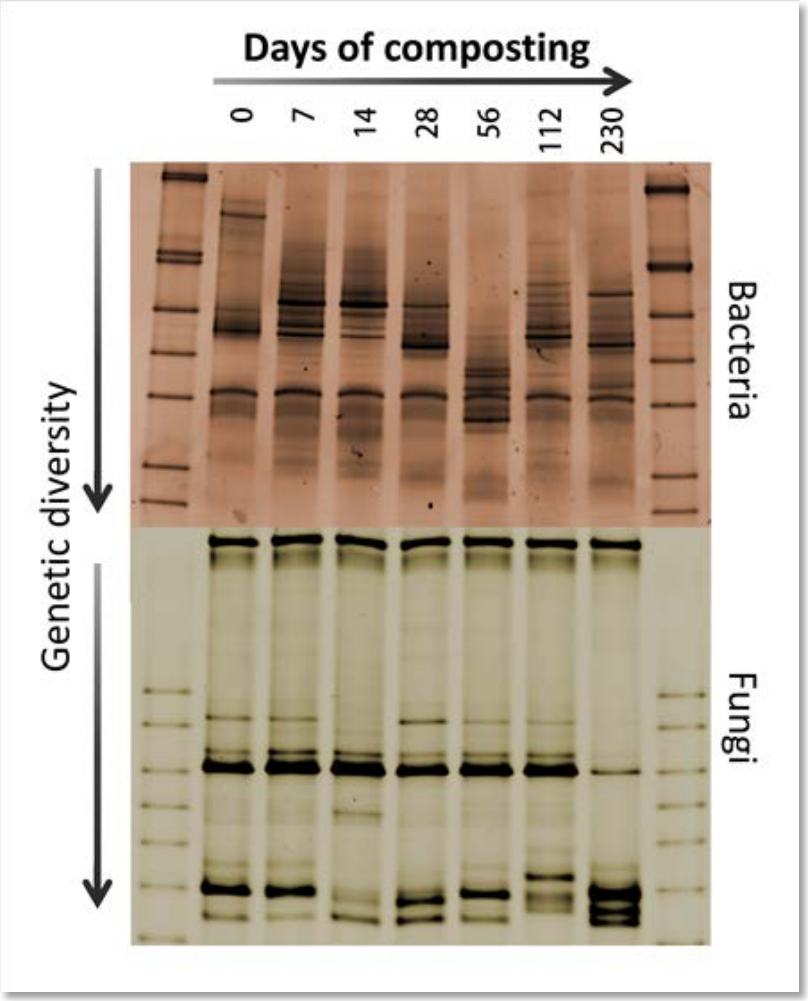
Day 7 Brain



Day 14 Brain



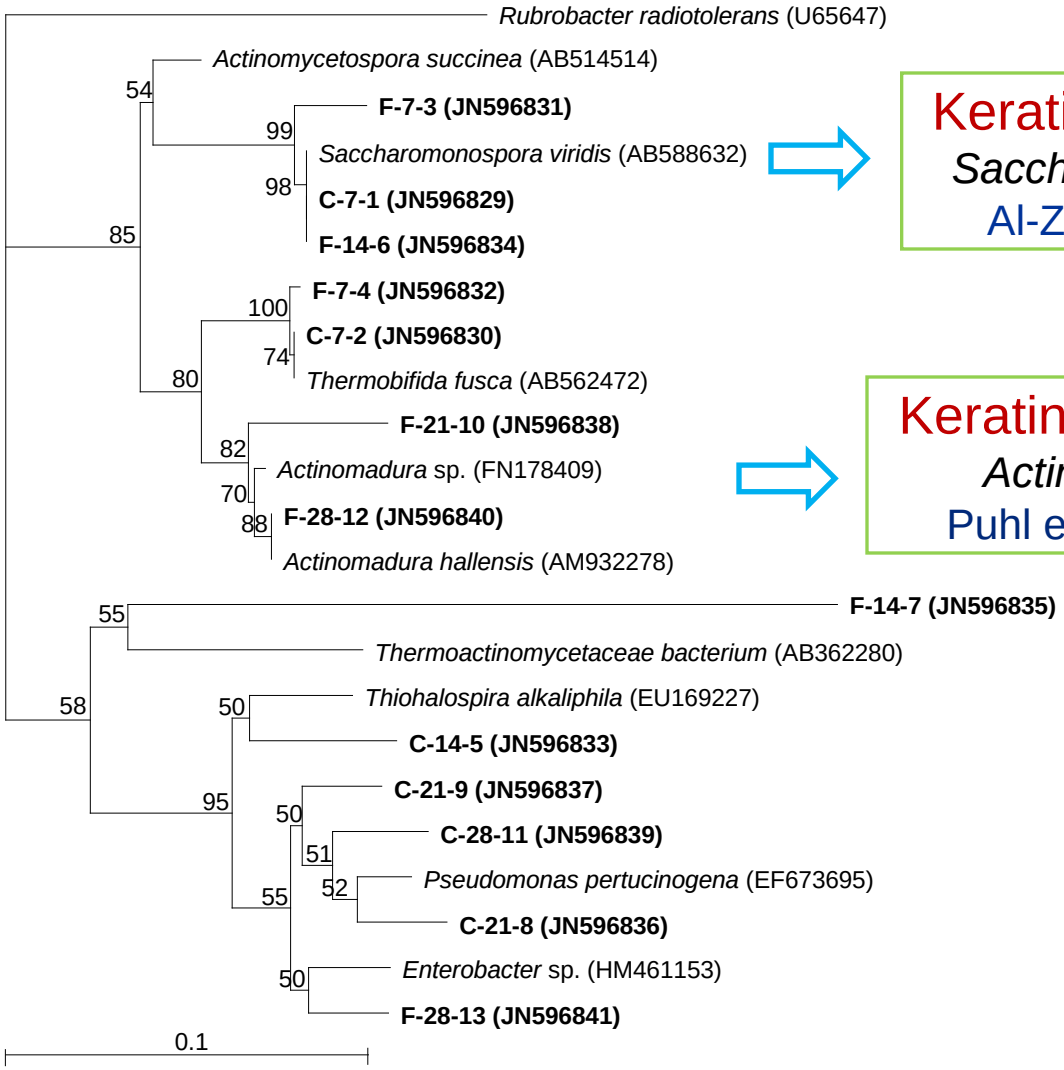
Microbial Diversity



Genetic Fingerprinting

- DNA Extraction
- PCR
- DGGE & Sequencing

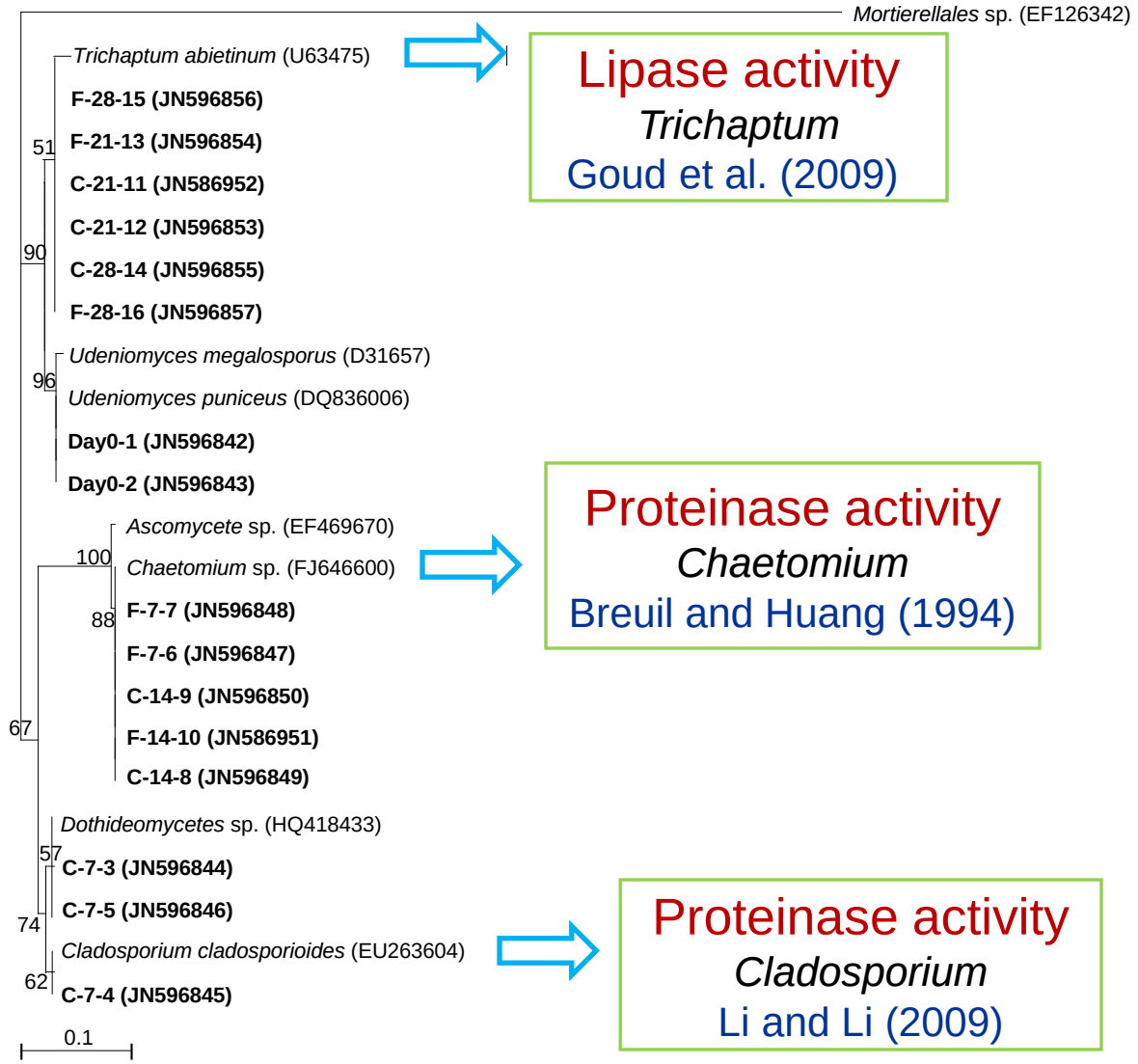
Bacterial Characterization



Keratinase activity
Saccharomonospora
Al-Zarban (2002)

Keratinase activity
Actinomadura
Puhl et al. (2009)

Fungal Characterization



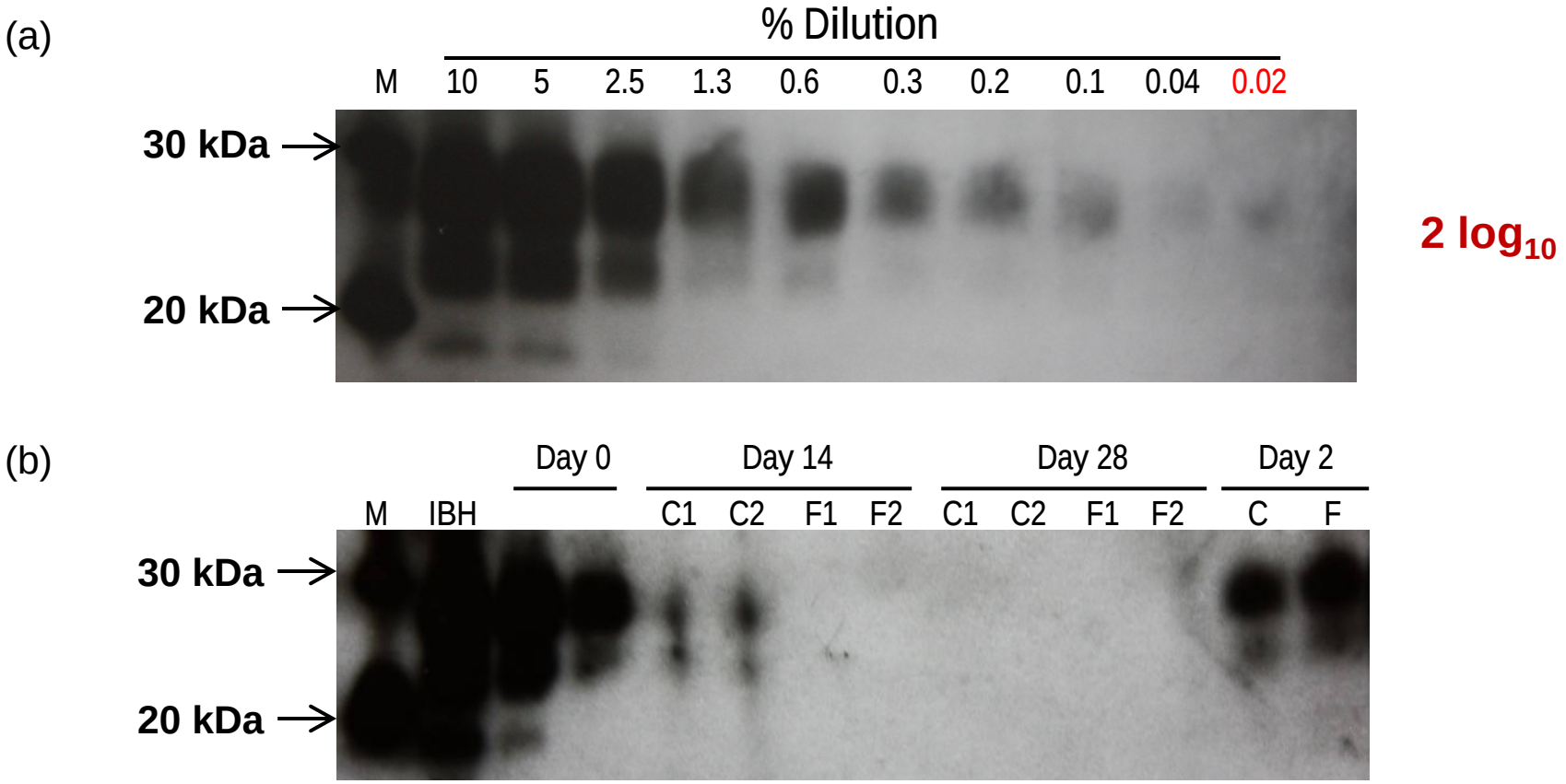
Lipase activity
Trichaptum
Goud et al. (2009)

Proteinase activity
Chaetomium
Breuil and Huang (1994)

Proteinase activity
Cladosporium
Li and Li (2009)

Scrapie 263K Degradation

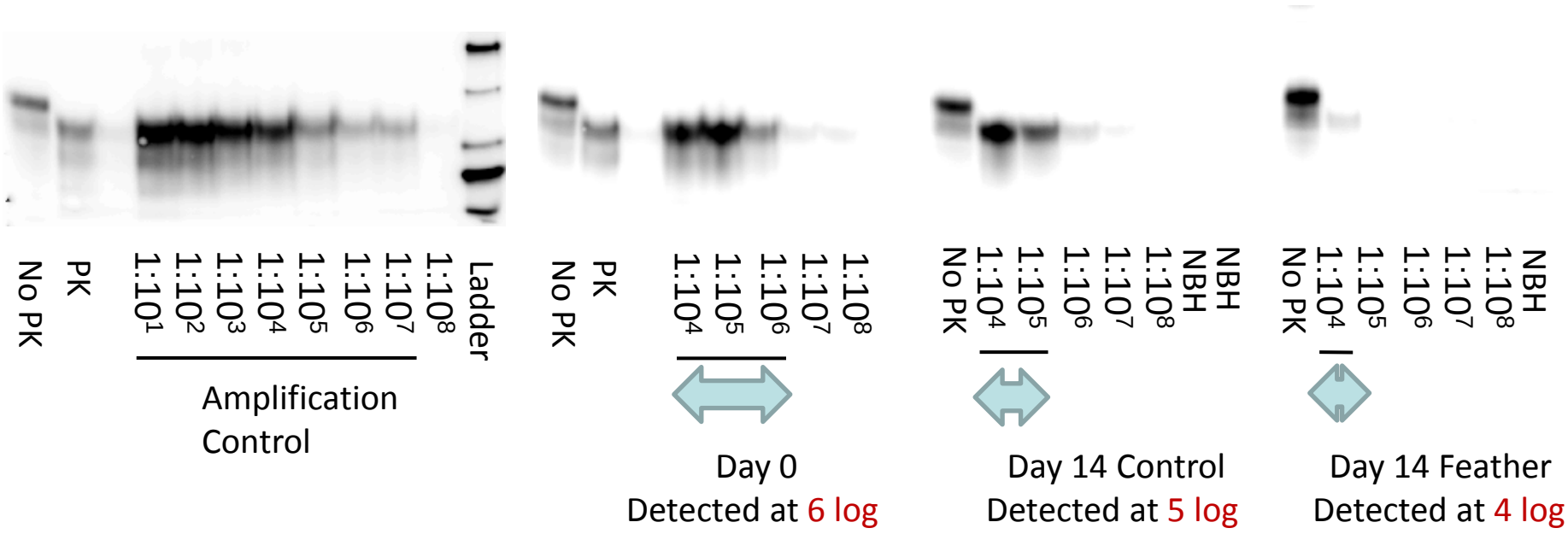
- Lab-scale compost – WB



Scrapie 263K Degradation

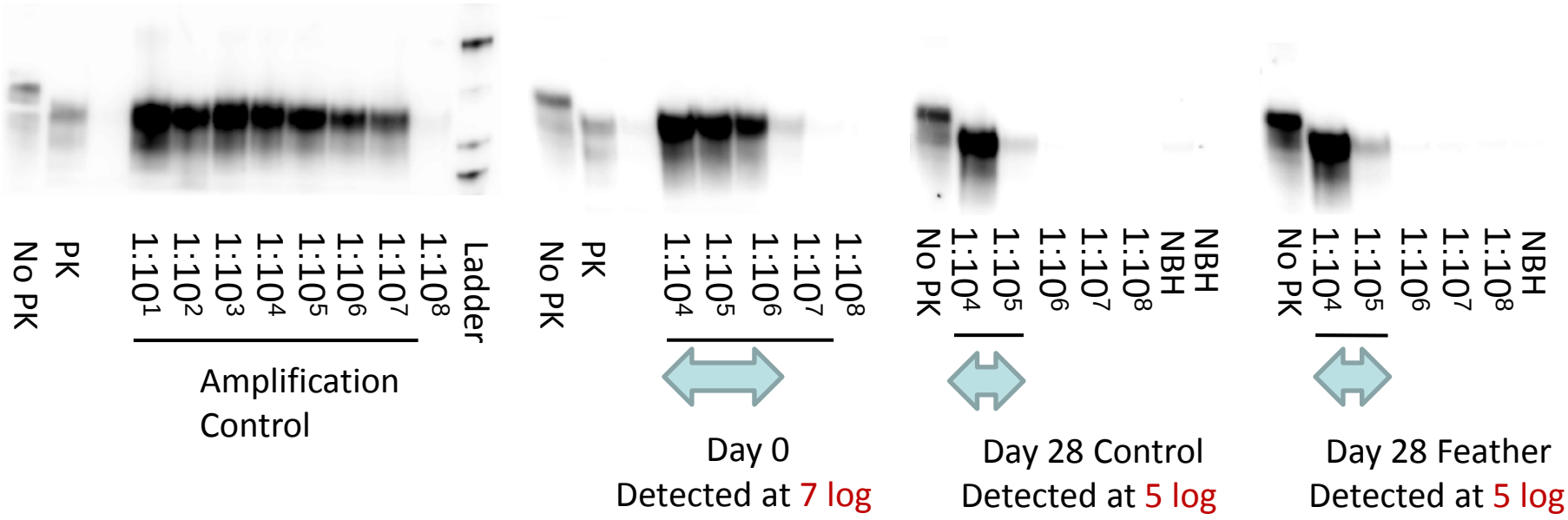
- Lab-scale compost – PMCA

Day 0 VS Day 14



Scrapie 263K Degradation

- Lab-scale compost – PMCA



Bioassay – Stainless Steel Bead

- Steel Bead IC implant

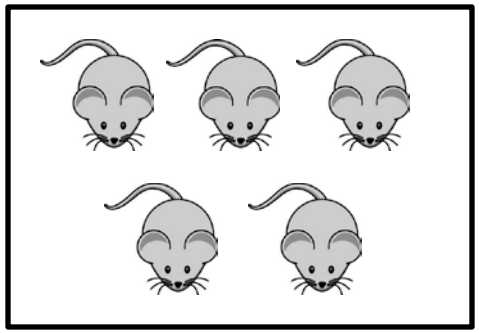
Dilution	Syrian hamsters	
	Clinical DPI (mean ± SD)	No. of sick hamsters/total no. of hamsters
10^{-1}	107 ± 2	3/3
10^{-2}	119 ± 6	5/5
10^{-3}	109 ± 4	4/4
10^{-4}	133 ± 10	4/4
10^{-5}	196 ± 38	3/4
10^{-6}	179 ± 32	3/5
10^{-7}	>330	0/5
10^{-8}	>330	0/4

Infectivity titre: 10^6 ID50 / bead = 100 ng brain tissue (Reed and Muench 1938)

Scrapie 263K Degradation

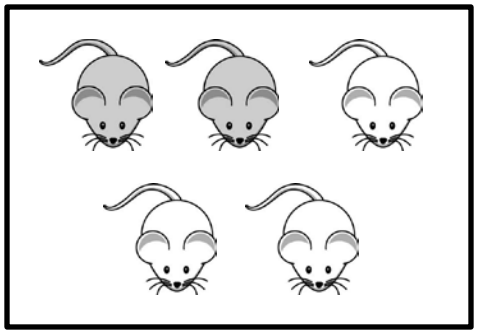
- Field-scale compost

Compost Day 0



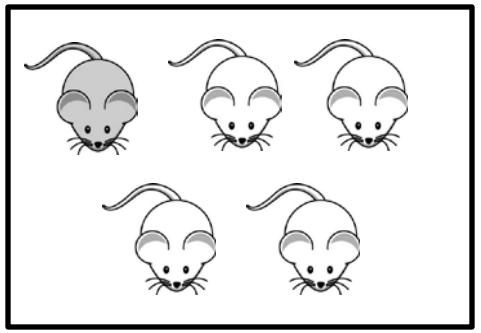
11/11 clinical disease
at 103 ± 3 dpi

Compost Day 112



2/5 clinical disease
at 123 ± 0 dpi

Compost Day 230



1/5 clinical disease
at 252 ± 0 dpi

Summary - Scrapie 263K

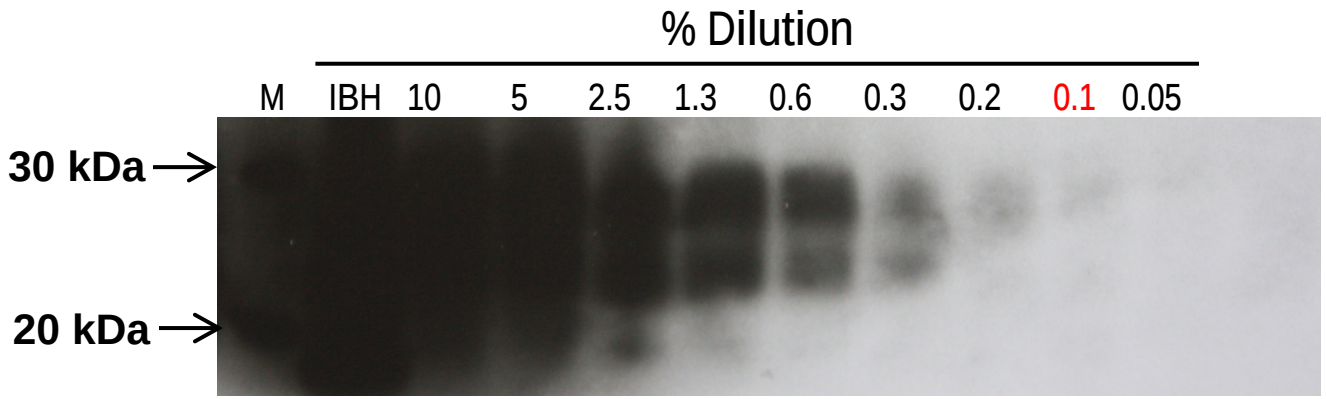
Lab-scale compost	Field-scale compost
• Total 28 days	• Total 230 days
• ≥ 55°C for 3 days	• ≥ 55°C for 75 days
• Feather > Control after 14 days	• Effective to reduce infectivity
• Approx. 2 log after 28 days	• 4.8 log* after 230 days

*Four-parameter logistic regression model (SAS)

CWD Degradation

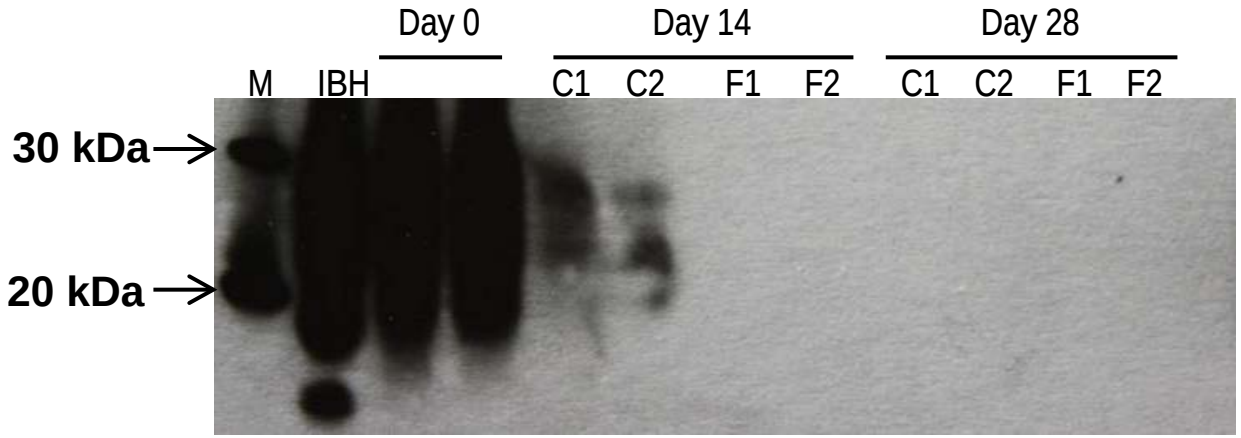
- Lab-scale compost – WB

(a)



1-2 $\log_{10}$

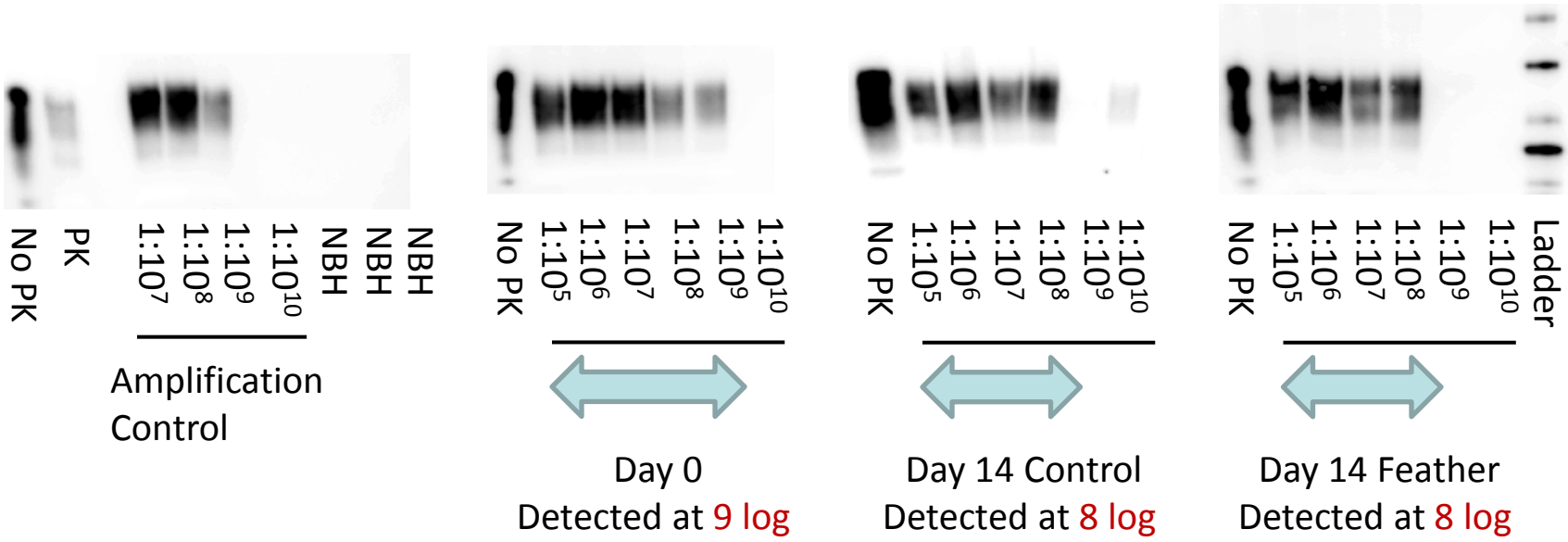
(b)



CWD Degradation

- Lab-scale compost – PMCA

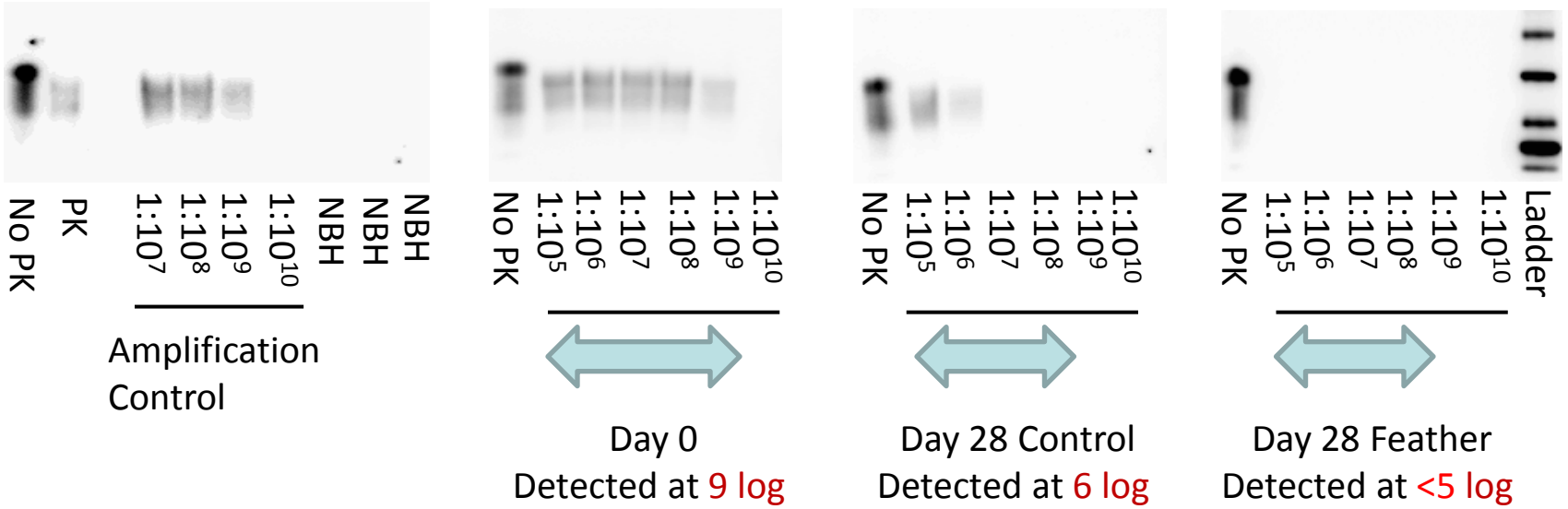
Day 0 VS Day 14



CWD Degradation

- Lab-scale compost – PMCA

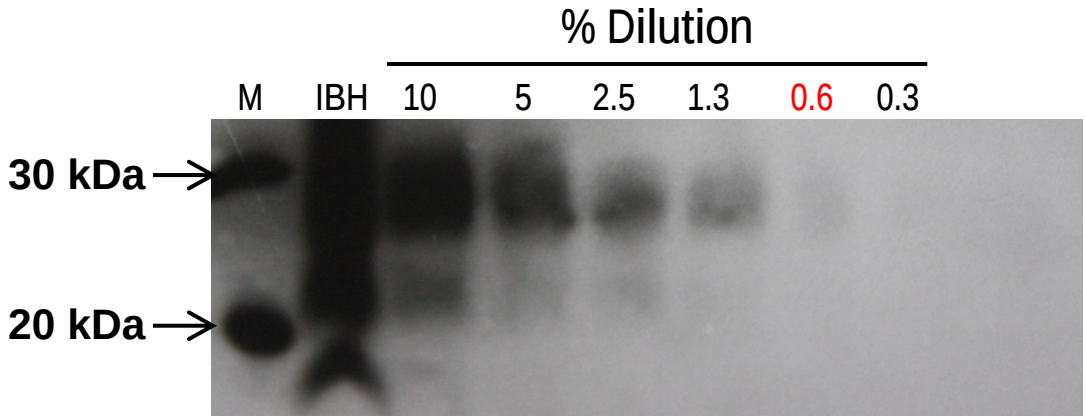
Day 0 VS Day 28



BSE Degradation

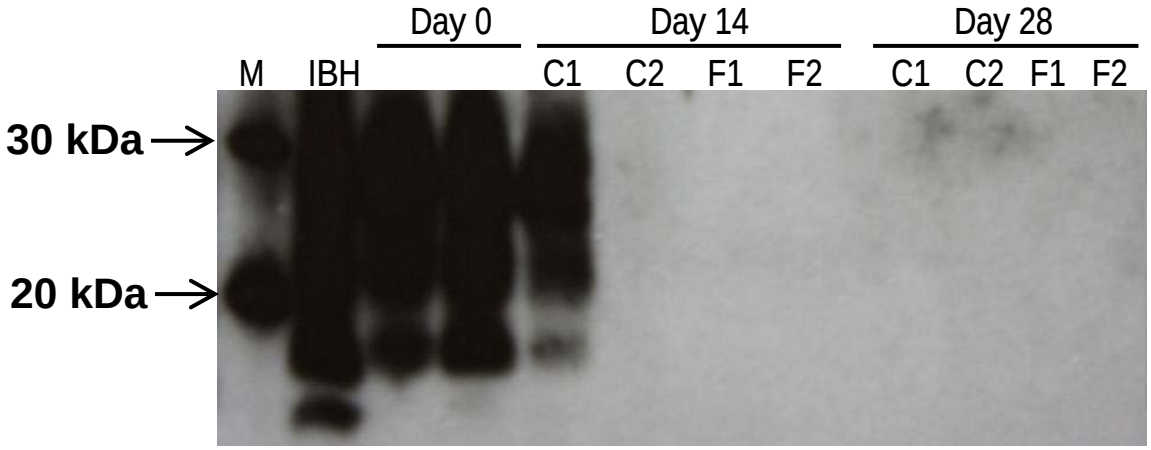
- Lab-scale compost – WB

(a)



1 $\log_{10}$

(b)



Future Directions

Composting degrades:

- SRM completely
- Prions at the logarithmic level
- CWD elk at field-scale after 230 days?
- BSE at lab-scale after 25 cycles (=230 days of field-scale composting)?

Acknowledgements

- Dr. Brandon Gilroyed – University of Guelph
- Drs. Norman Neumann, Mike Belosevic, Jerry Leonard – University of Alberta
- Drs. Aru Balachadran, Stefanie Czub, Catherine Graham – Canadian Food Inspection Agency
- Alberta Prion Research Institute
- Natural Sciences and Engineering Research Council of Canada
- Agriculture and Agri-Food Canada

Agriculture et Agroalimentaire Canada
Agriculture and Agri-Food Canada

Natural Sciences and Engineering Research Council of Canada
Conseil de recherches en sciences naturelles et en génie du Canada

ALBERTA PRION RESEARCH INSTITUTE
prion

Thank you

Way to go. . .

COMPOSTING

Large Compost Construction

Lab-scale Composter Layout