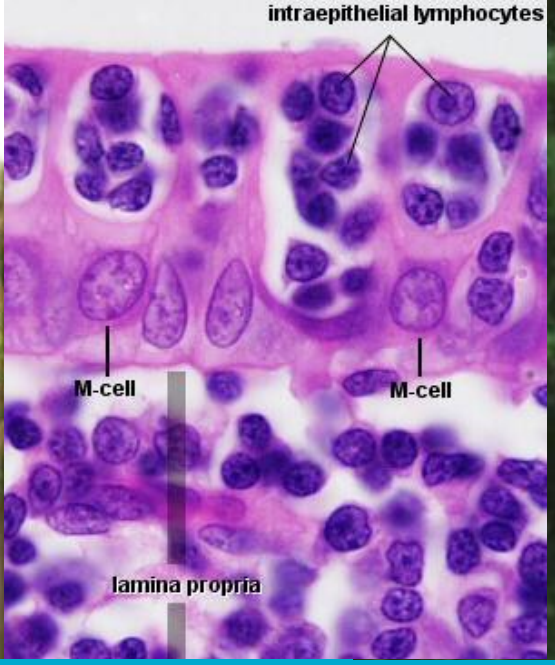

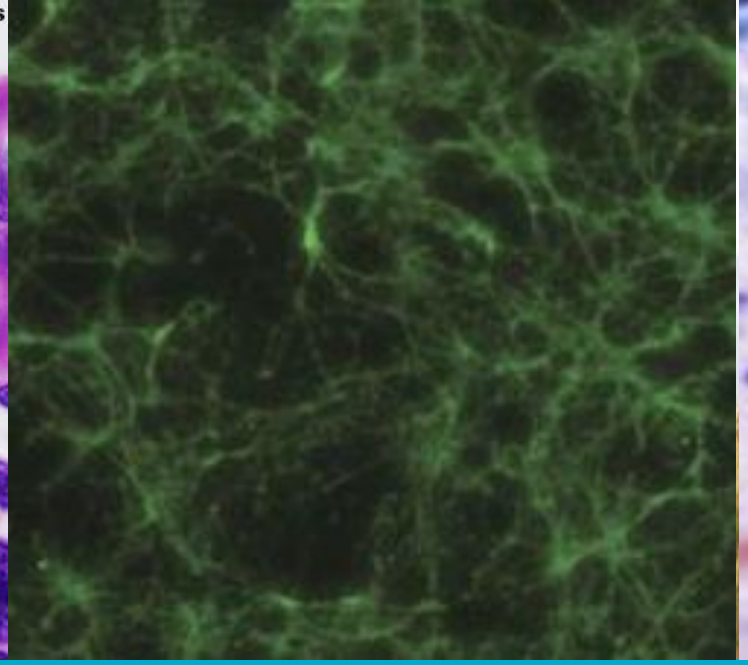
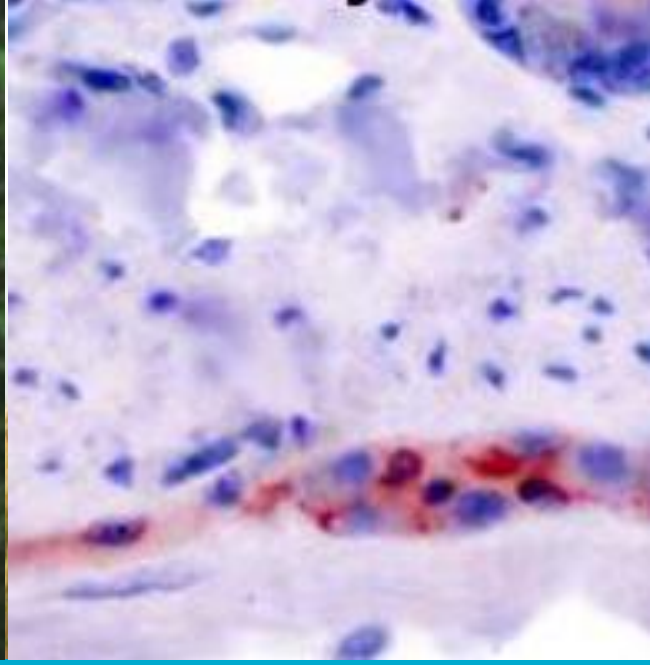

Prions

Prion biology and the pathogenesis of prion diseases

prion

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Special issue co-edited by Drs. Neil Cashman and Yury Chernoff

Issue highlights:

Profiles & Legacies: *The end of the road* by Charles Weissmann

The Creutzfeldt-Jakob Disease Foundation by Florence Kranitz

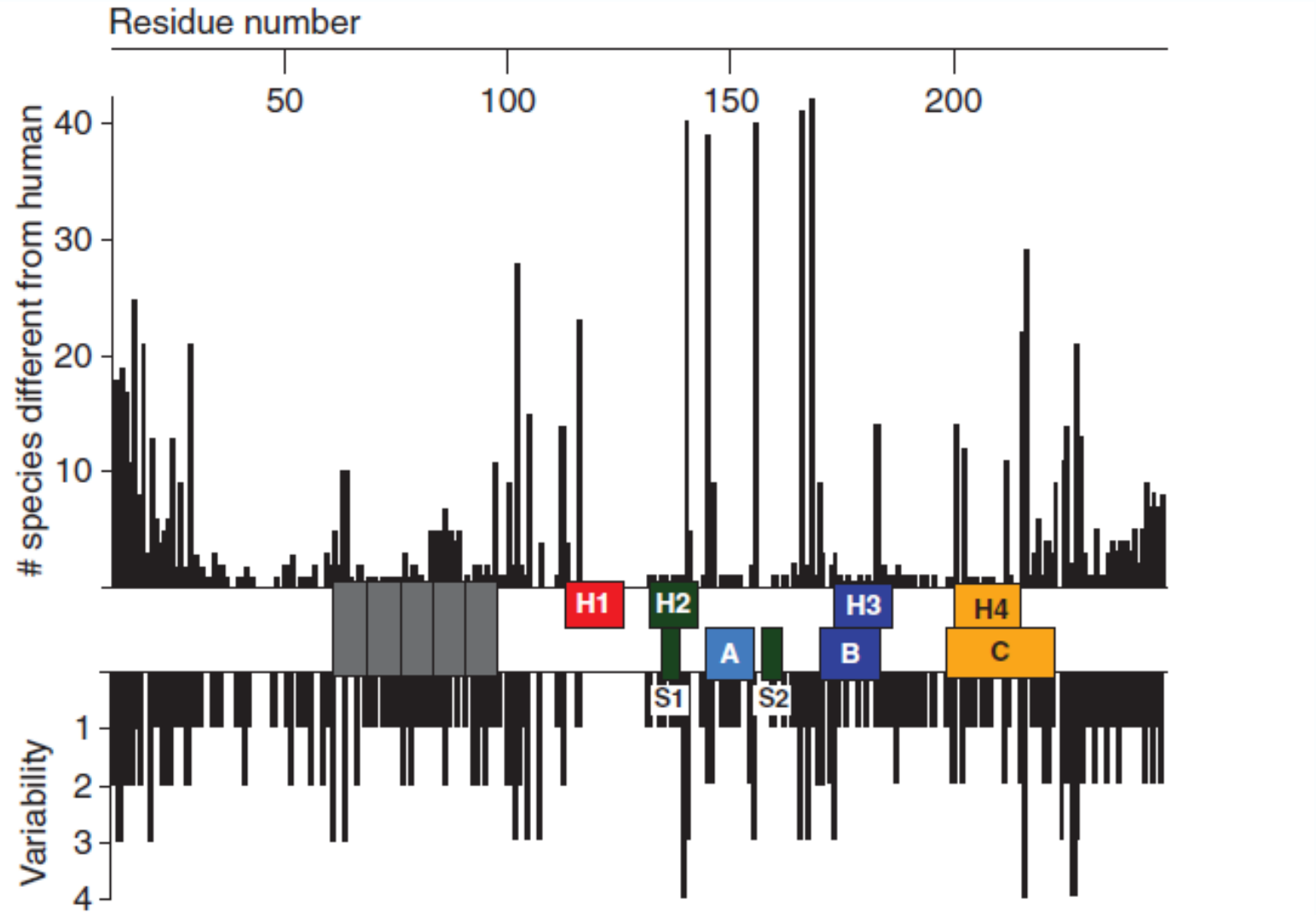
Reviews and research papers on material of the Prion 2011 meeting in Montreal

LANDES BIOSCIENCE

Prion diseases….in a nutshell

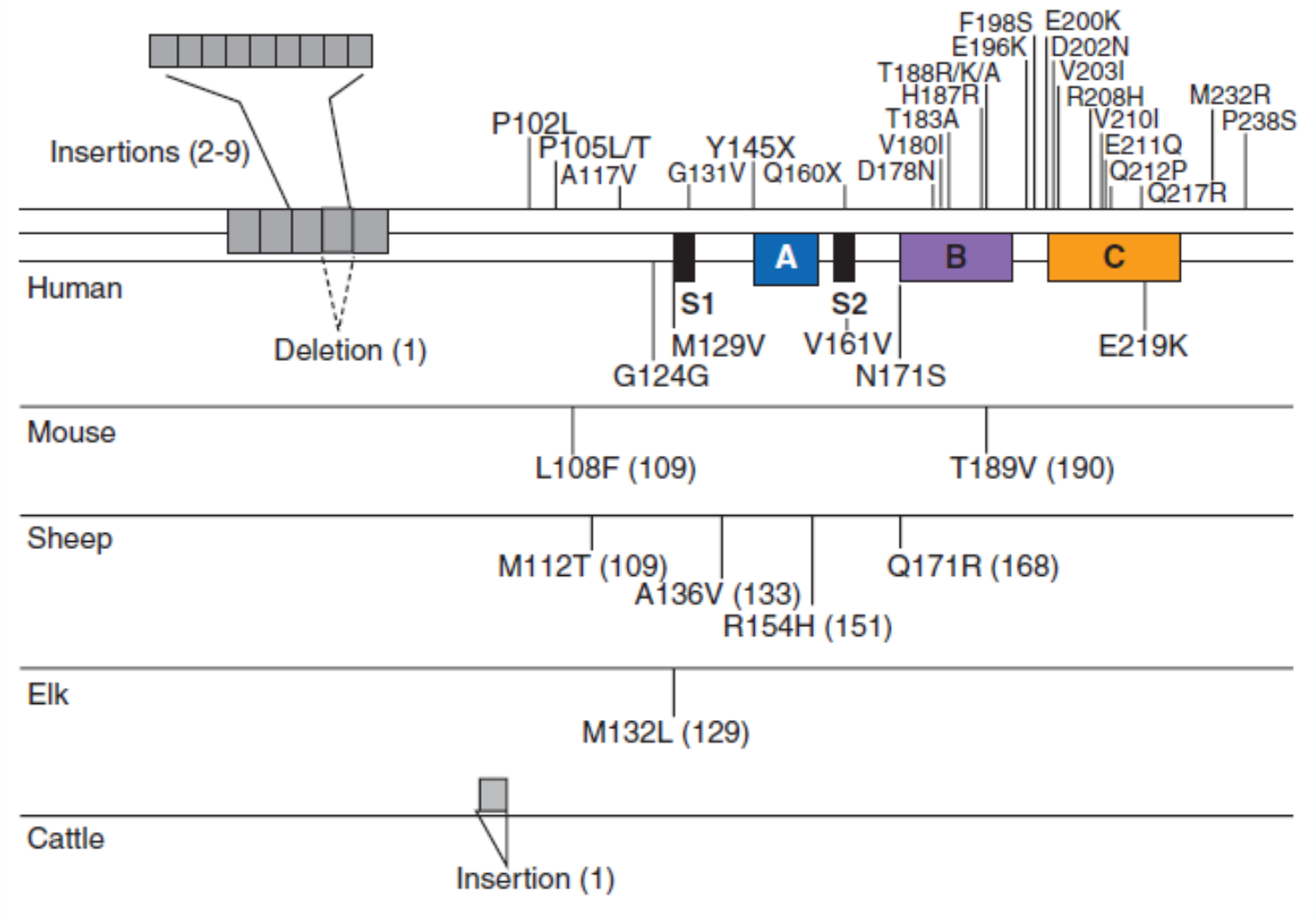
- Neurodegeneration and lethality
  - Vacuolation, gliosis , PrP deposition
  - Predominantly grey matter
  - Location and morphology of vacuoles and PrP deposits varies with strain and host.
- Conformational event
  - Recruitment of the normal, cellular isoform (PrP^{C}) and conversion into the disease-causing isoform (PrP^{Sc})
  - Circumvents innate and adaptive immunity
- No exogenous genetic material involved in transmission

The PrP gene – species variation



Colby & Prusiner 2012

The PrP gene – polymorphisms and mutations



Colby & Prusiner 2012

Infection, or is it just in your genes?

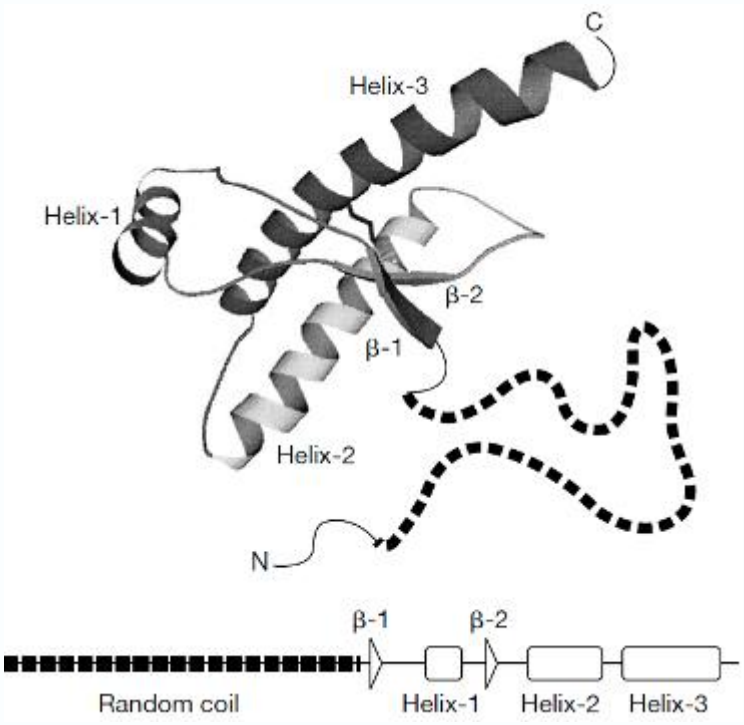
Mutations

- Heritable tendency for destabilisation of PrP
- Transmissible to non-human primates
- Sporadic CJD
  - Somatic mutation?
  - Spontaneous low-frequency mis-folding event?

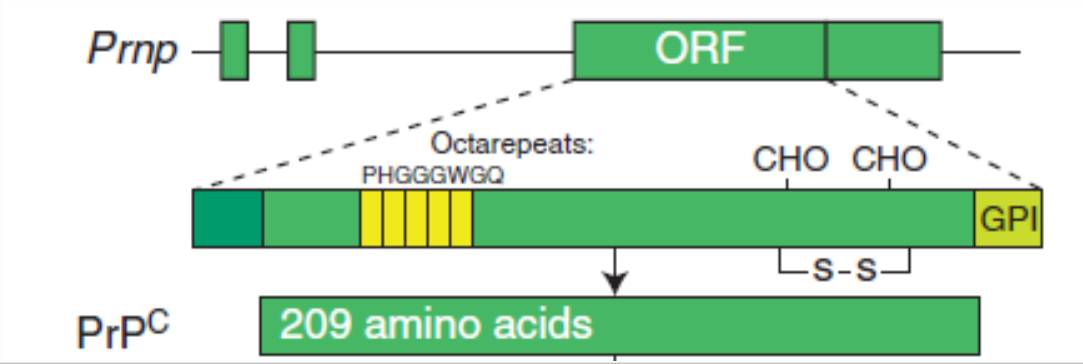
Infections

- Kuru
- Iatrogenic CJD (HGH, equipment)
- Variant CJD
- Classical BSE (FSE, EUE)
- Classical scrapie
- Chronic Wasting Disease (mule deer, white tailed deer, elk)

The prion protein



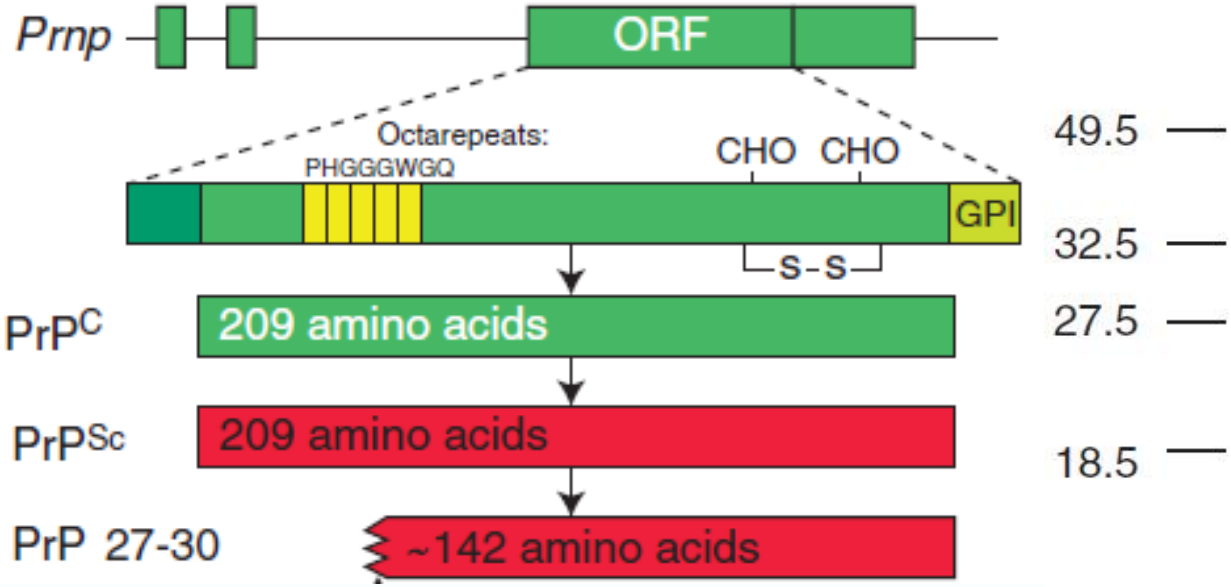
- Cellular isoform PrP^C
  - Two glycosylation sites
- Elusive physiological function
  - Expressed in most adult tissues except liver
  - Hjghest in brain
- Neuroprotective
  - PrP knockouts are normal
  - Tg with mutant protein develop neurodegenerative disease (in wm!)
- Post-translational processing removes peptide at either end

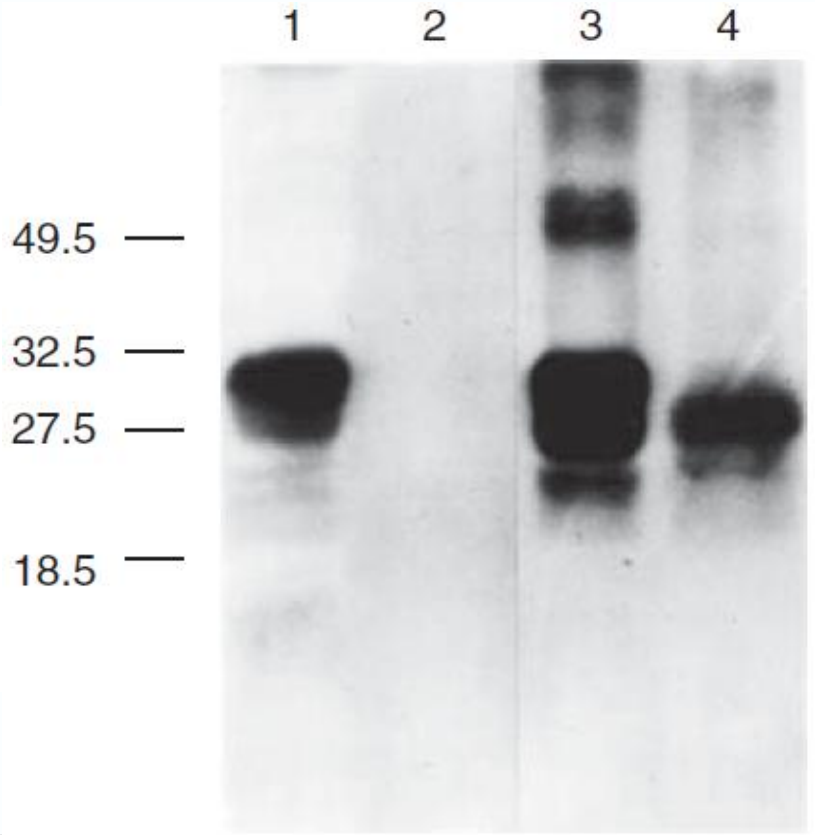


Colby & Prusiner 2012

The prion protein – abnormal isoform

- Conversion to PrP^{SC} likely occurs at cell surface
  - Protease digestion produces residual resistant molecule PrP^{Sc} 27-30
    - Basis of diagnostic detection systems





Colby & Prusiner 2012

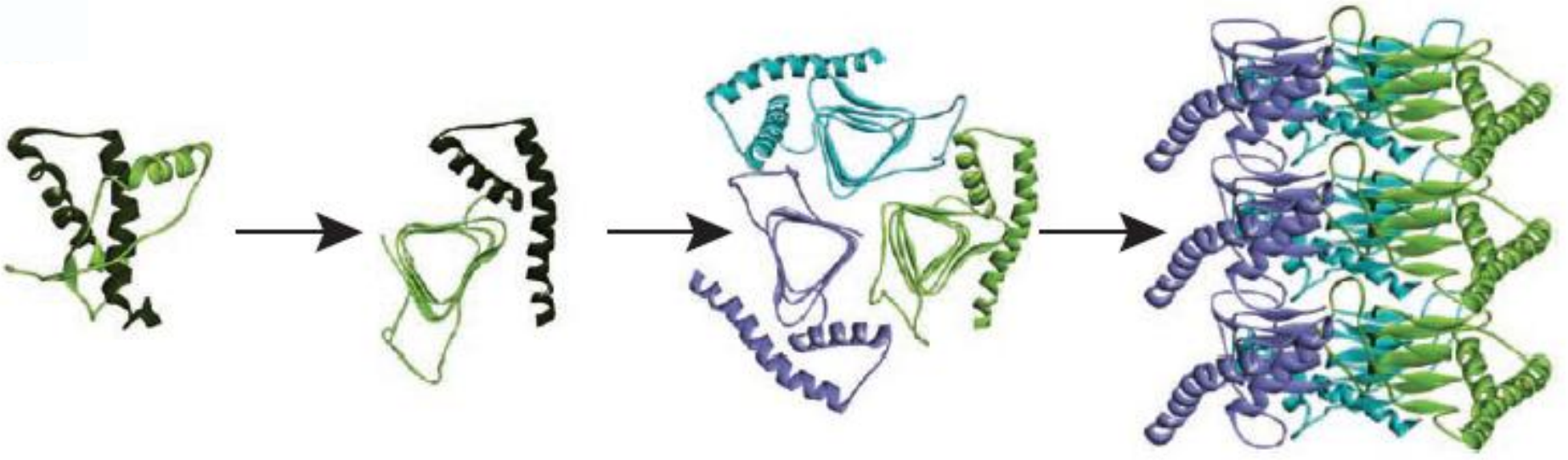
PrP^{C} vs PrP^{Sc}

Enriched in β-sheets

- Major refolding of N-terminal region
- Similar structure to an amyloid – aggregated and insoluble

Polymer

Toxic to cells – mechanism largely unknown

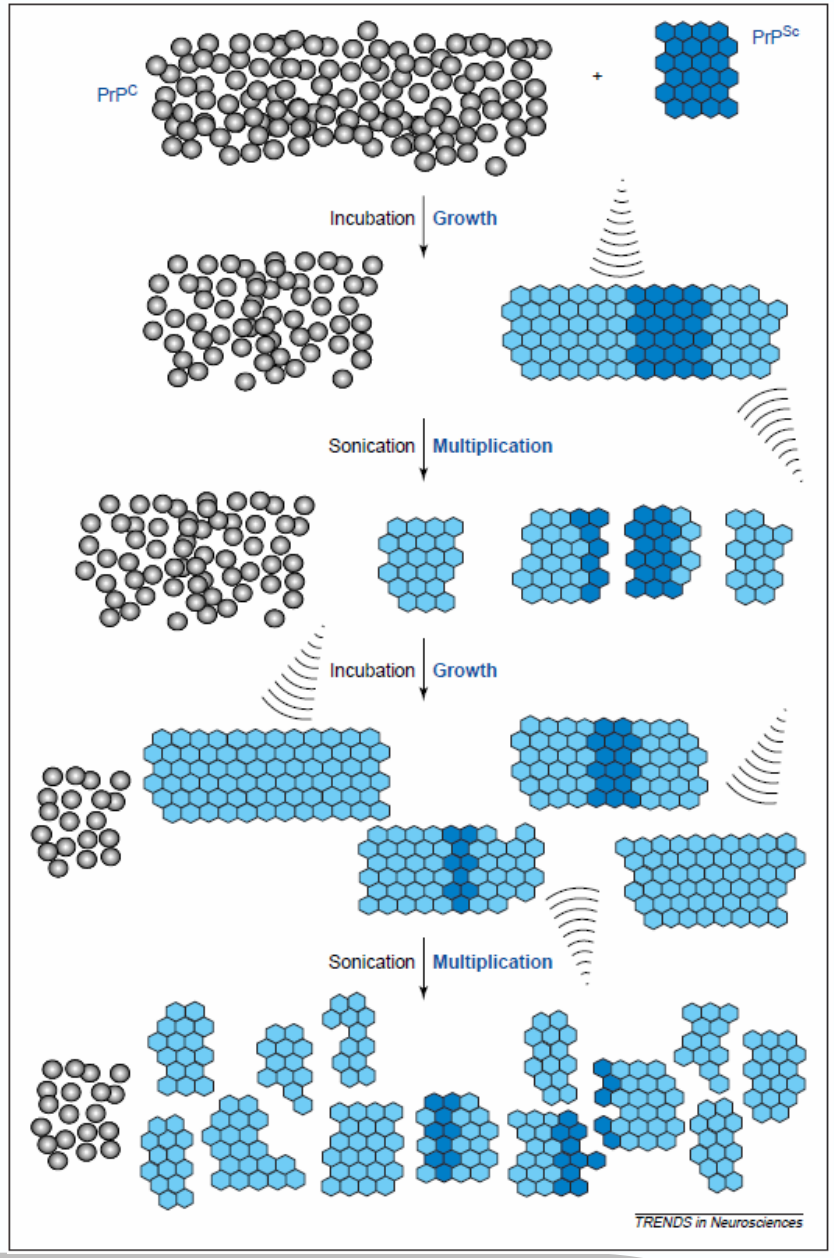


Diaz-Espinoza *et al* 2012

The prion hypothesis

A **prion** is an infectious agent composed of protein in a misfolded form without nucleic acids (RNA,DNA, or both)

Protein **M**isfolding **C**yclic **A**mplification



Soto *et al* 2002

The prion hypothesis

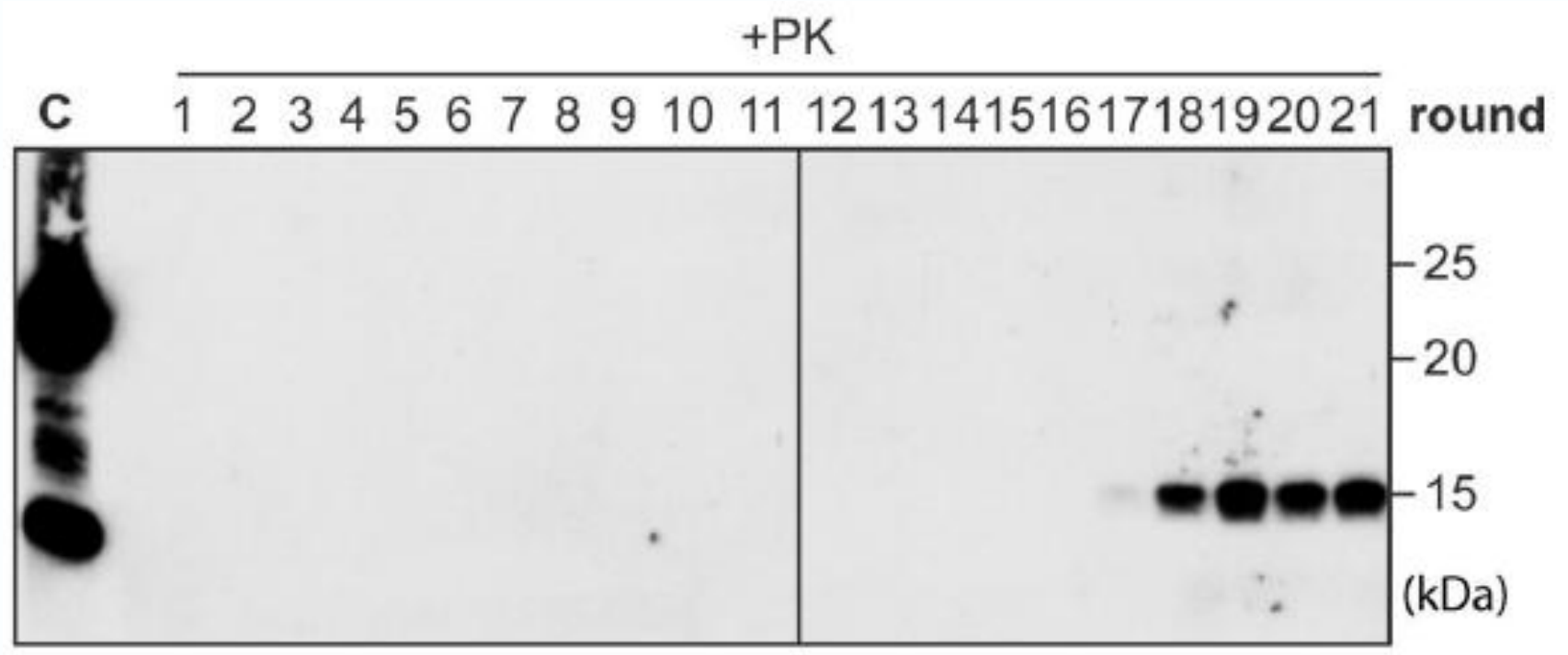
Recombinant PrP (*E.coli)*

+

Non-coding mouse liver RNA + lipids

PMCA

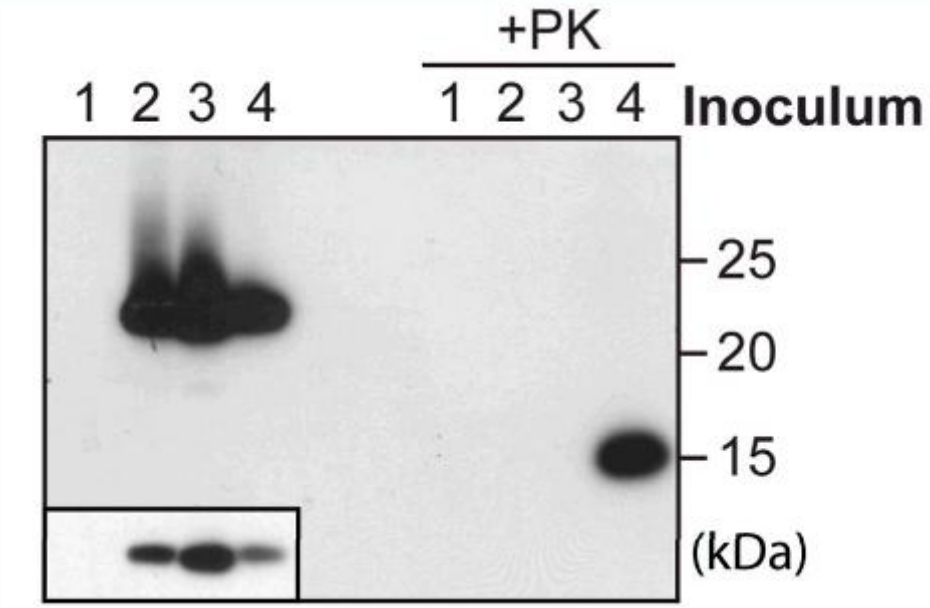
→ 15 kDa PK resistant band

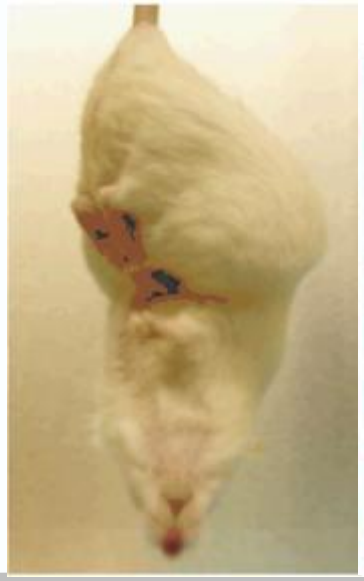


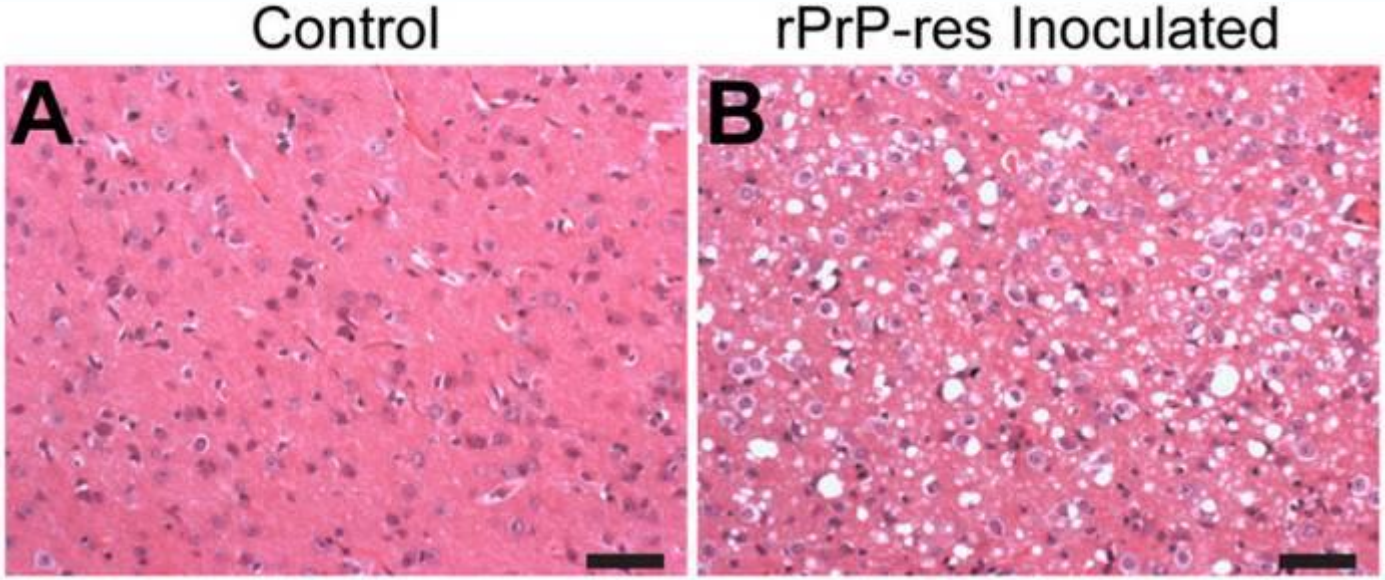
Wang *et al* 2010

The prion hypothesis

- Disease in wild-type mice
- Transmissible on serial passage







Wang *et al* 2010

Prion disease

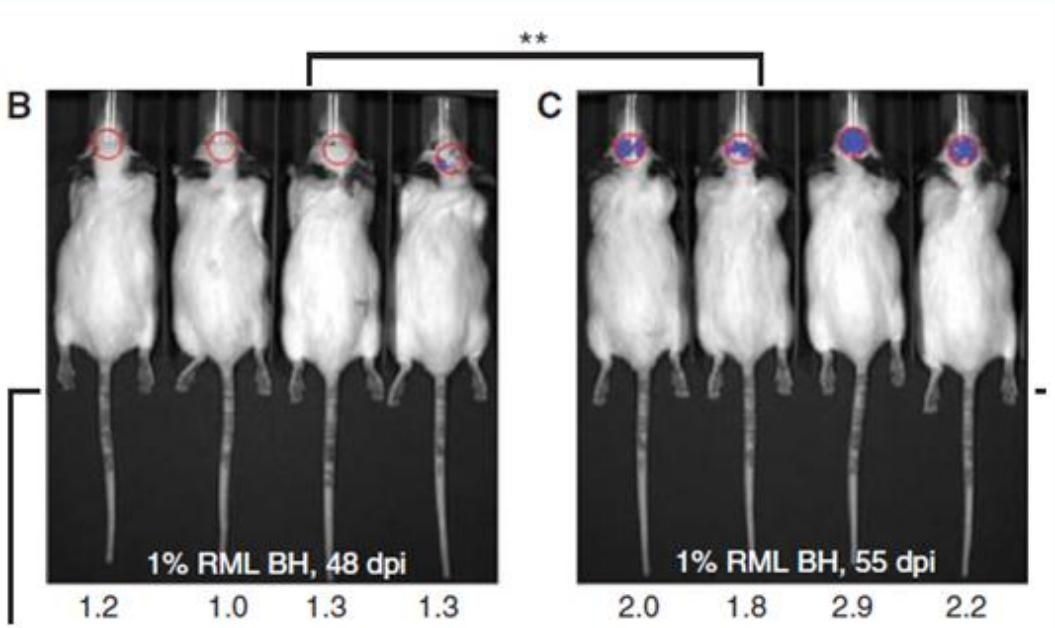
Astrocyte reaction

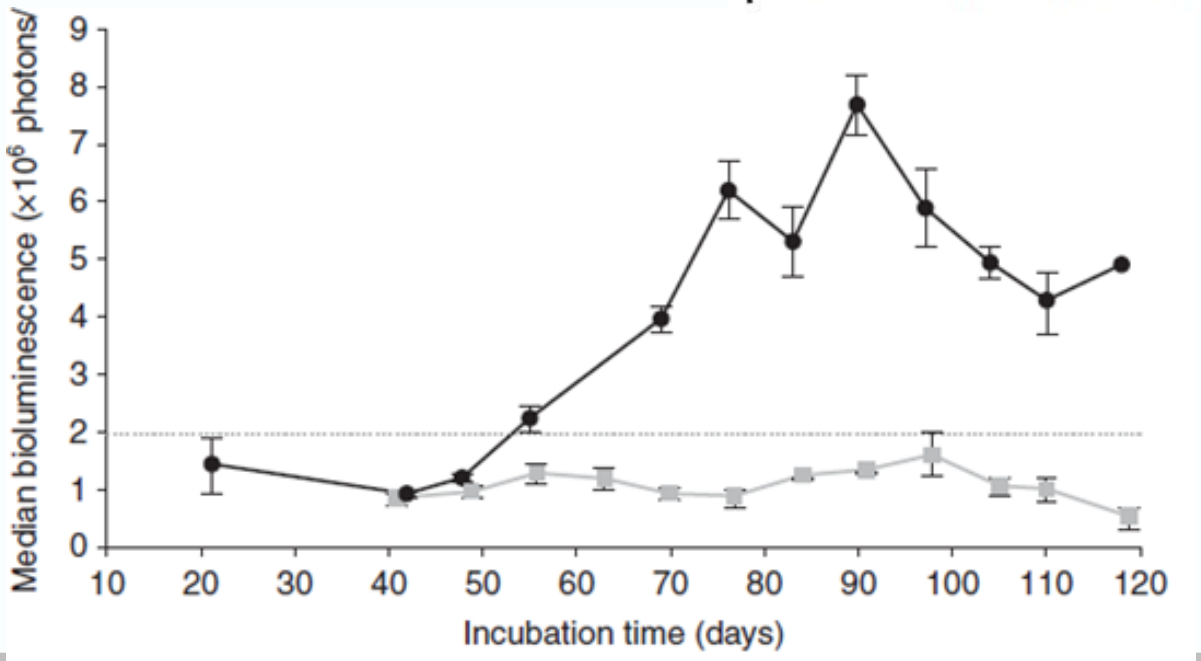
- proliferation
- hypertrophy

direct effect of PrP

IL-1 + IL-6 from microglia

Co-localises with PrP^{sc}



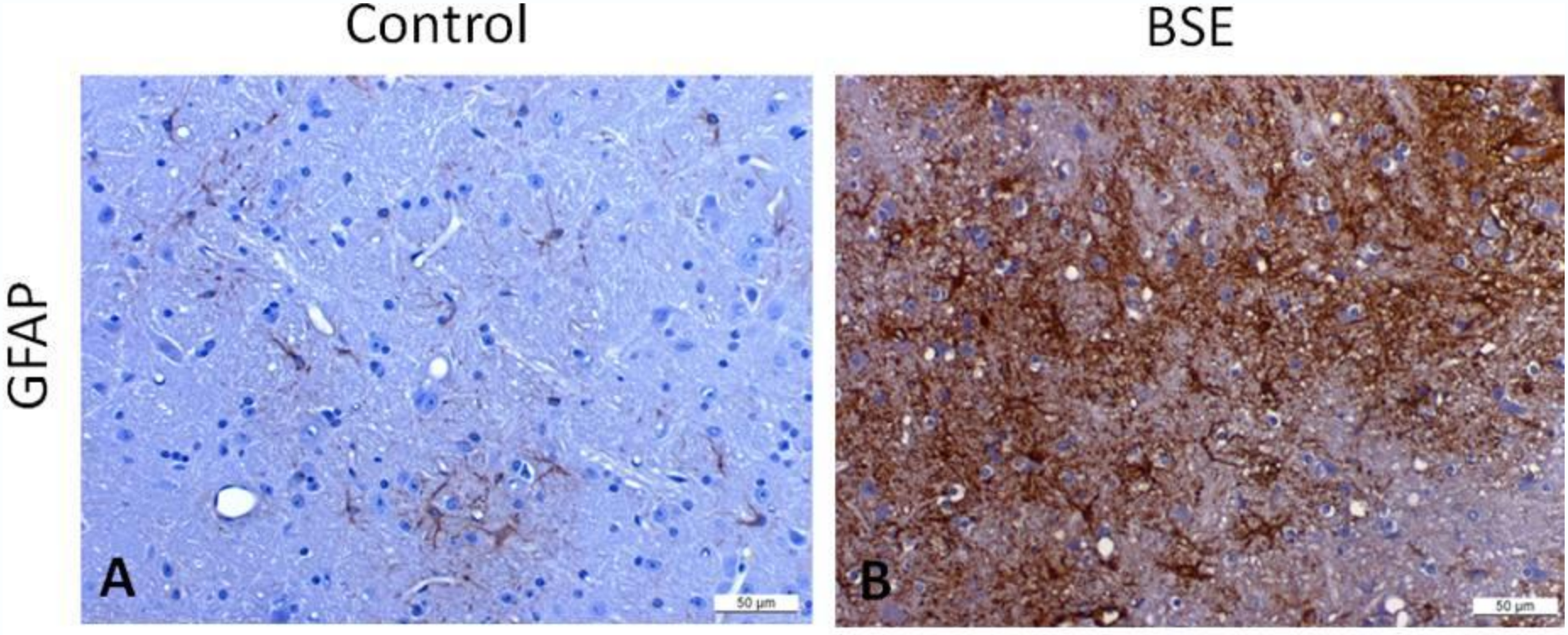


Tg mice expressing luciferase under GFAP promoter

Colby & Prusiner 2012

Prion disease

Brain sections of boTg110 terminal stage mice (275 dpi) and mock inoculated matched controls



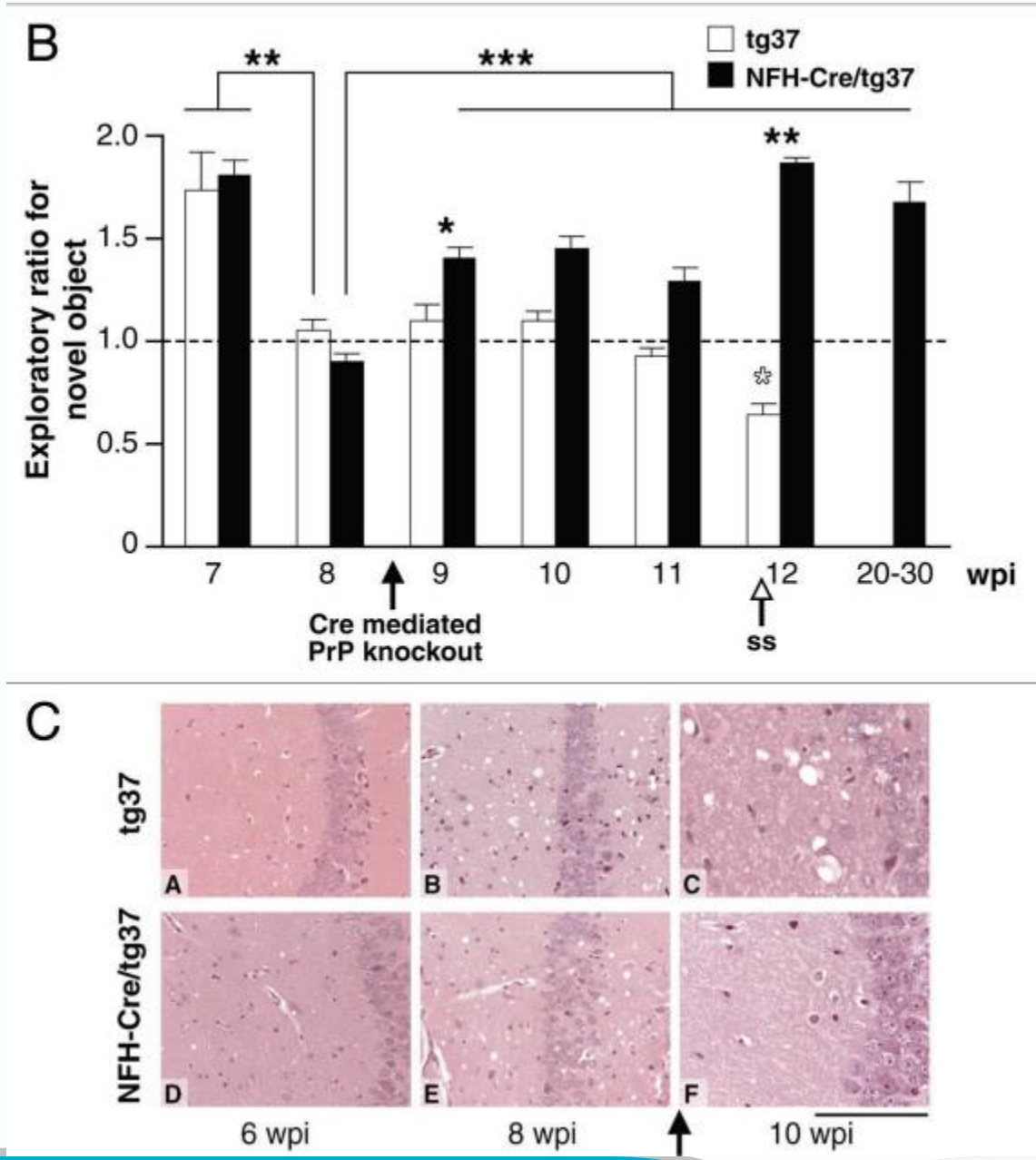
Tortosa *et al* 2011

Prion disease

- Neuronal degeneration 2^0 synaptic damage ➡ neuronal loss
  - Dendritic vacuoles and impaired pre-synaptic axonal function
    - mRNA/miRNA screens – Δ gene regulation of pre- and post-synaptic proteins
  - Synaptic pathology and dendritic dysfunction precede PrP deposition
    - Transient neurotoxic species is produced within neurons on PrP^C conversion to PrP^{Sc}
  - NFH-Cre/tg37 neuronal PrP knockout mice
    - accumulate extra neuronal PrP^{Sc} (presumably in astrocytes)
    - no impairment of memory function or synaptic responses

Prion disease

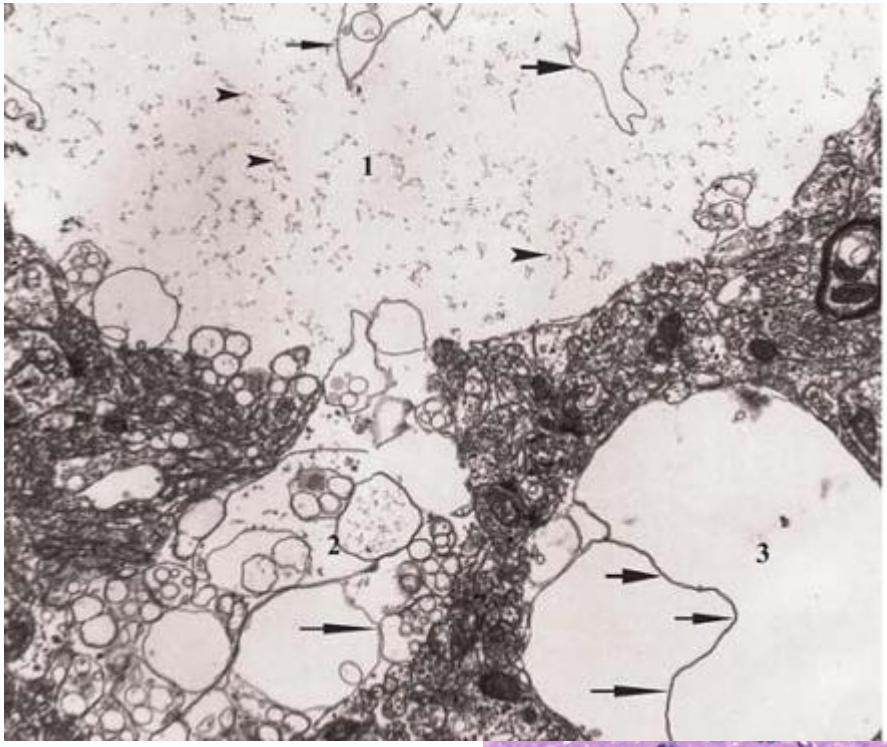
Early synaptic dysfunction and spongy change that precede synaptic and neuronal loss may be reversible

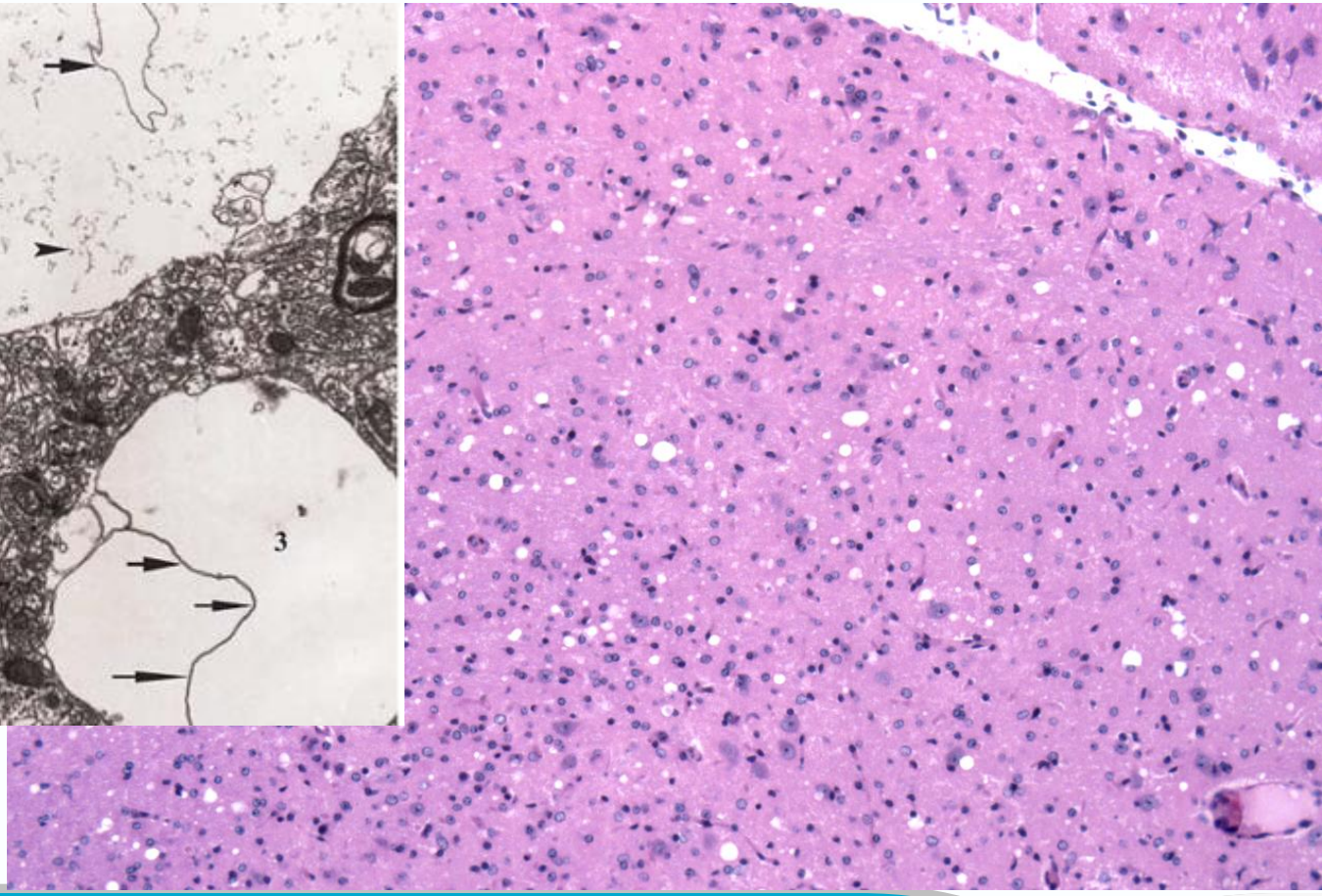


Malluci 2009

Prion disease

Spongiform change

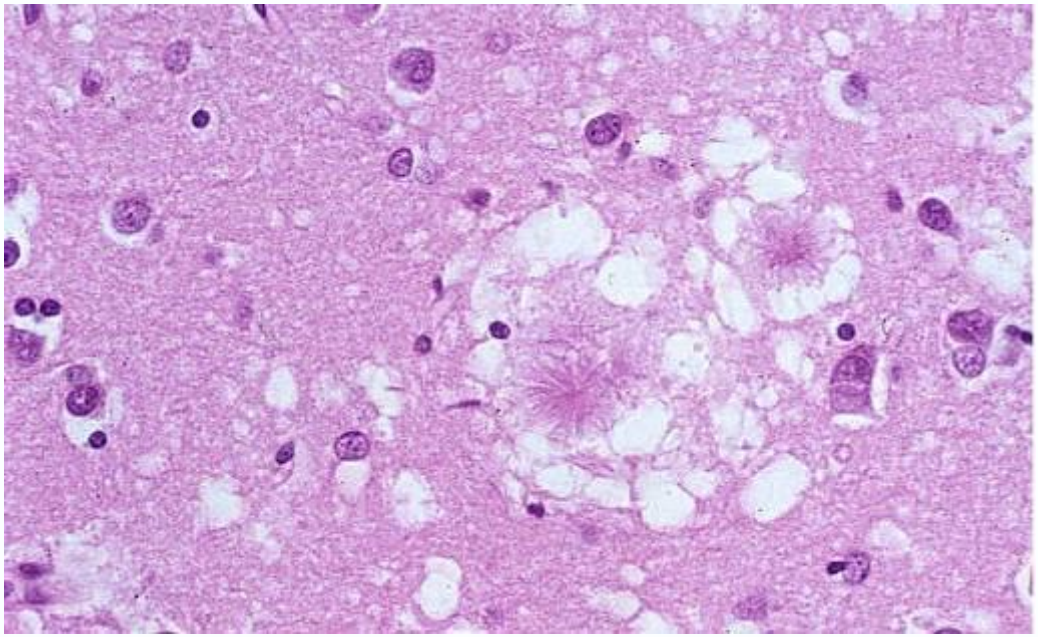


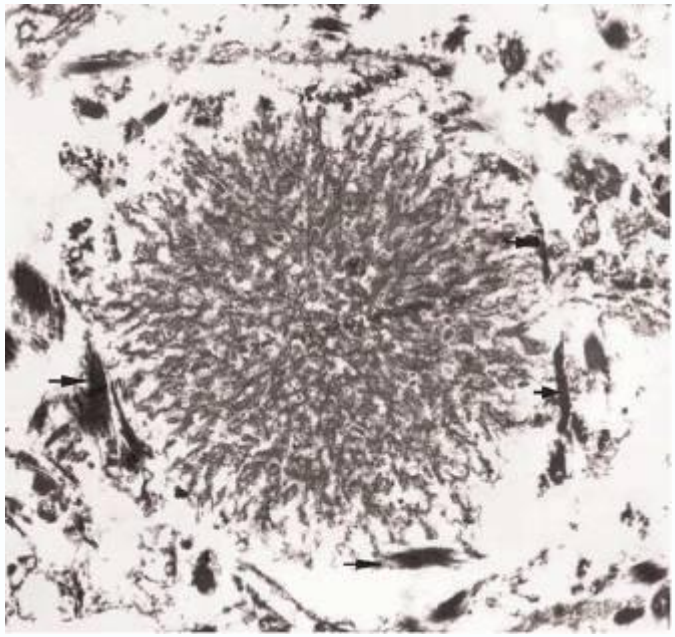


Liberski *et al* 2010

Prion disease

- PrP amyloid
  - Intimately associated with astrocytes and microglia
  - Human deposits tend to be neat compact structures cf sheep and mice
    - Kuru, variant CJD, 87V scrapie
    - Surrounded by halo of spongy degeneration (“florid” plaques)





Liberski *et al* 2010

Scrapie

Neurological disease

- erratic involuntary movements
- ataxia
- excessive scratching

Transmission

- 1934 sheep to sheep
- 1961 sheep to mouse

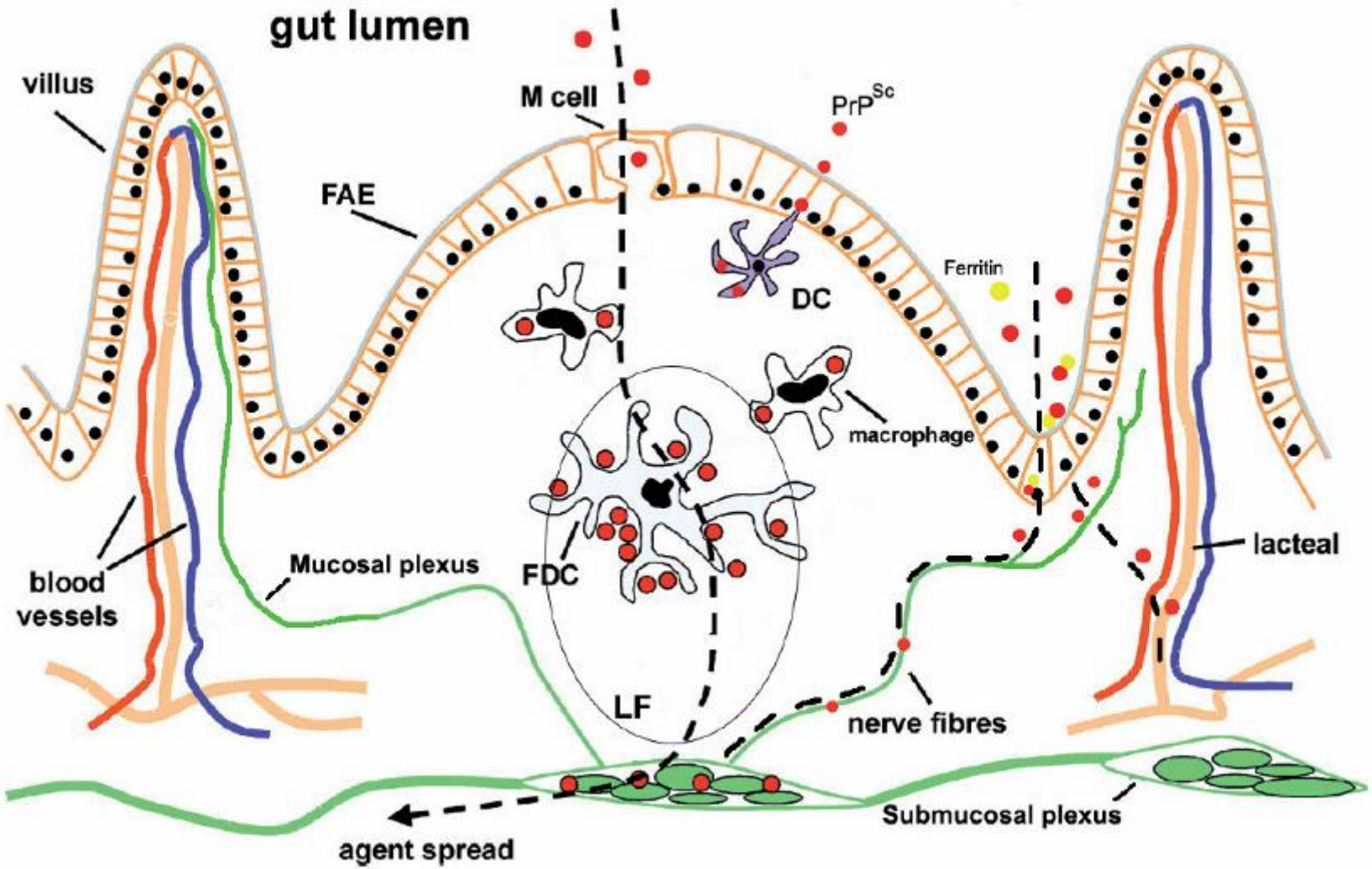
PrP gene polymorphisms

- modulate breed susceptibility
- guide eradication programs

No demonstrable connection with CJD

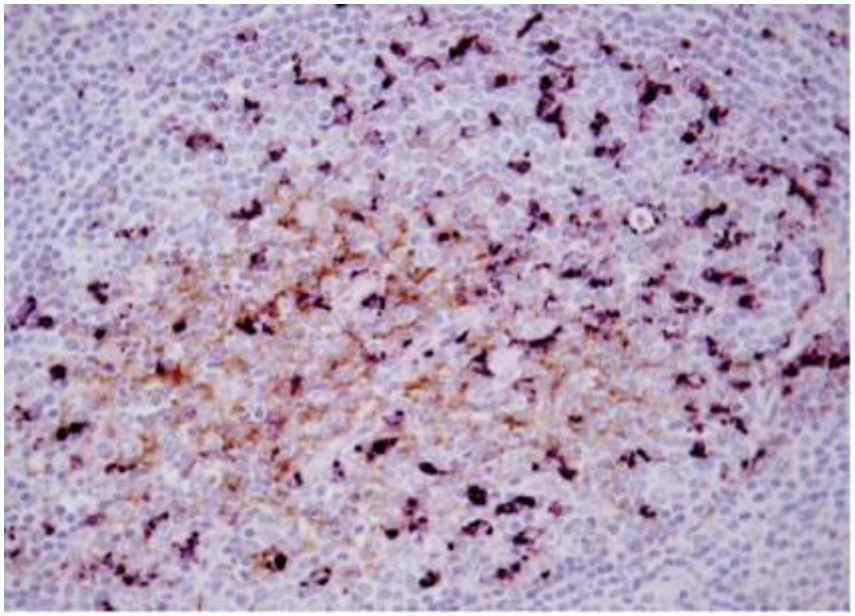
Mouse^{Sc} and hamster^{Sc}

Scrapie pathogenesis

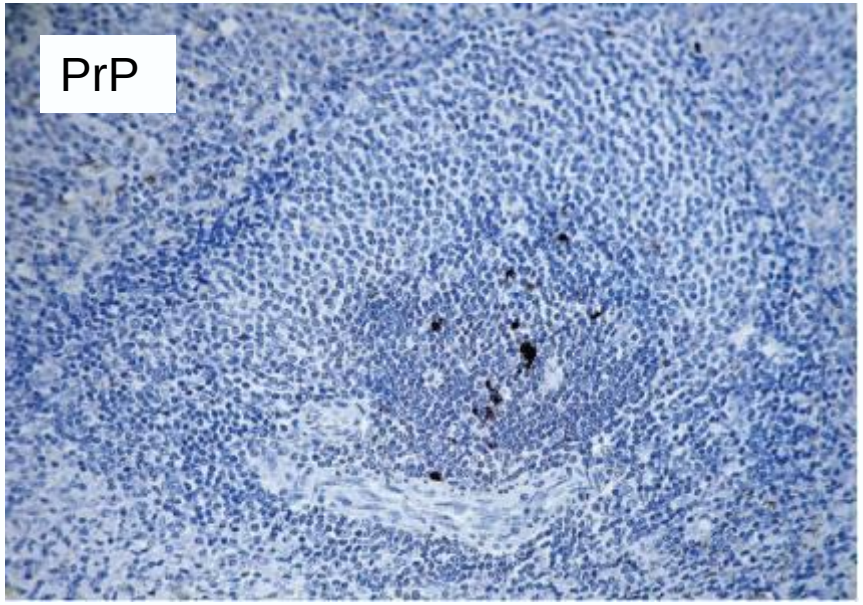


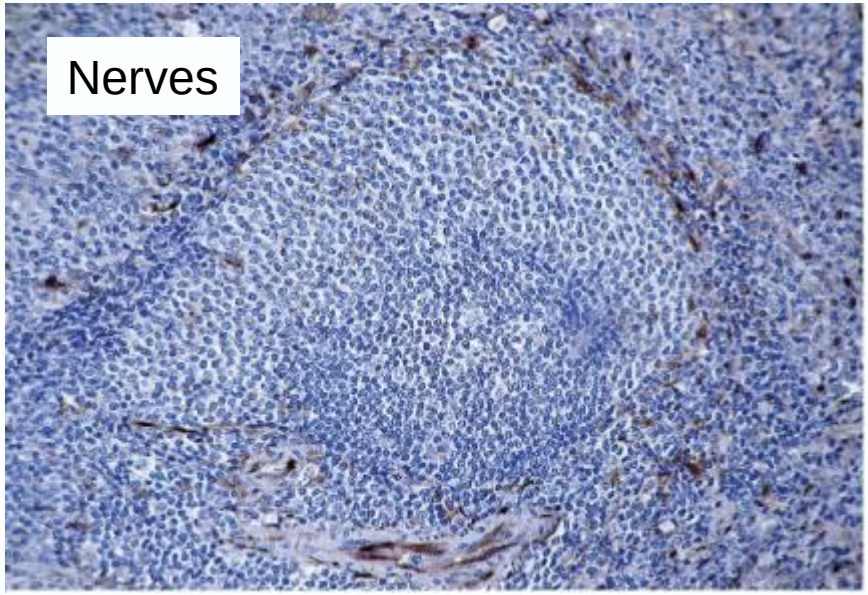
Van Keulen *et al* 2008

Scrapie pathogenesis



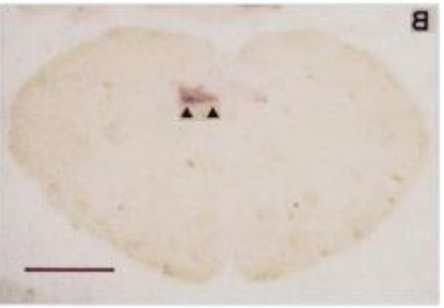
Peyers Patch – TBM/FDC



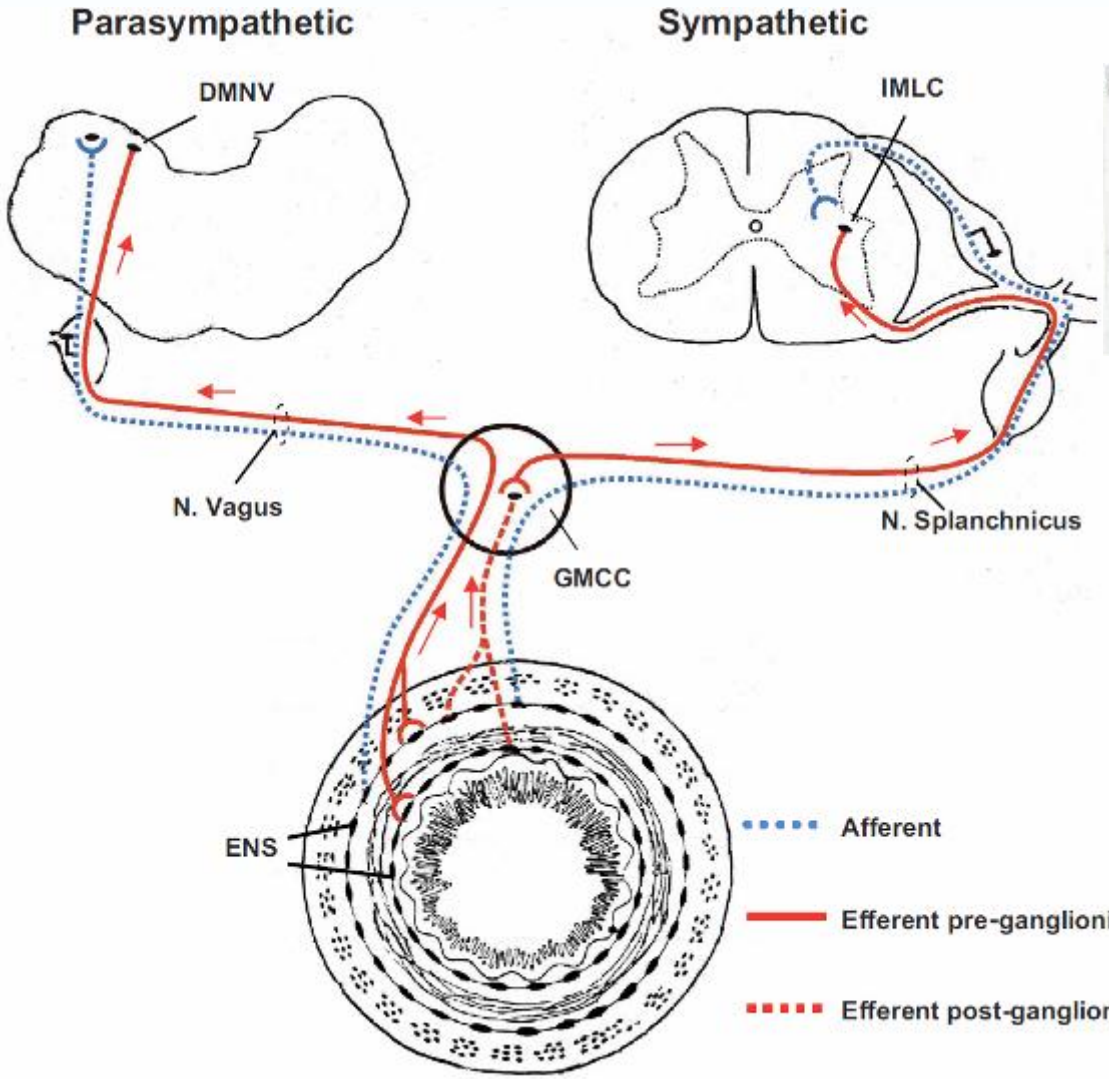


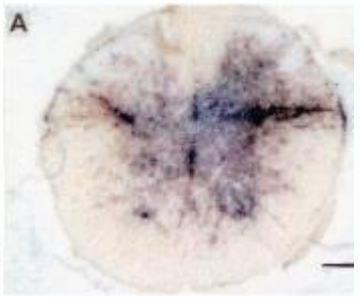
Siso *et al* 2010

Scrapie pathogenesis



McBride *et al* 2001

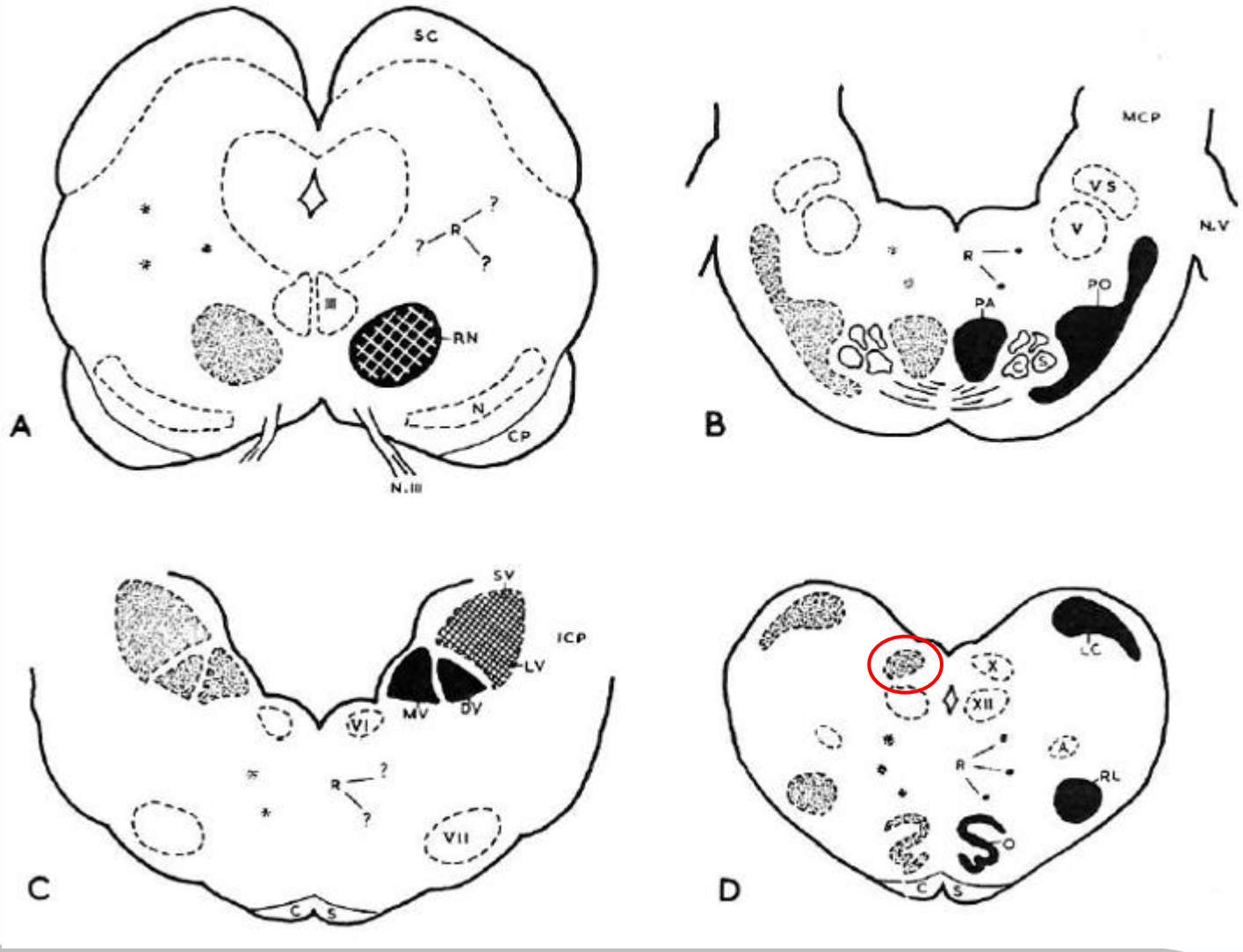




McBride *et al* 2001

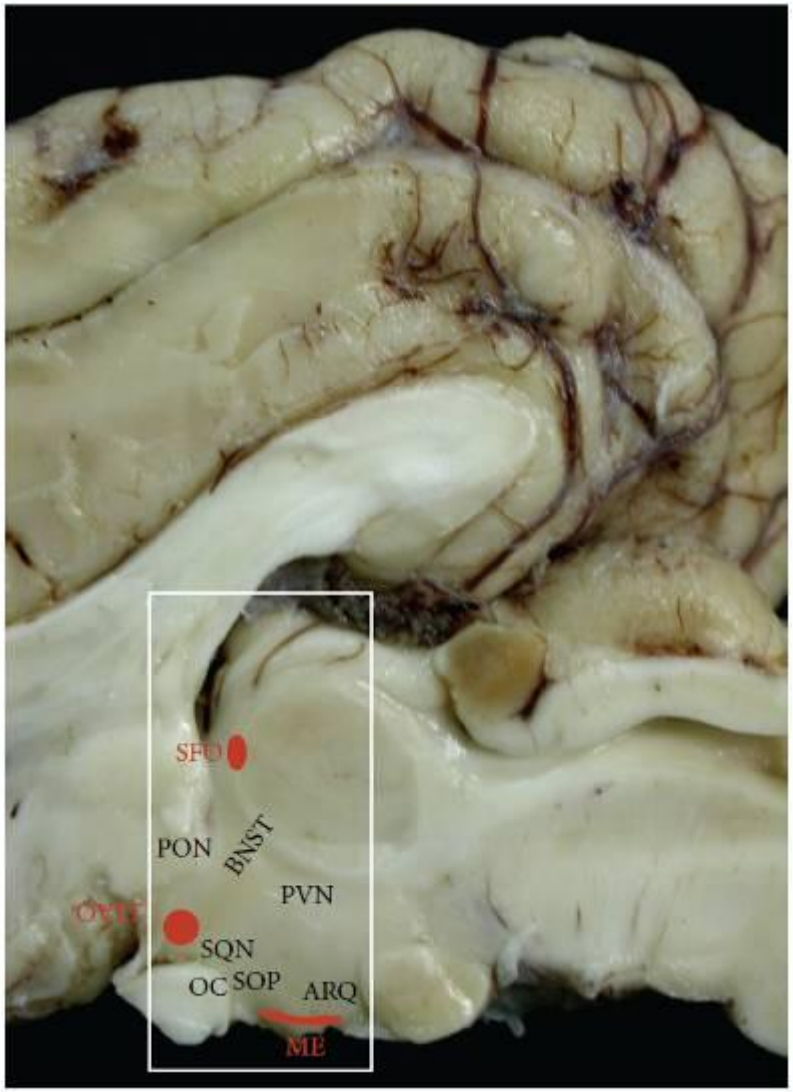
Van Keulen *et al* 2008

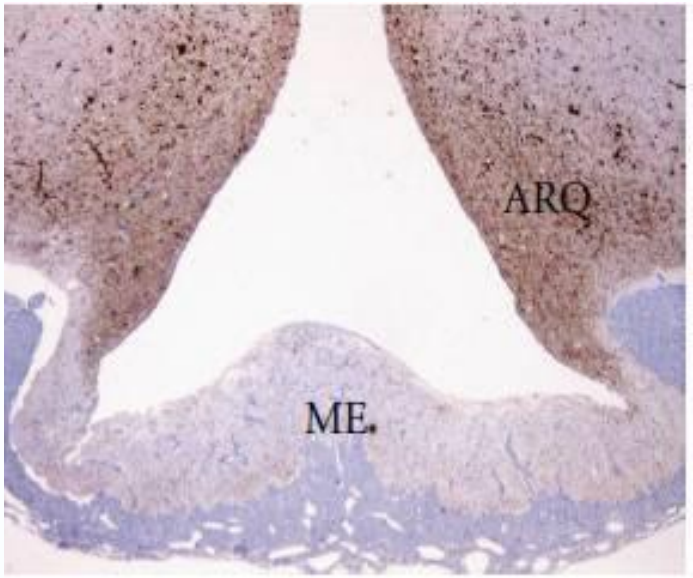
Scrapie pathogenesis

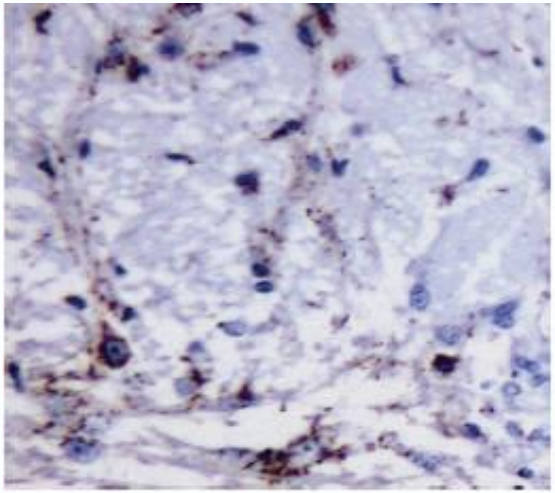


from *Scrapie disease in sheep - Parry 1983*

Scrapie pathogenesis – circumventricular organs







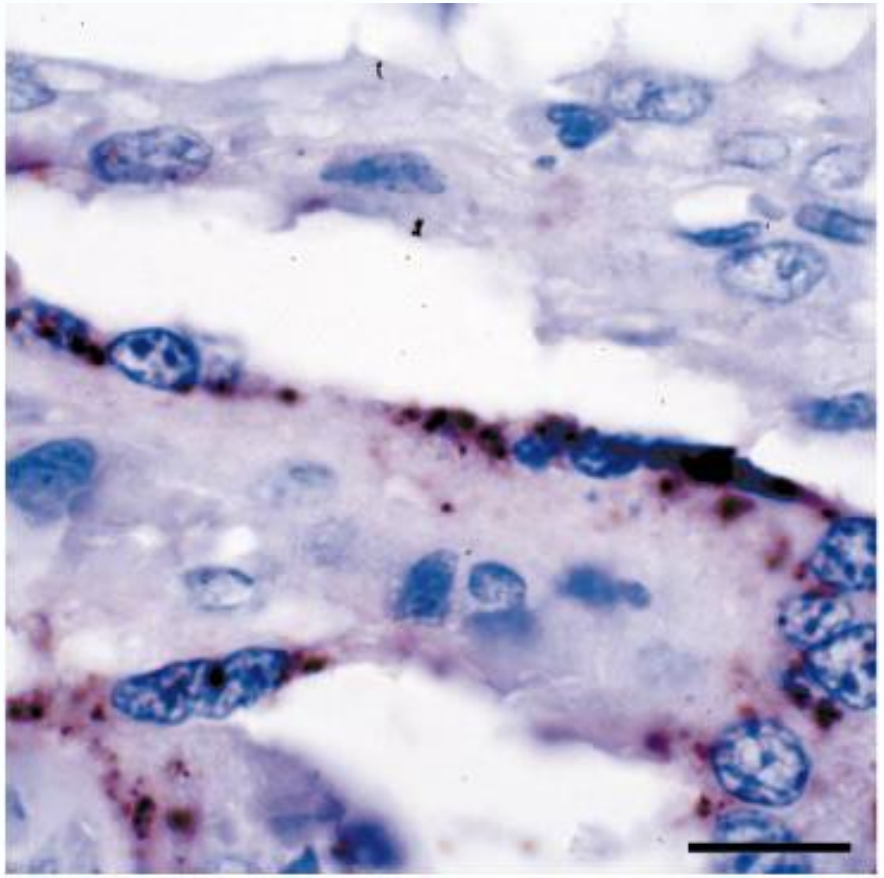
Siso *et al* 2010

Scrapie transmission

Correlates with LRS involvement

- Horizontal transmission
  - oral cavity
    - saliva - salivary gland
  - renal papillae*
  - feces
  - skin
  - **milk - mammary lymphoid tissue**
  - **fetal placenta (trophoblasts)**
    - **via maternal blood**

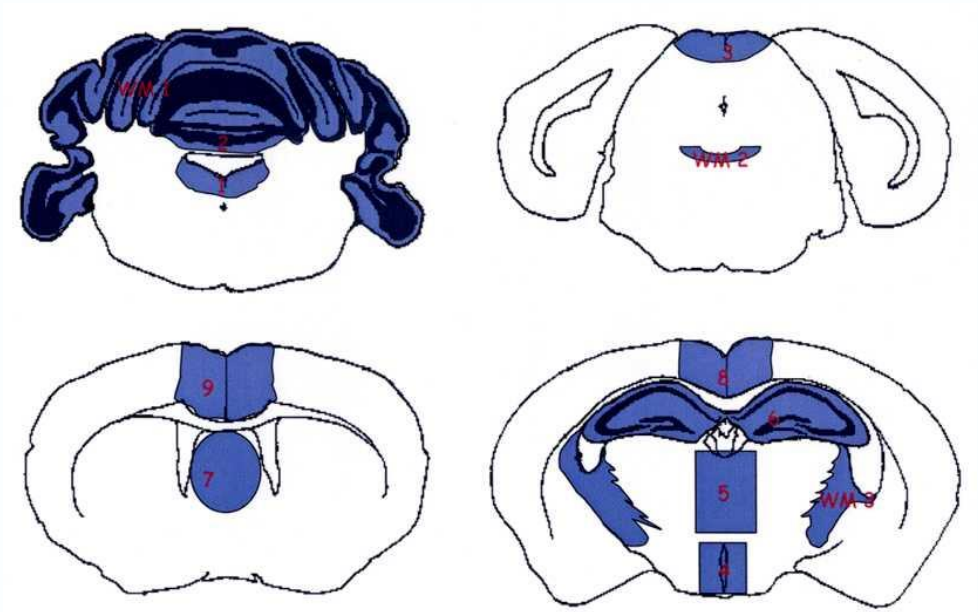
- Environmental reservoirs
  - soil ?

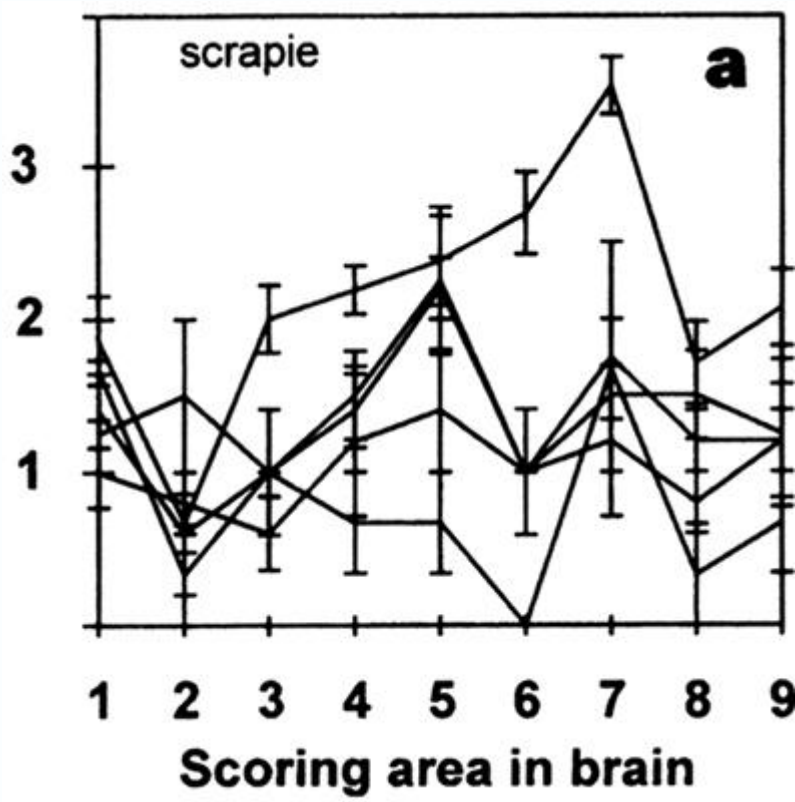


Andreolotti *et al* 2002

Prion strains

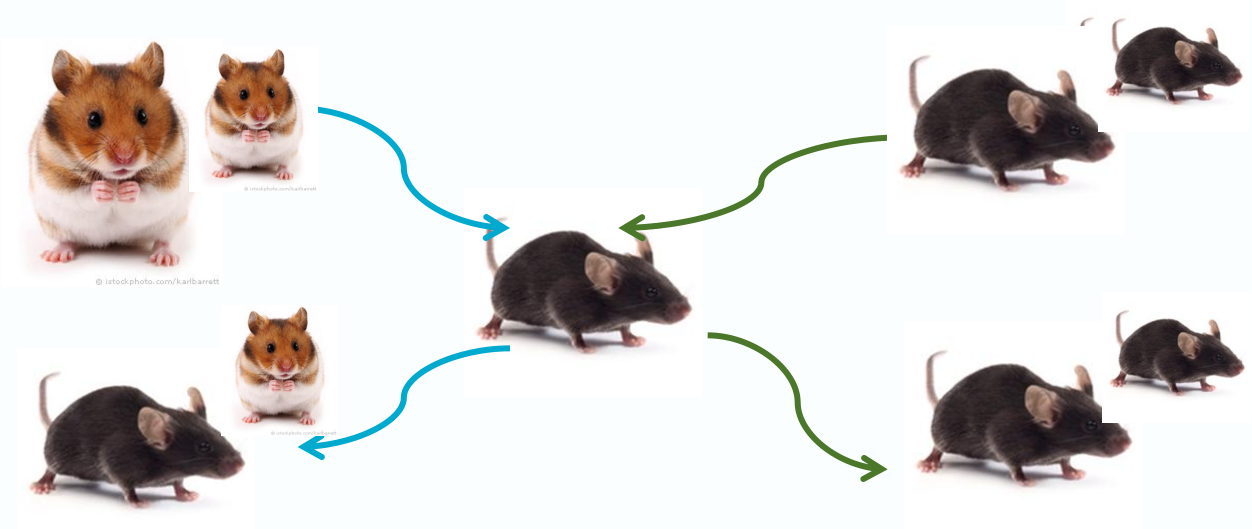
- Defined by incubation period and pathology
  - Goat scrapie – “hyperactive” and “drowsy” forms
    - Inbred mouse strains – C57Bl6 and VM





Prion strains

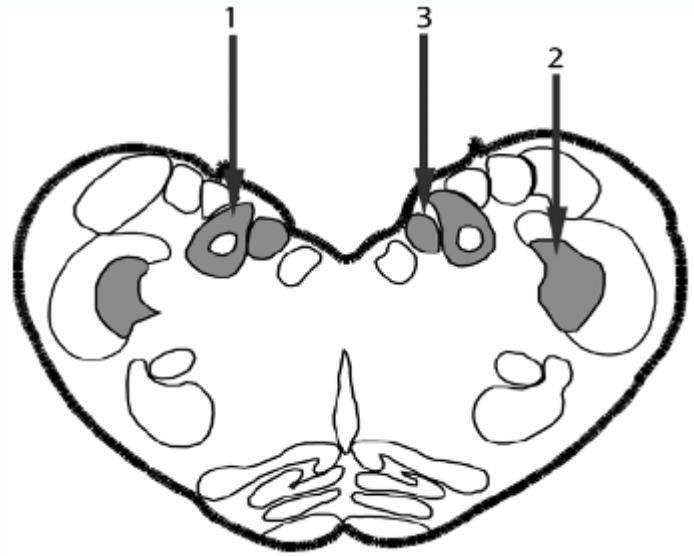
- Characteristics are conserved on serial transmission
  - Identical sequence to host → short IP…….. “species barrier”
- Enciphered on the PrP^{Sc} structure
  - Competition and selection …….transformation and adaptation in new hosts
  - Tg mice expressing both SHaPrP and MoPrP

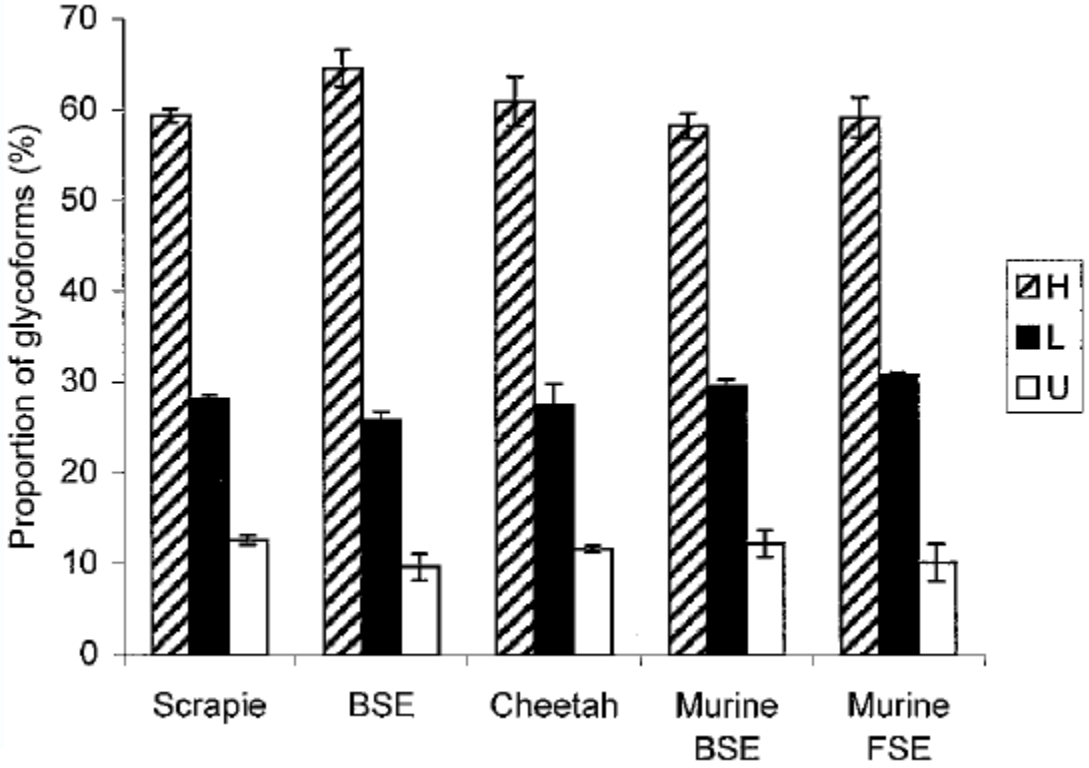


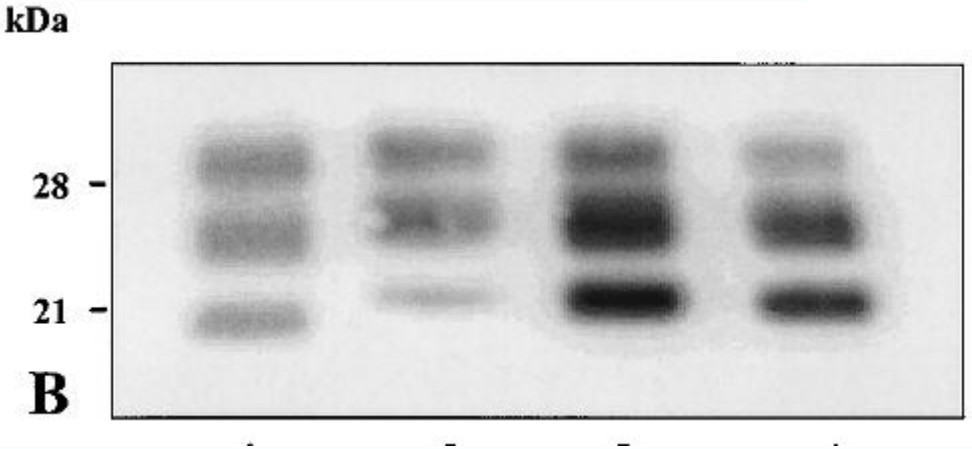
BSE

- 1986 – BSE described in UK cattle
- 1988 – linked to feeding MBM to dairy calves
- Altered rendering methods in late ‘70s
  - Higher fat content
- > 180,000 cases, IP 5 yrs
  - > 3M entered human food chain
- ? Origin

BSE







- Similar glycoform to some scrapie strains
- Unglycosylated band lower

- Single strain type in mice

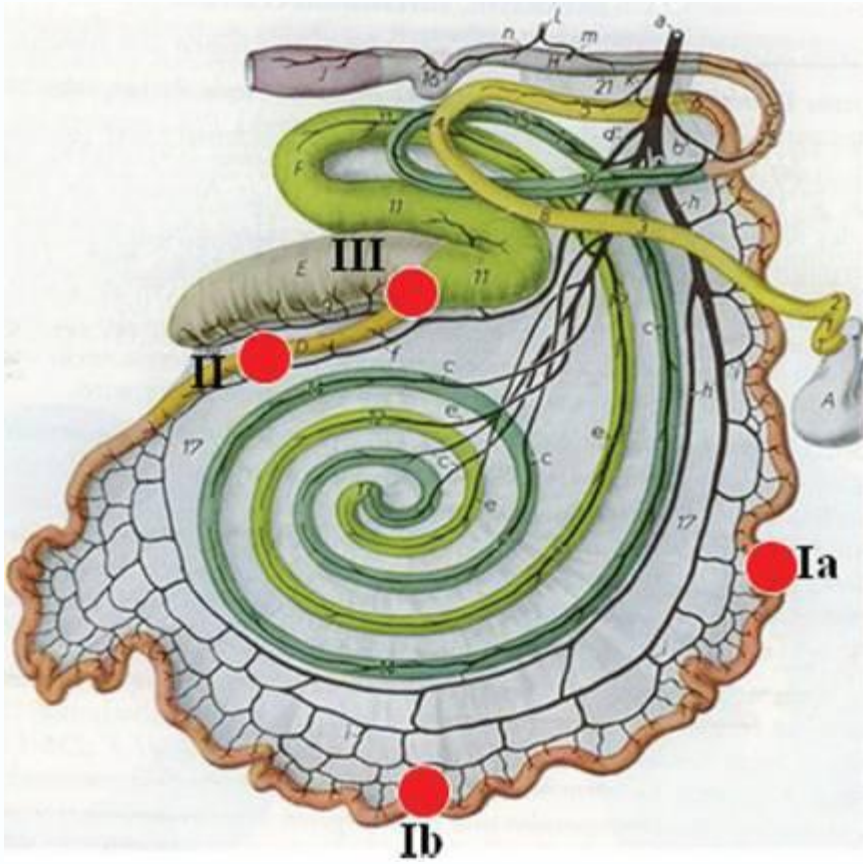
Baron and Biacabe 2001

Baron *et al* 1999

BSE pathogenesis

Mouse assay inbred / bovinised Tg

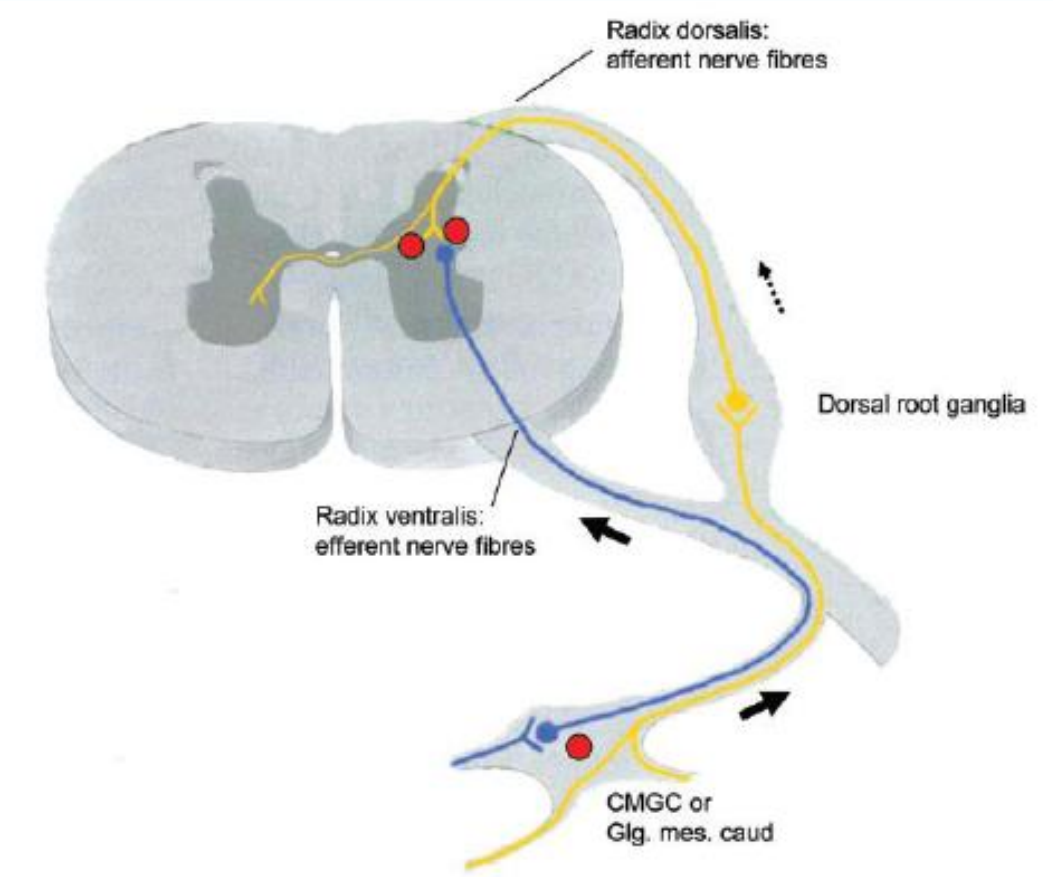
- 4-10m pi
  - Palatine tonsils, enteric lymphoid tissue
- 12m pi
  - TBM, FDC (less than scrapie)
- 16mths pi
  - Enteric nervous system / ANS
  - Not in blood/lymph issues
- 27mths PI
  - Amplification in PNS and CNS
    - brain and **sciatic nerve**
  - M. semitendinosus (Tgbov XV mice)
    - **? Sciatic n terminals**
  - No evidence for neuroinvasion via CVOs



Hoffman *et al* 2011

BSE pathogenesis

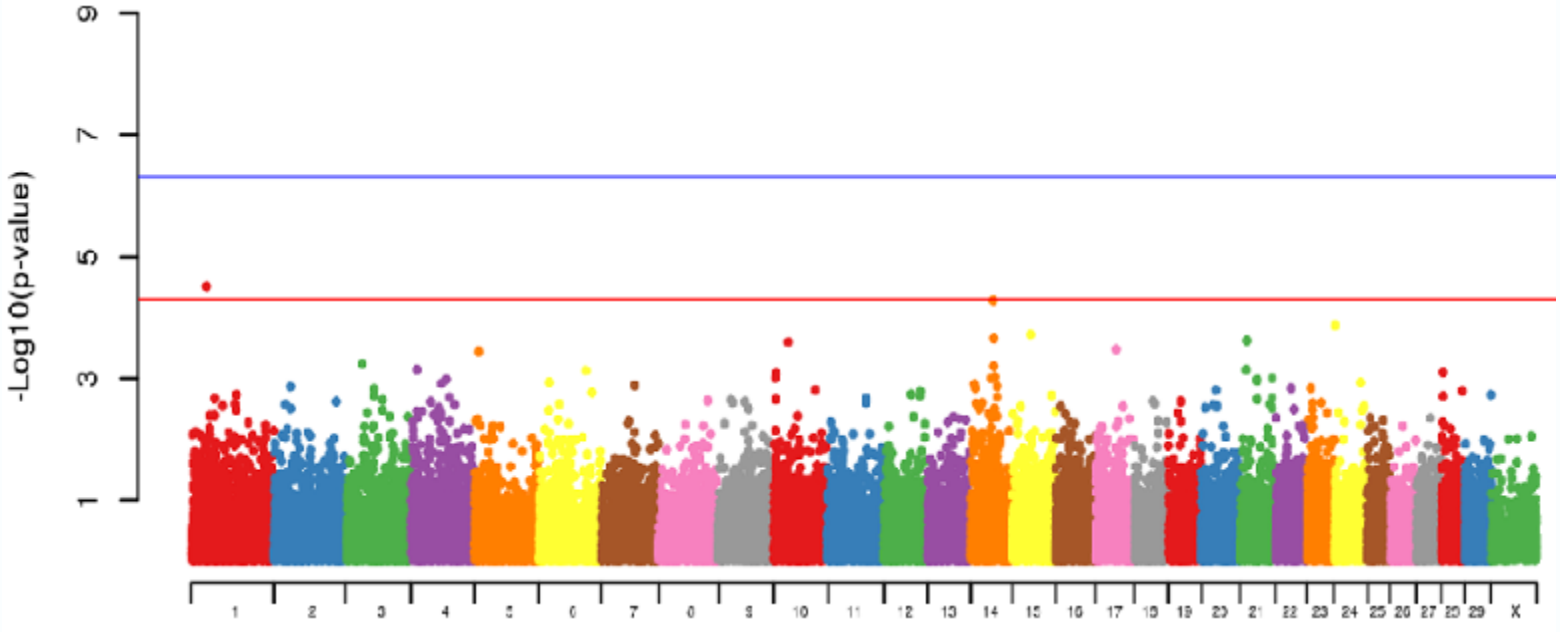
- Centrifugal spread late in disease
  - DRG, tongue, nasal mucosa
    - DRG not involved in IP
    - Coincides with clinical phase



Hoffman *et al* 2007

Genetic predisposition to BSE

- No association with PrnP gene sequence variations
- Whole genome case-control study
  - Age matched half sibs, same calving season, same farm
  - SNP on chromosome 1 encoding a protein involved in basic cellular processes (protein folding)



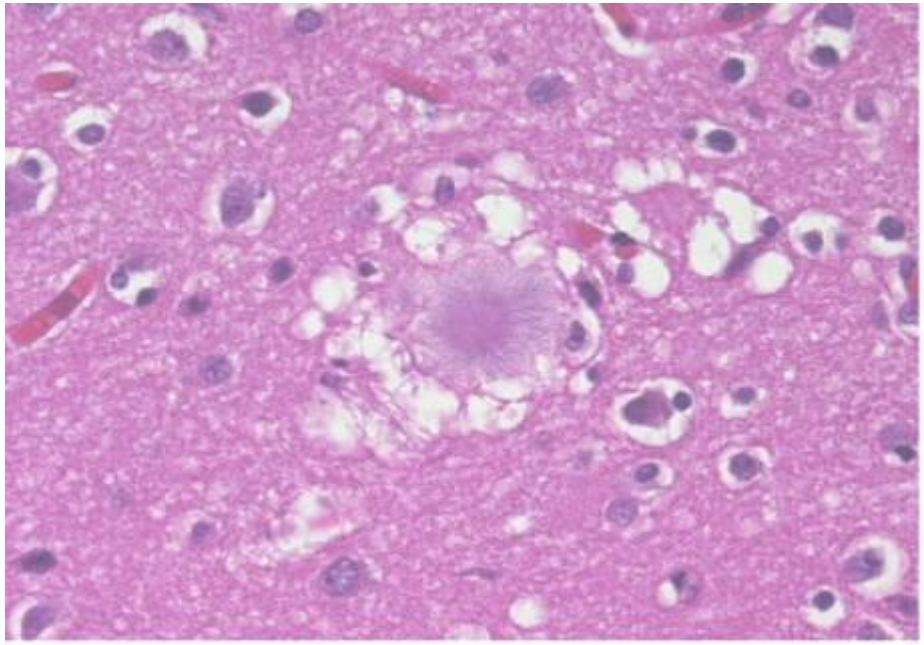
Murdoch *et al* 2011

Emergence of variant CJD - 1996

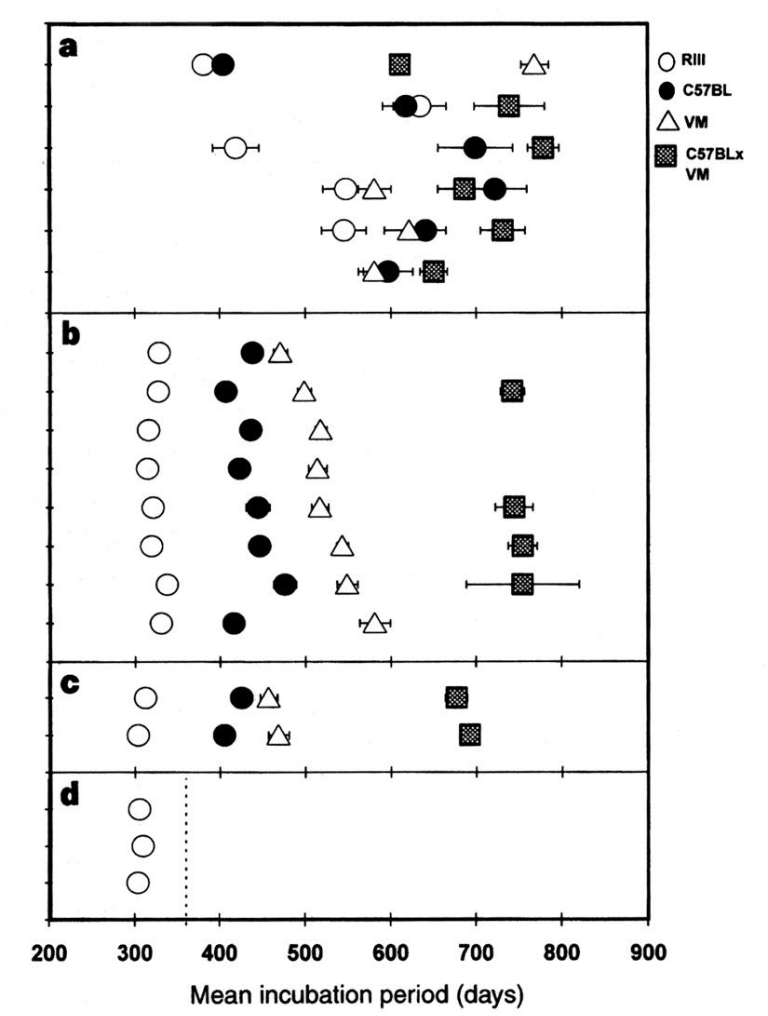
- 175 cases in UK
- 49 cases elsewhere
  - Europe, Japan, North America, Saudi Arabia
  - many epidemiologically linked to UK residence
- 5 suspects still alive

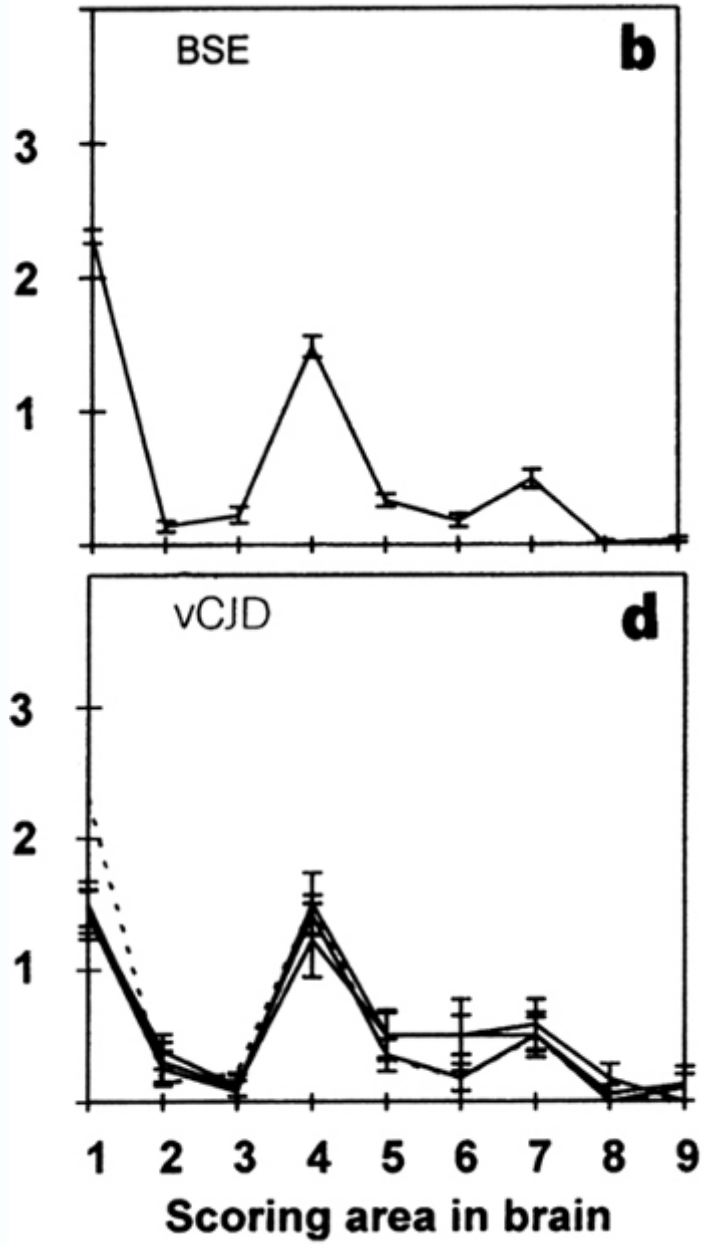
Emergence of variant CJD – spCJD comparison

- Young people - median age at death 28 vs 68 years
- Longer clinical phase – 14mths vs 4.5mths
- Different clinical signs – psychiatric , unsteadiness, involuntary movements, immobile and mute
- Different pathology



Emergence of variant CJD – strain typing





Queniborough, Leicestershire

Control of human exposure

Table IA: High-infectivity tissues

Tissues	Cattle BSE		Sheep & goats Scrapie	
	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}
Brain	+	+	+	+
Spinal cord	+	+	+	+
Retina	+	NT	NT	+
Optic nerve[2]	+	NT	NT	+
Spinal ganglia	+	+	+	+
Trigeminal ganglia	+	+	NT	+
Pituitary gland[3]	-	NT	+	+
Dura mater[5]	NT	NT	NT	NT
Lymphoreticular tissues				
Spleen	-			
Lymph nodes	-			
Tonsil	+			
Nictitating membrane	+			
Thymus	-			
Alimentary tract[5]				
Esophagus	-			
Fore-stomach[6] (ruminants only)	-			
Stomach/ abomasum	-			
Duodenum	-			
Jejunum[7]	-			
Ileum[7]	+			
Appendix	NA			
Colon/caecum[7]	-			
Rectum	NT			

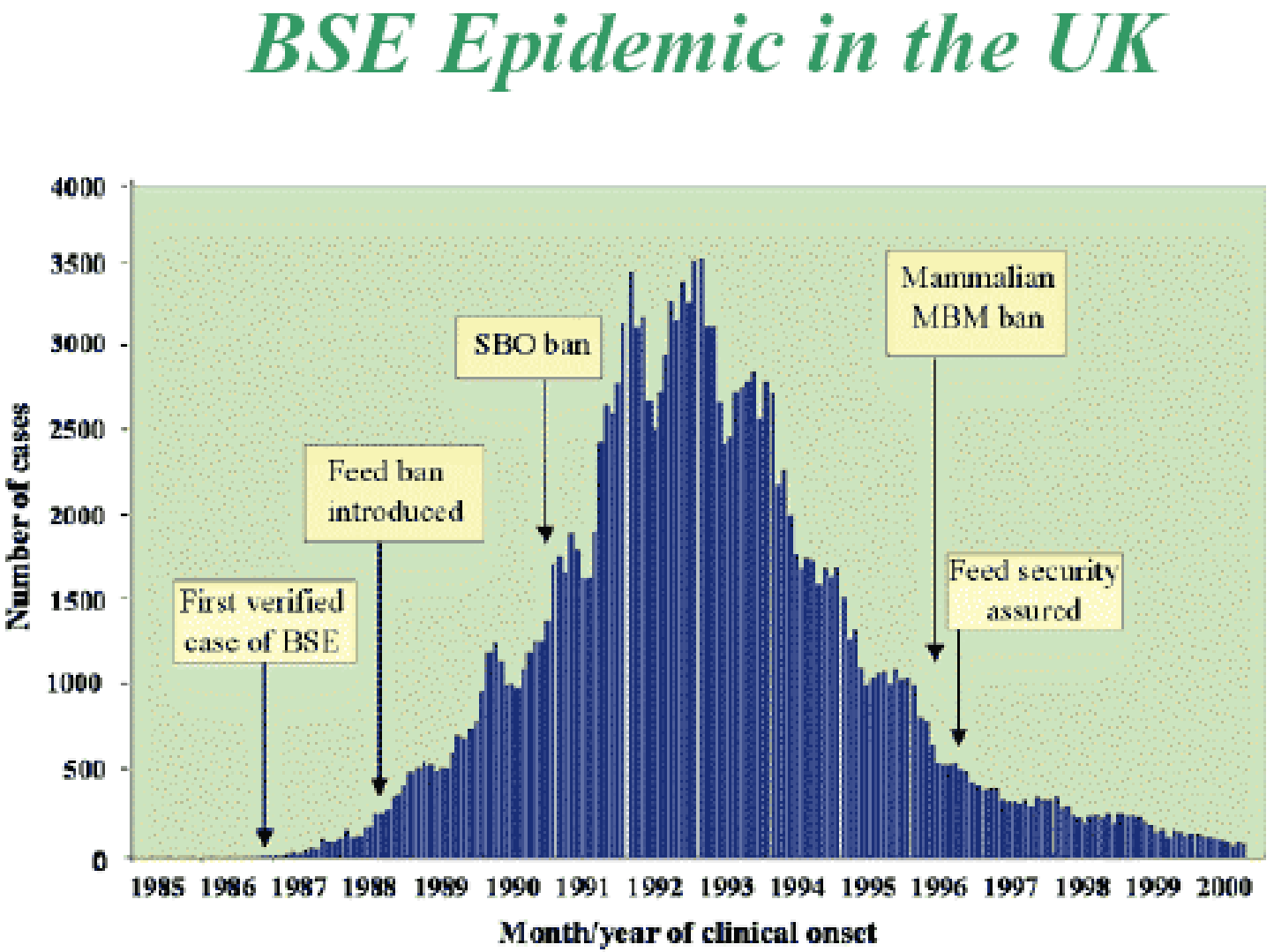
WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies

Control of human exposure

- LRS in variant CJD
  - 3 transfusion – related cases
    - RBC + leucocytes
    - Purified Factor VIII
  - Pharmaceutical industry
    - No bovine materials
    - No natural hosts for TSEs
  - UK imports plasma since 1999
    - Leucocyte depletion
  - Donor deferral scheme in Oz

http://www.justice4andy.com/documents_61.html

Control of animal disease – BARB cases



…….and in Australia – TSE FAP

Ruminant feed bans

- *Ruminant derived MBM (1996)*
- *Mammalian materials (1999)*

Disease surveillance scheme

OIE International Animal Health Code

Prohibited imports

- *Stockfeed of animal origin (1966)*
- *Live cattle from UK, Ireland (1988)*
- *Beef and beef products from UK (1996)*
- *Beef and beef products from Europe (2001)*

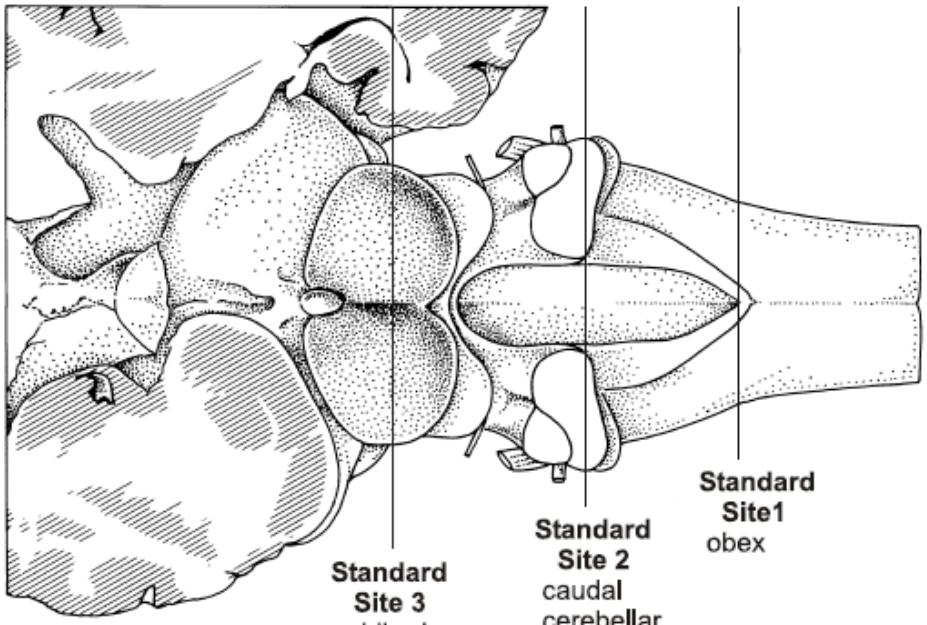
TSE Surveillance - Australia

- OIE BSE *Negligible Risk*
  - OIE Type B surveillance
  - One BSE case per 50,000 in adult cattle at a confidence level of 95%.
    - 150,000 surveillance points during a seven-year moving window
      - Investigation of clinical suspects
      - Fallen and casualty slaughter subpopulations are tested (300 pa)
- Scrapie
  - 99% confidence if 1% neurological cases
  - 438 sheep brains

Surveillance subpopulation			
Routine slaughter[1]	Fallen stock[2]	Casualty slaughter[3]	Clinical suspect[4]
Age ≥1 year and <2years			
0.01	0.2	0.4	N/A
Age ≥2 years and <4 years (young adult)			
0.1	0.2	0.4	260
Age ≥4 years and <7 years (middle adult)			
0.2	0.9	1.6	750
Age ≥7 years and <9 years (older adult)			
0.1	0.4	0.7	220
Age ≥9 years (aged)			
0.0	0.1	0.2	45

TSE Surveillance - Australia

- Histology
  - Focus on medulla at level of obex

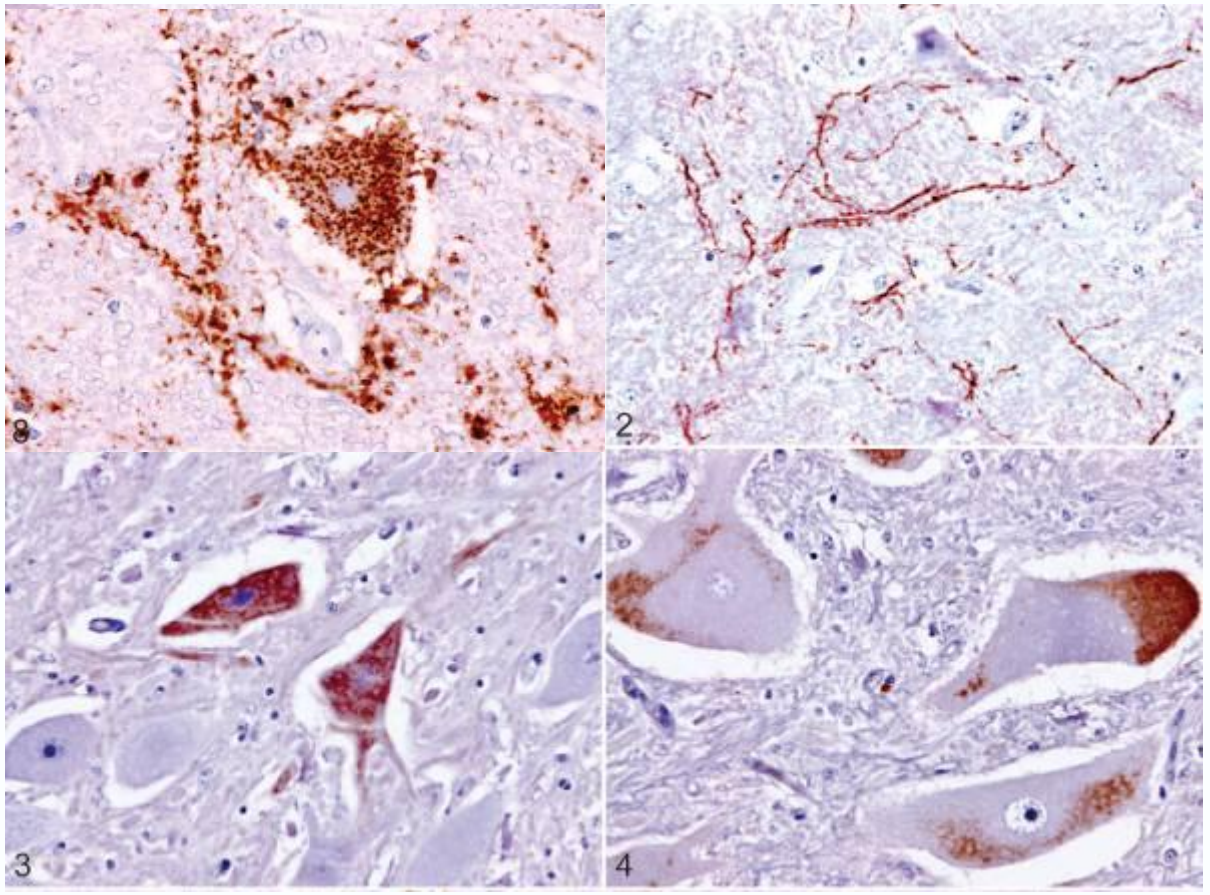


Dorsal view of bovine brainstem and the three standard sites.

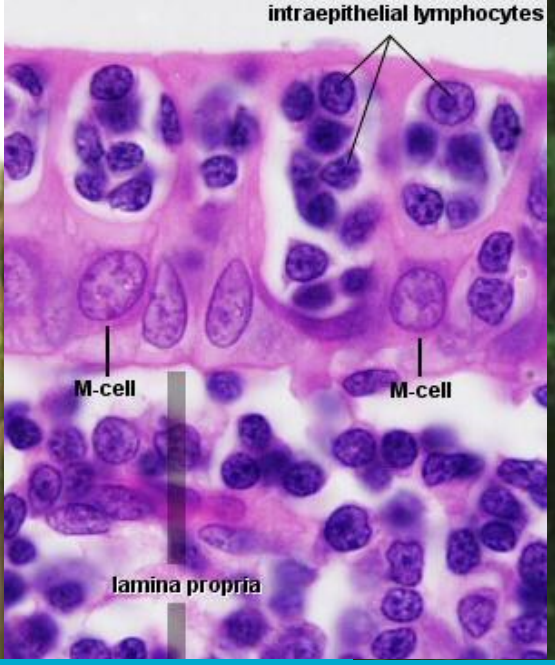
TSE Surveillance - Australia

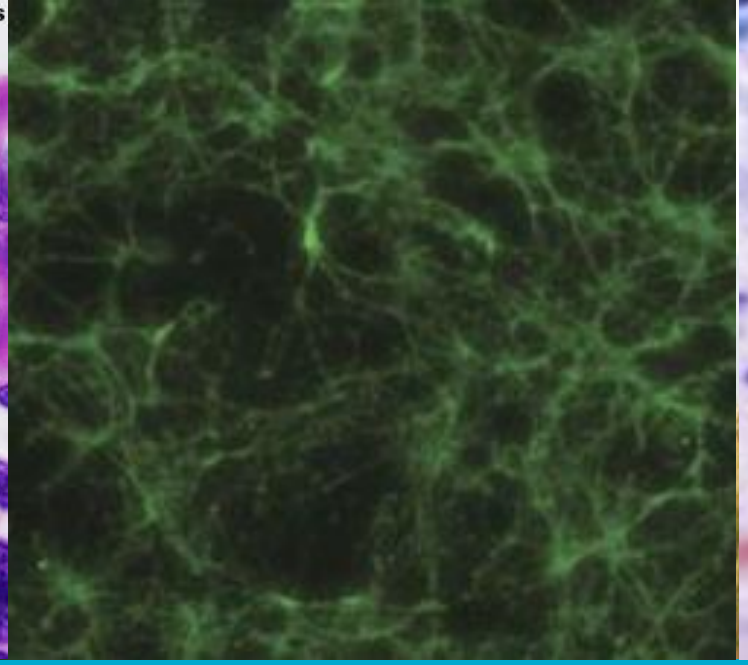
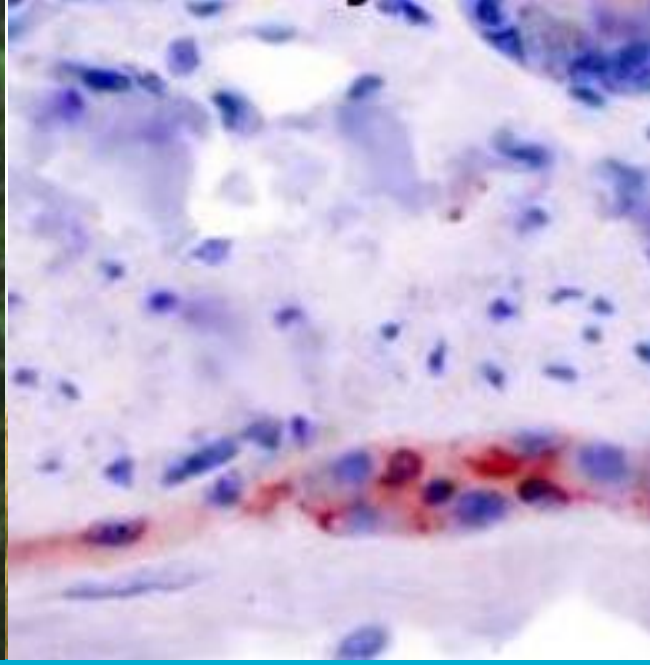
- Immunohistochemistry

Biorad TeSeE, Prionics WB



Simmons *et al* 2010



Prions

Atypical scrapie, atypical BSE, and the future

Rapid testing at slaughter in the EU

Cases of scrapie with unusual features in Norway and designation of a new type, Nor98

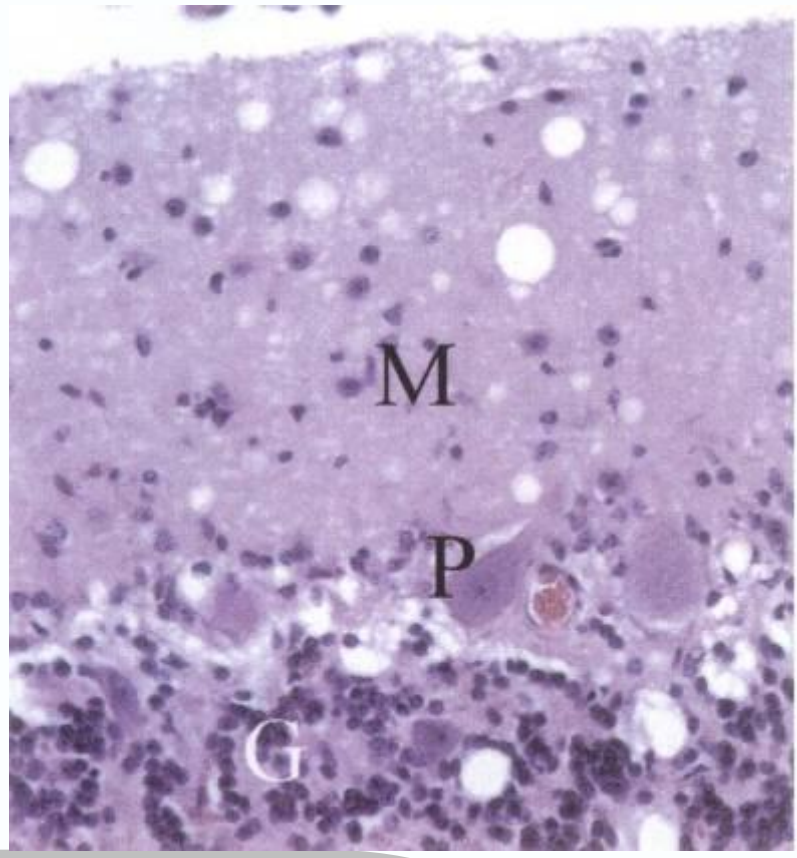
S. L. Benestad, P. Sarradin, B. Thu, J. Schönheit, M. A. Tranulis, B. Bratberg

The **Veterinary Record**, August 16, 2003

- Field cases: Progressive ataxia, anxiety, loss of condition
  - occurred in flocks without scrapie
  - older sheep
  - often only one case in a flock

- Vacuolation
  - cerebral and cerebellar cortices
  - basal ganglia,
  - thalamus
  - less in midbrain
    - substantia nigra

- No lesions at obex
- Few neuronal vacuoles
  - PK sensitive PrP^{Sc}

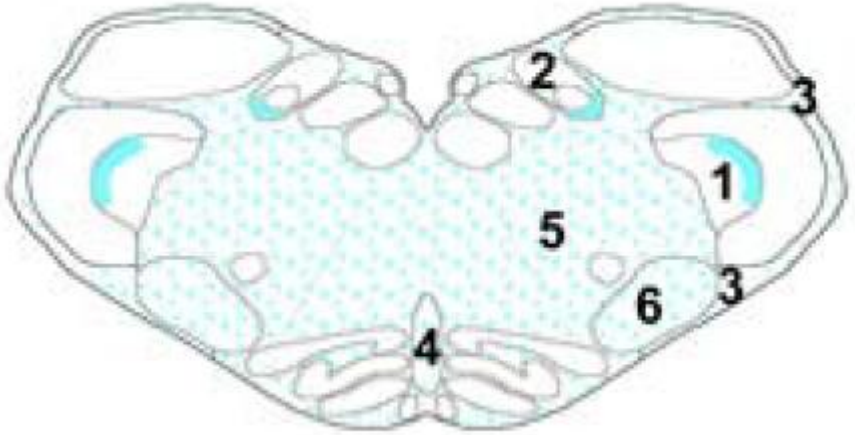


Atypical scrapie - PrP^{Sc}

- Cerebral and cerebellar cortices
  - white matter
- Not intracellular
  - neurones
  - glia
- DMNV not affected.

- Biorad ELISA +ve
- Prionics WB –ve on medulla
- OIE WB +ve

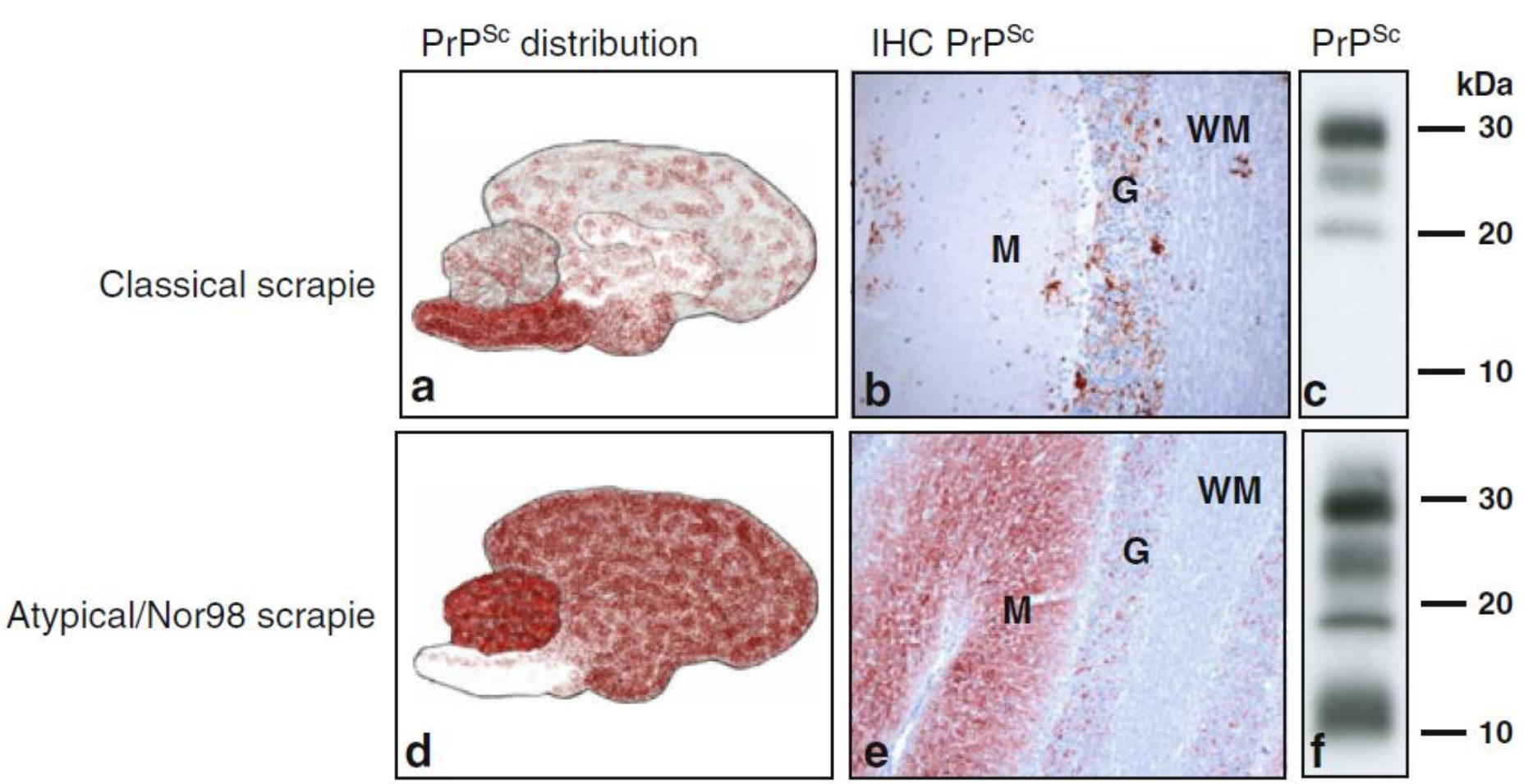
Number	Neuroanatomical area
1	Spinal tract of trigeminal nerve
2	Solitary tract
3	Spinocerebellar tract
4	Nucleus raphe magnus
5	Reticular formation
6	Spinothalamic tract



Lymphoid tissues negative for PrP^{sc}

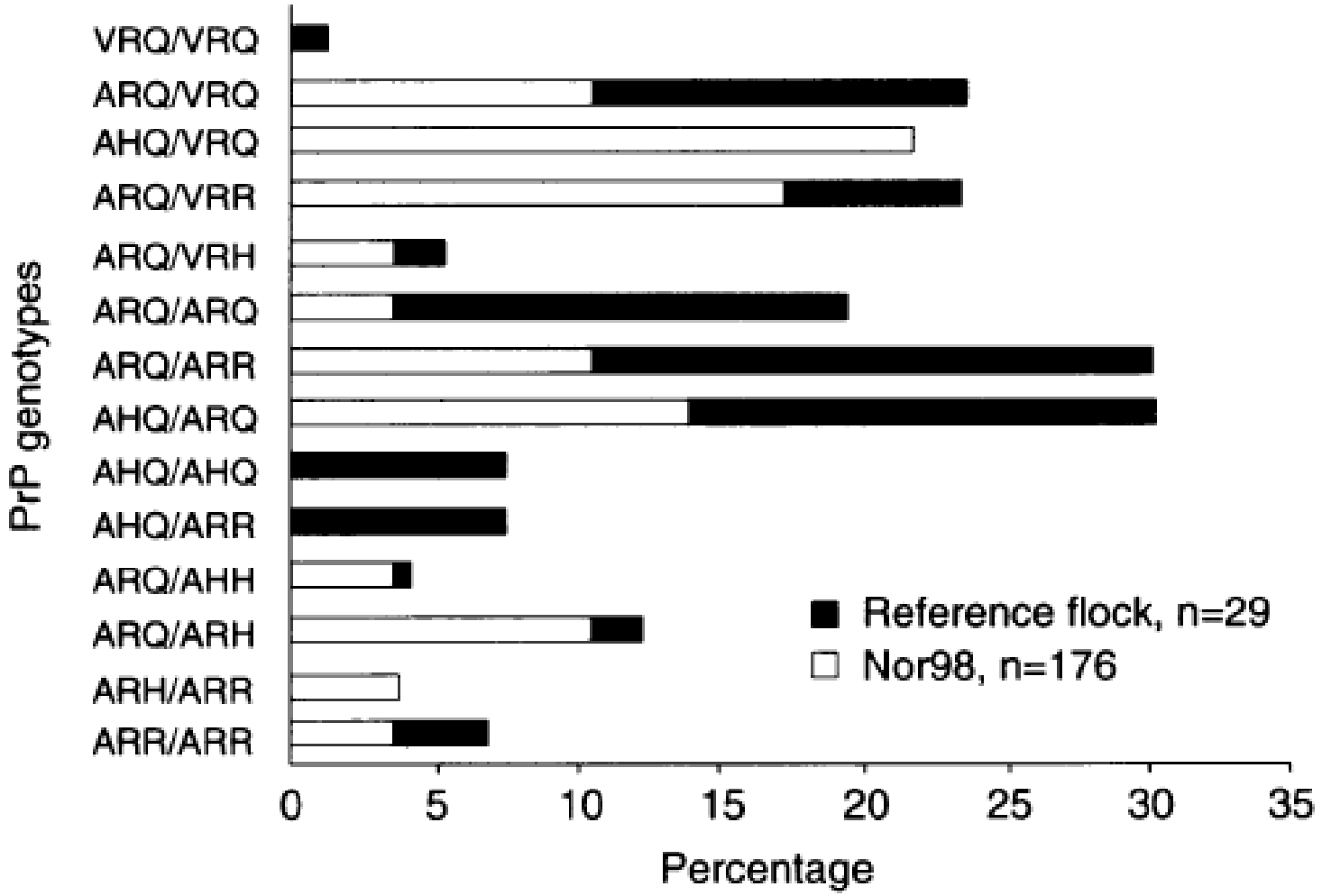
Moore *et al* 2008 Acta Neuropath

Atypical scrapie - PrP^{Sc}



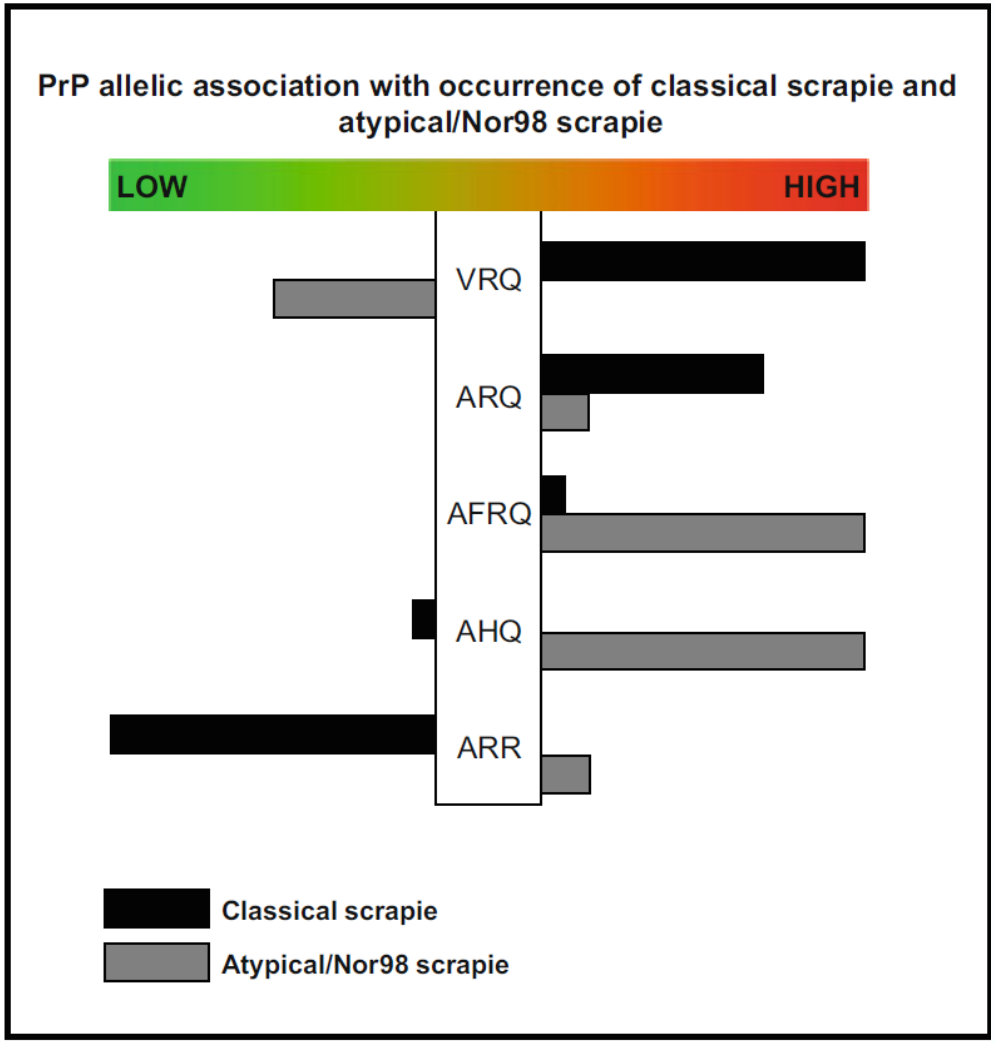
Tranulis *et al* 2011

Atypical scrapie – flock genotype



Benestad *et al* 2003

Atypical scrapie – animal genotype



Tranulis *et al* 2011

Atypical scrapie – transmission

No

- Inbred mice RIII, C57Bl, VM I/C

Yes

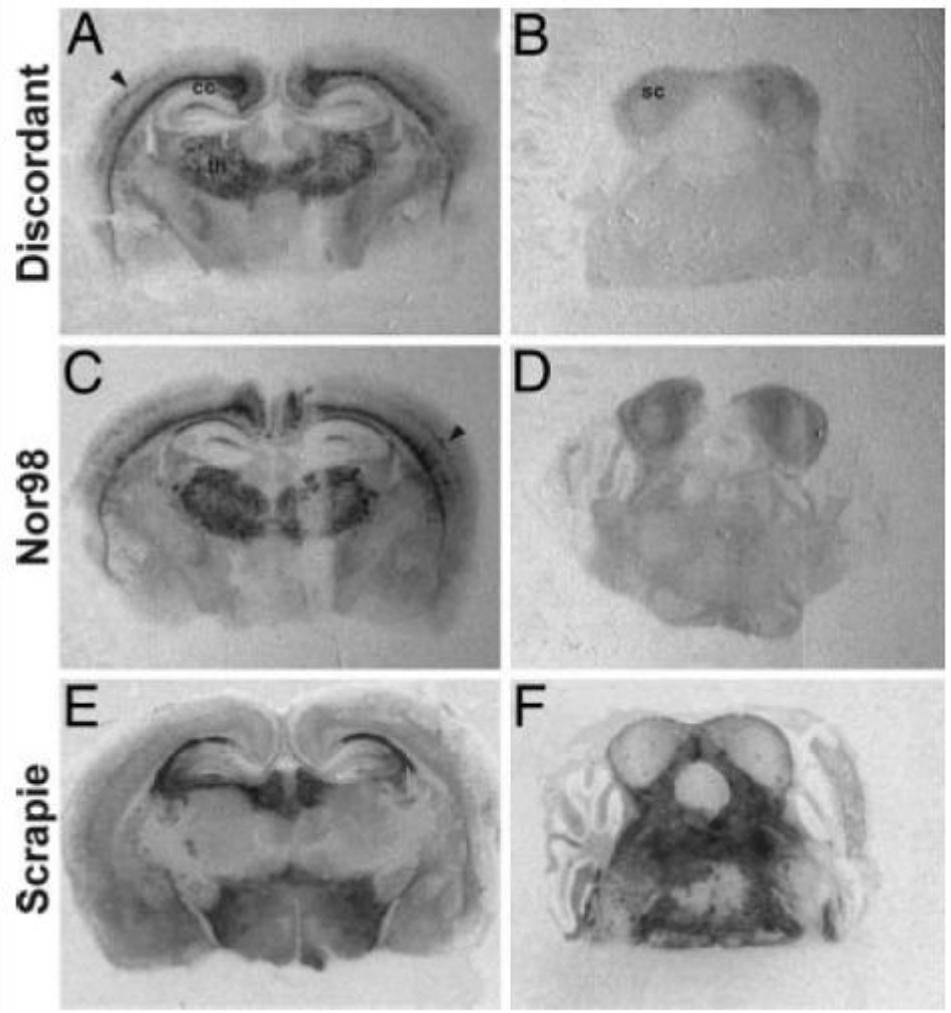
- Ovinised mice
- I/C and orally to sheep

One strain type

- not BSE
- few cases well studied

Challenge to selective breeding programs

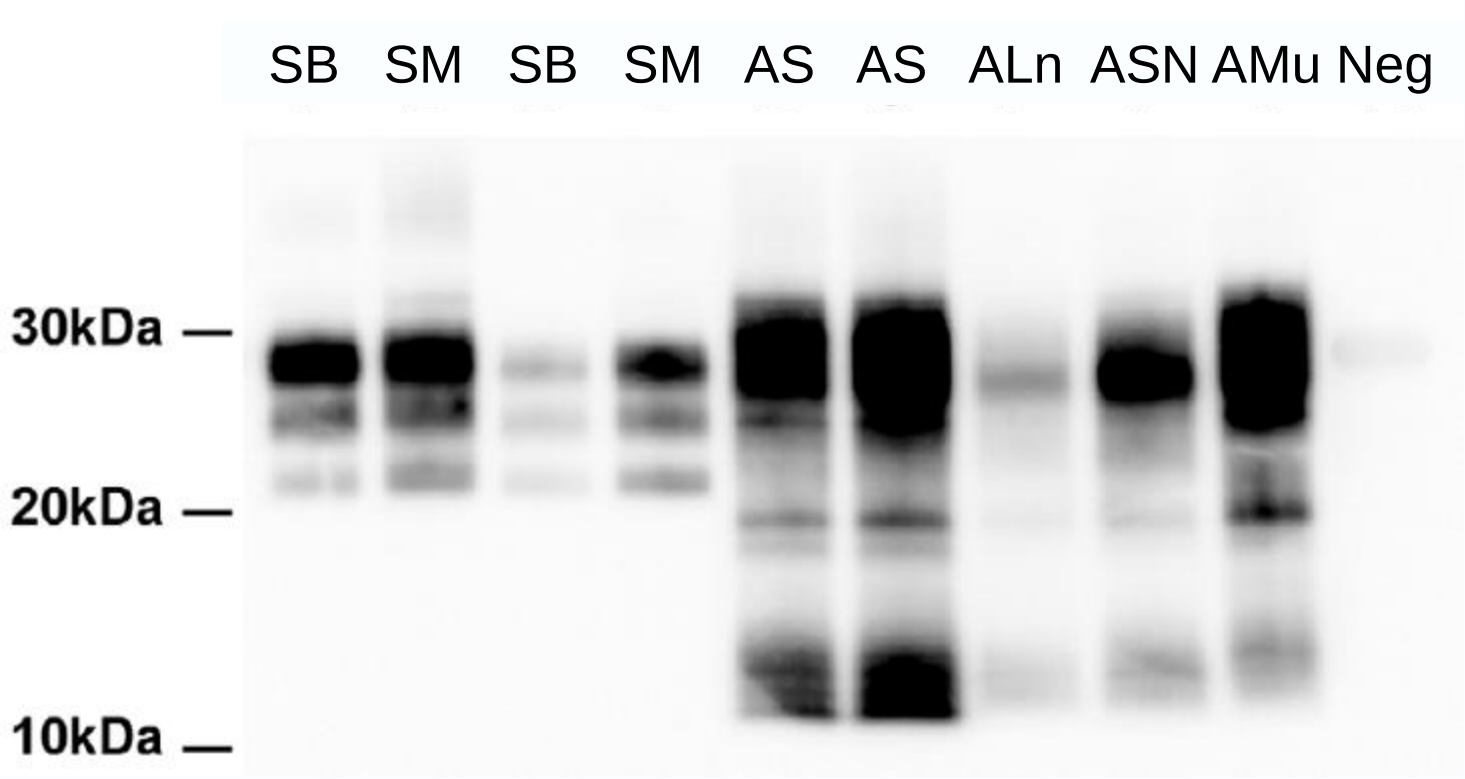
- ? zoonotic risk



LeDur *et al* 2005

Atypical scrapie – non-nervous tissues

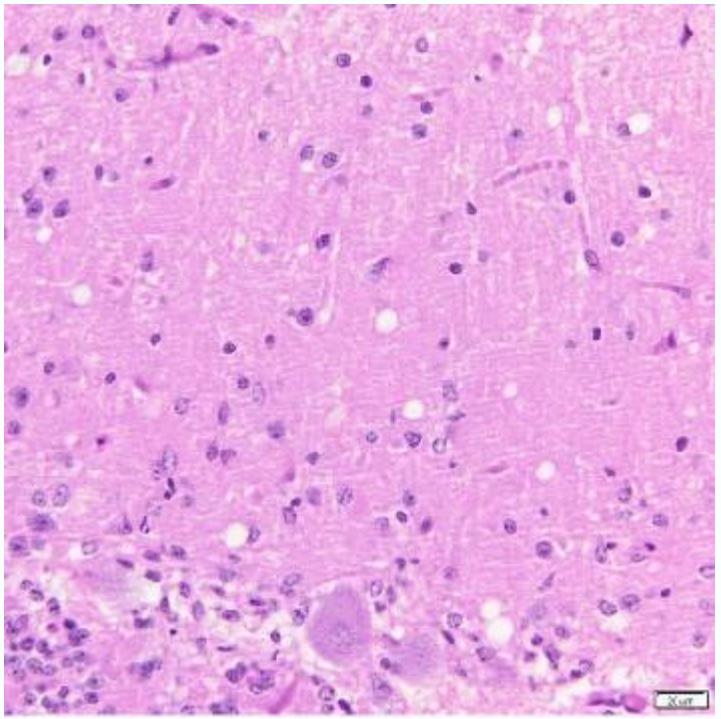
- Ovinised mice
  - PrP^{Sc} not detected in donor sheep tissue
    - PrP^{Sc} dependent surveillance Pathogenesis

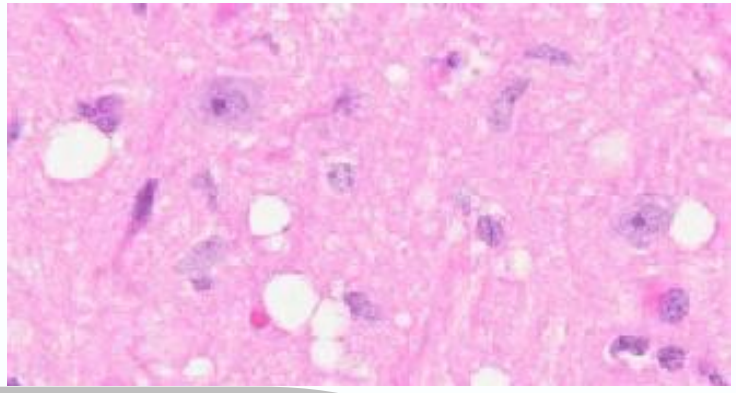


Andreolotti *et al* 2011

Atypical scrapie – surveillance in Australia

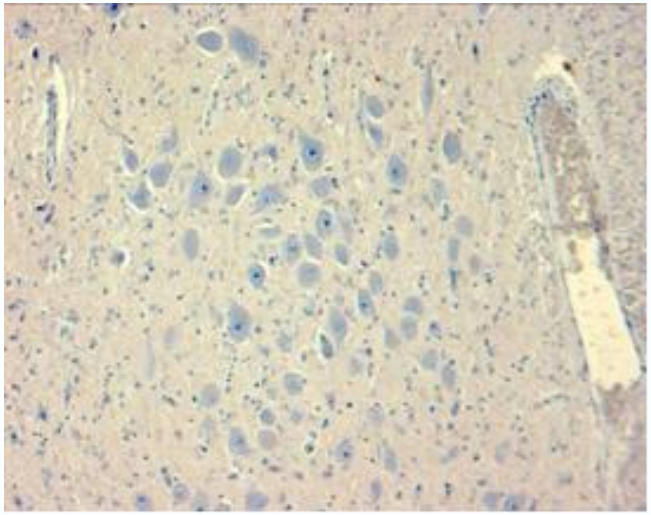
- May be overlooked if
  - surveillance is obex-focussed
  - IHC used to confirm +ve ELISA
- Case 1
  - WA – merino wether for live export “circling”
    - Vacuolation in cerebellar cortex
- Case 2
  - Vic – 5yo ewe- ataxia, loss of condition
    - Vacuolation in cerebellar cortex, cerebellar wm and substantia nigra
- Not an OIE listed disease

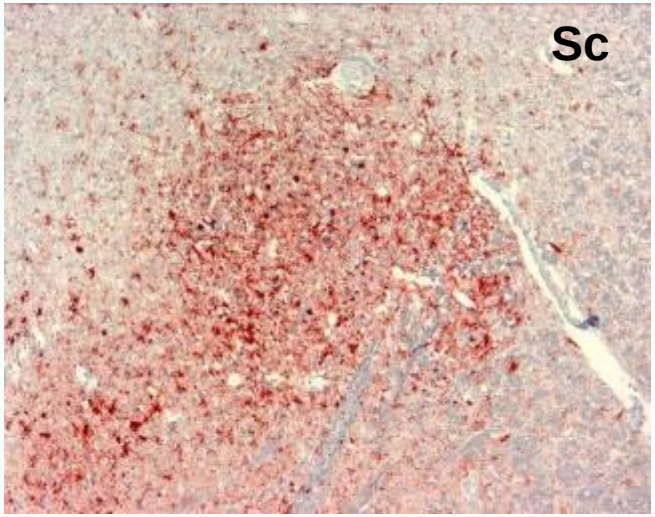




Atypical scrapie – surveillance in Australia

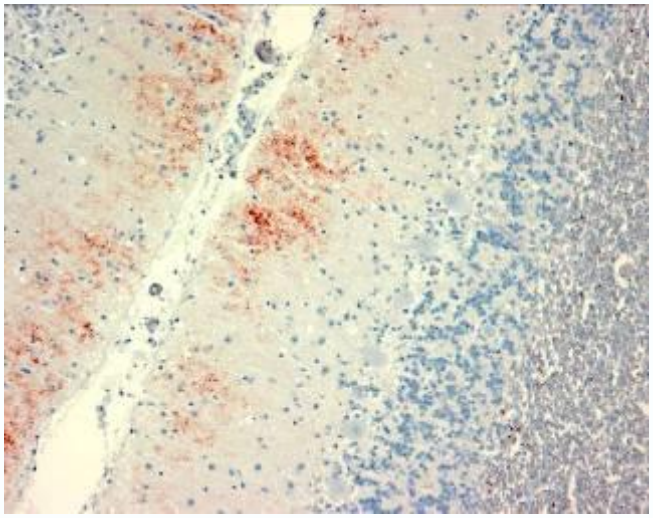
Case 1 DMVN

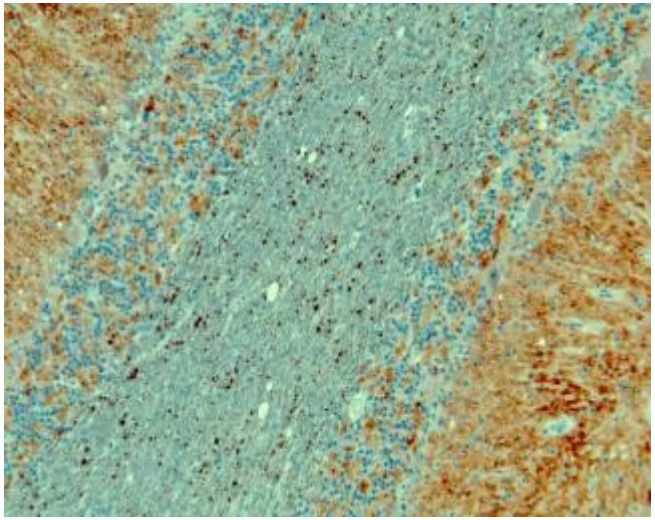




Case 2

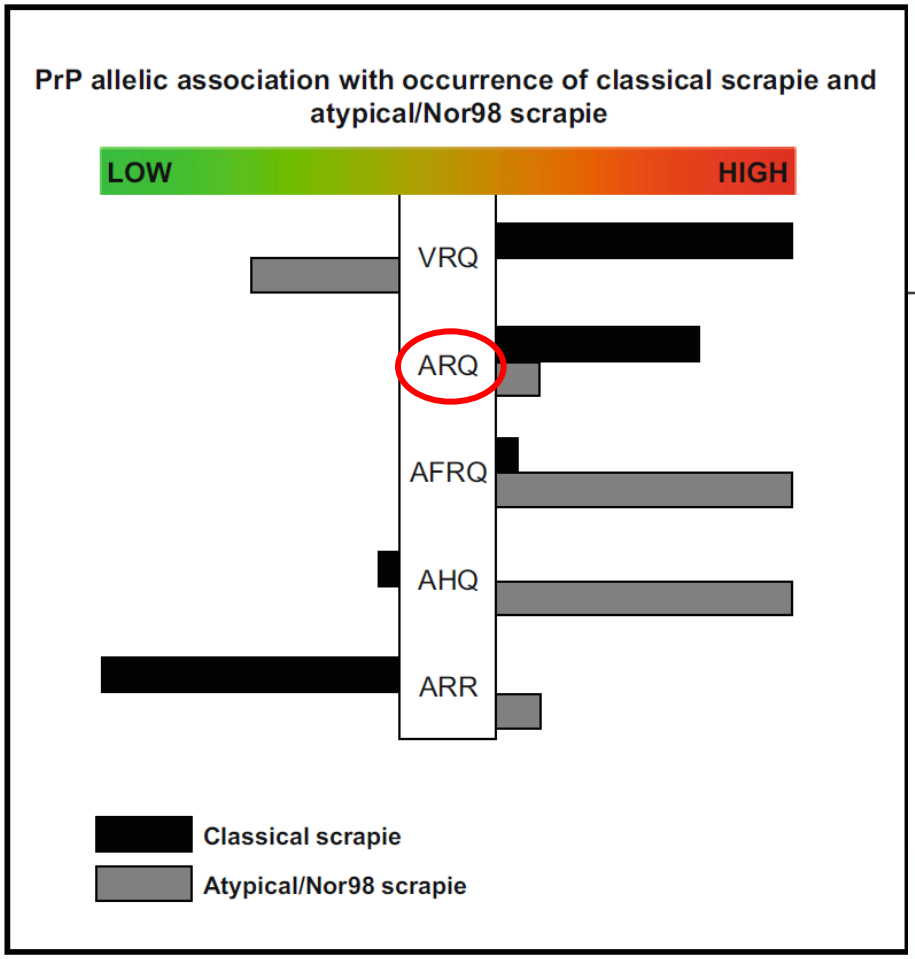
Cerebellum





Atypical scrapie – genetic susceptibility

PrP codon			Aus/M1 (%)
136	154	171	
VA	RR	QQ	5
AA	RR	QQ	46
AA	HR	QQ	8
AA	RR	RQ	28
AA	HR	RQ	8
AA	RR	RR	5
Number of animals			39



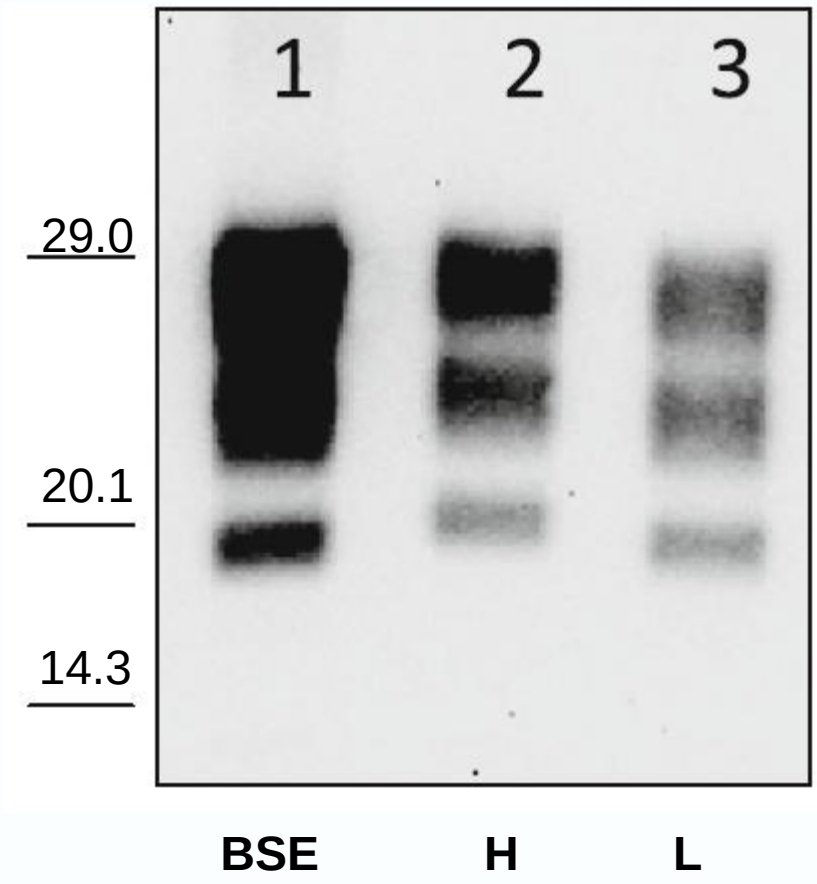
Hunter and Cairns 1998

Tranulis *et al* 2011

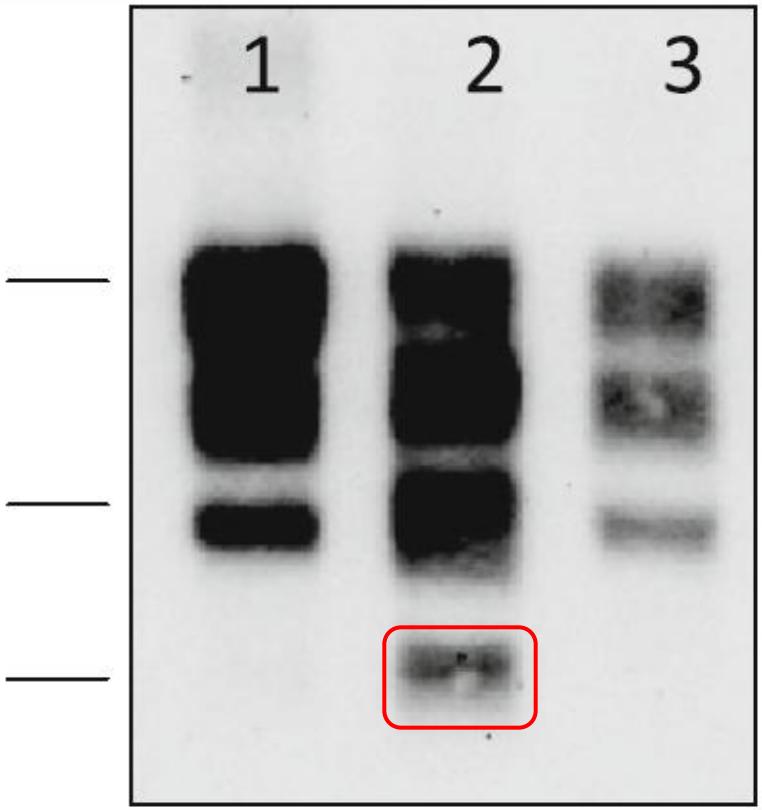

Atypical BSE

Atypical BSE and $\sigma_x \sigma_p \geq \frac{\hbar}{2}$

Epitope aa 156-163



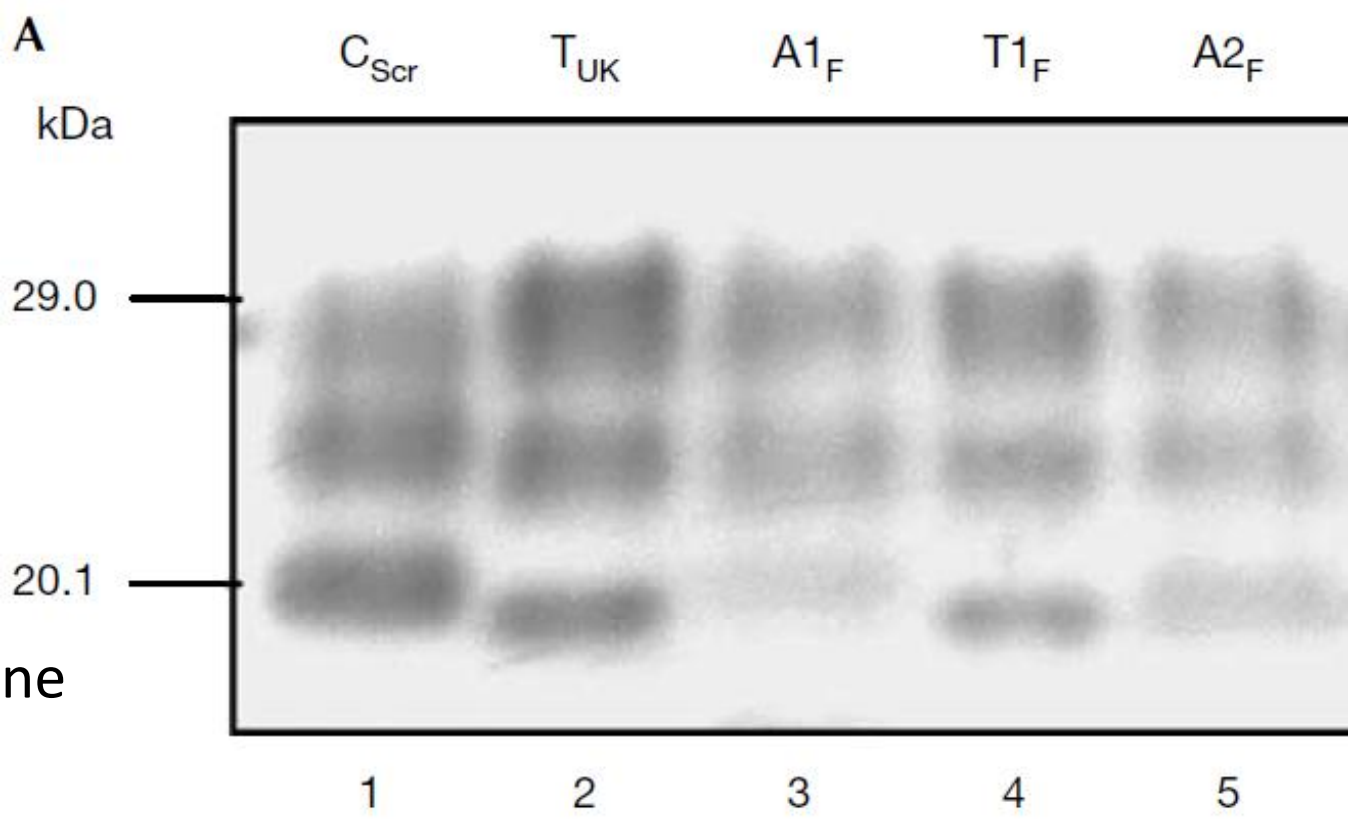
Epitope aa 175-180



Tranulis *et al 2011*

Atypical BSE – H type

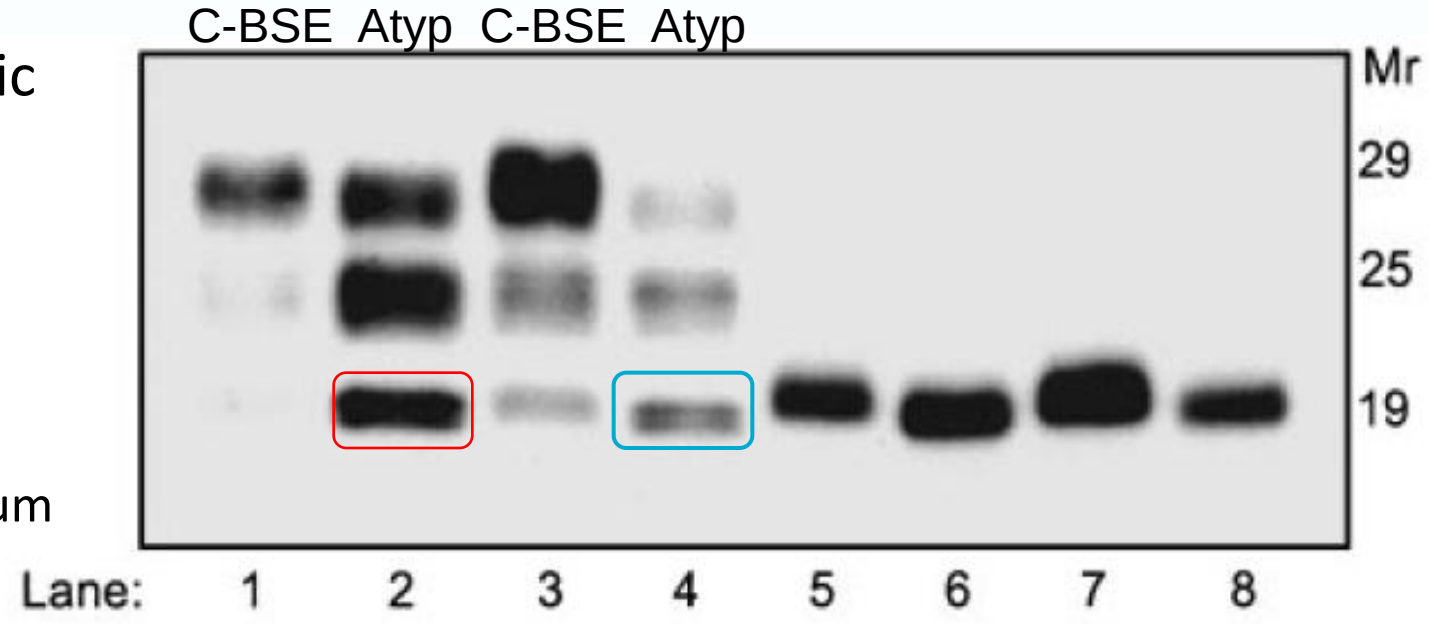
- Aged
- Asymptomatic
- Fallen stock
- Brainstem
  - Biora ELISA +ve
  - Prionics WB +ve
- Normal Prn-P gene



Biacabe *et al* 2004

Atypical BSE – L type

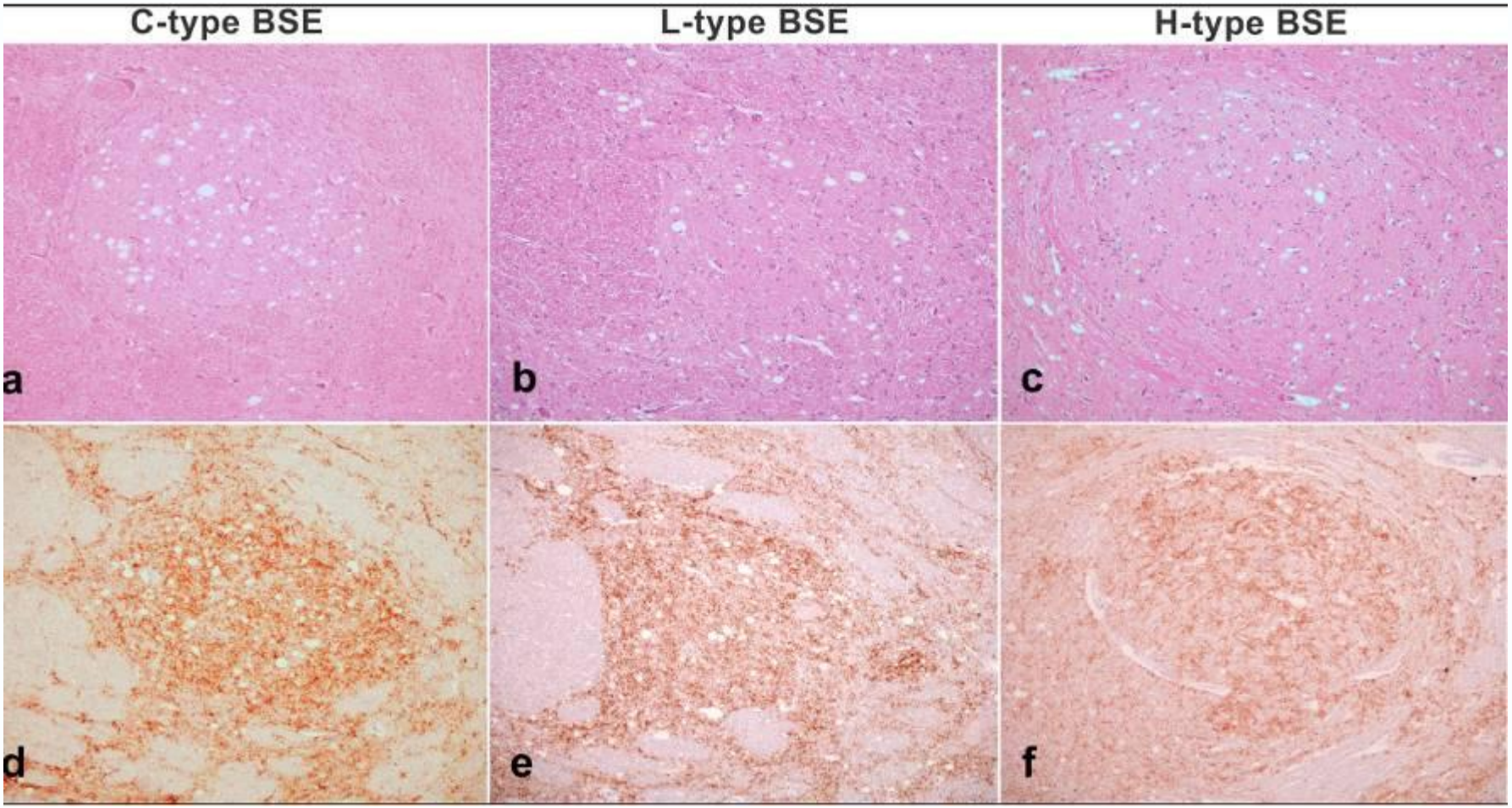
- Aged
- Asymptomatic
- Brainstem
  - WB +ve
- PrP amyloid
  - supratentorium
  - DMVN –ve
- Inconsistent spongiosis
  - Thalamus
- Normal Prn-P gene



Casalone *et al* 2004

Transmission of L and H forms to cattle

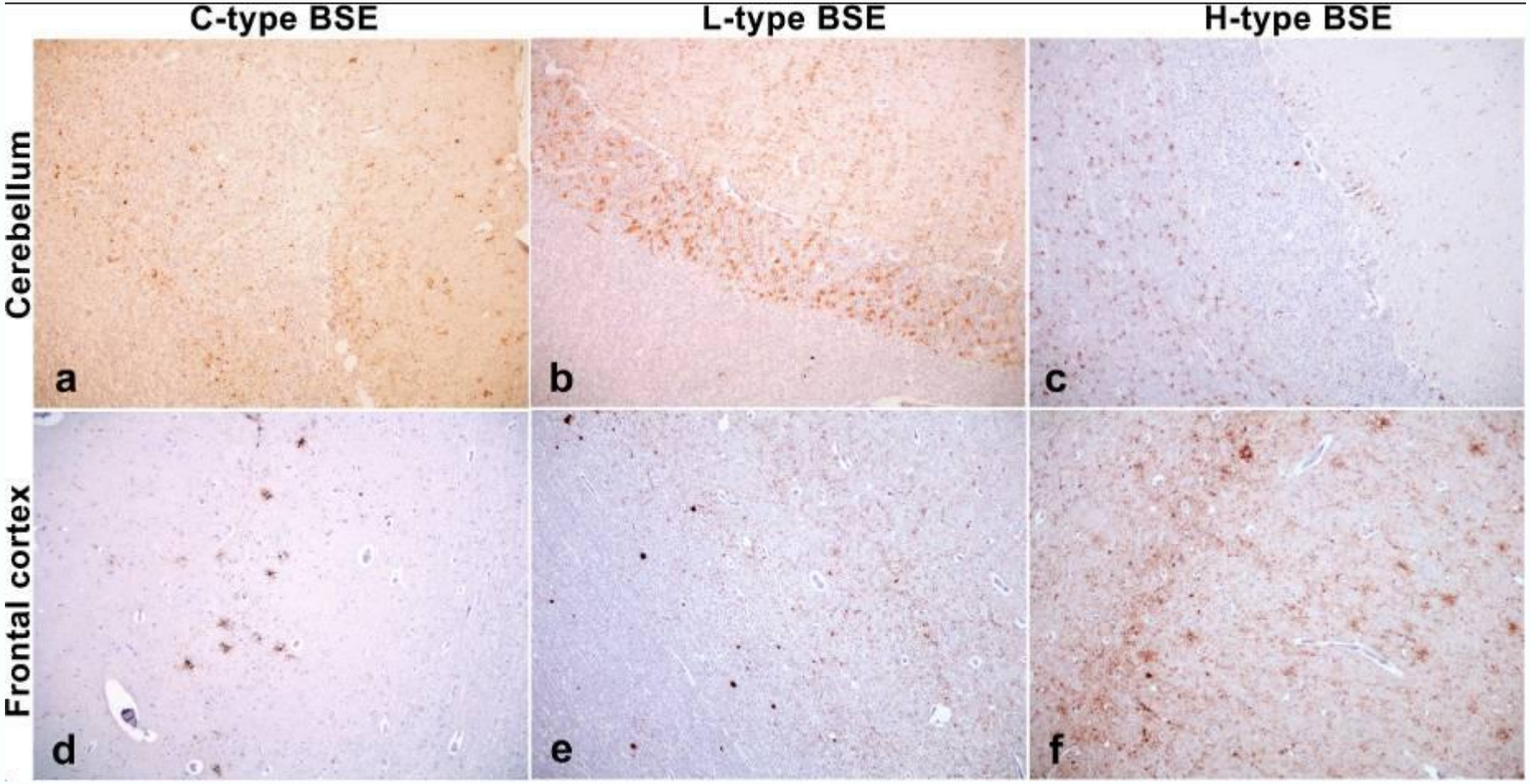
Diagnostic distinction not clear at obex



Solitary tract nucleus

Konold *et al* 2012

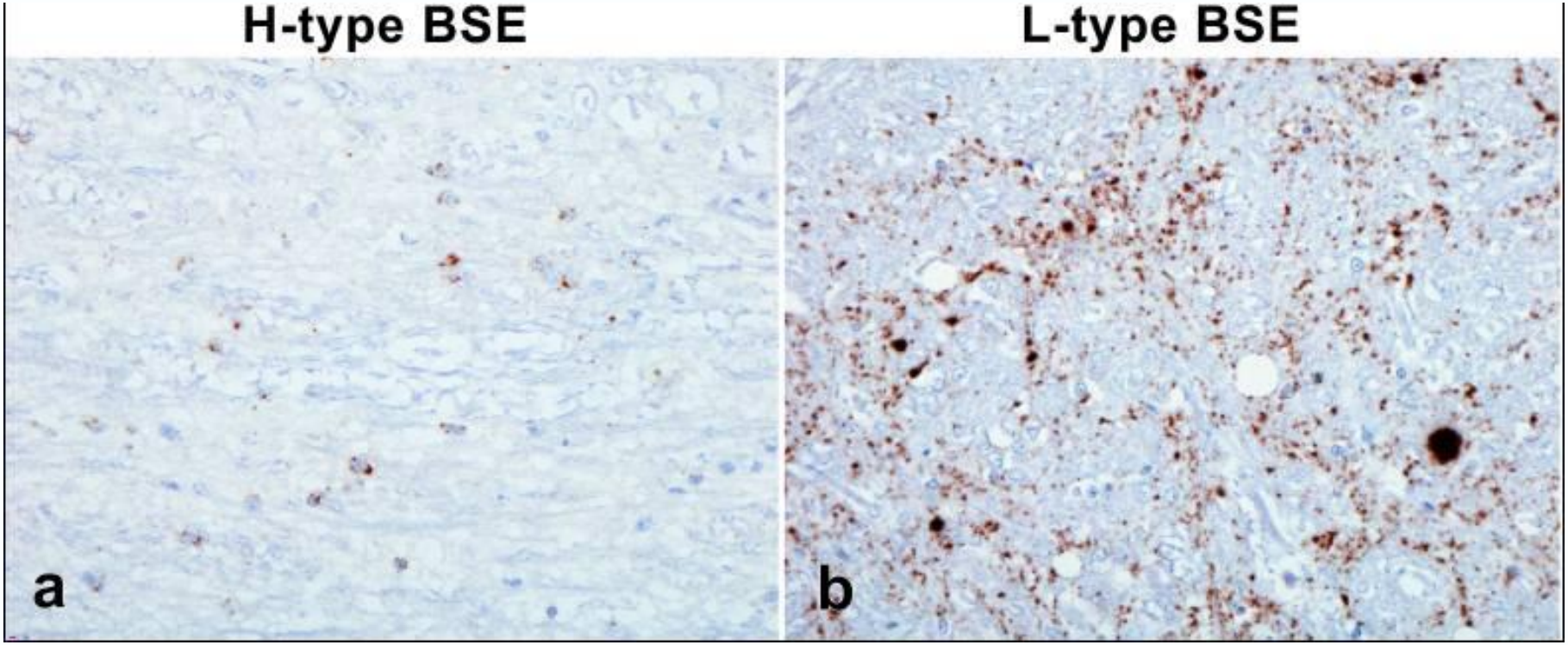
Transmission of L and H forms to cattle



Konold *et al* 2012

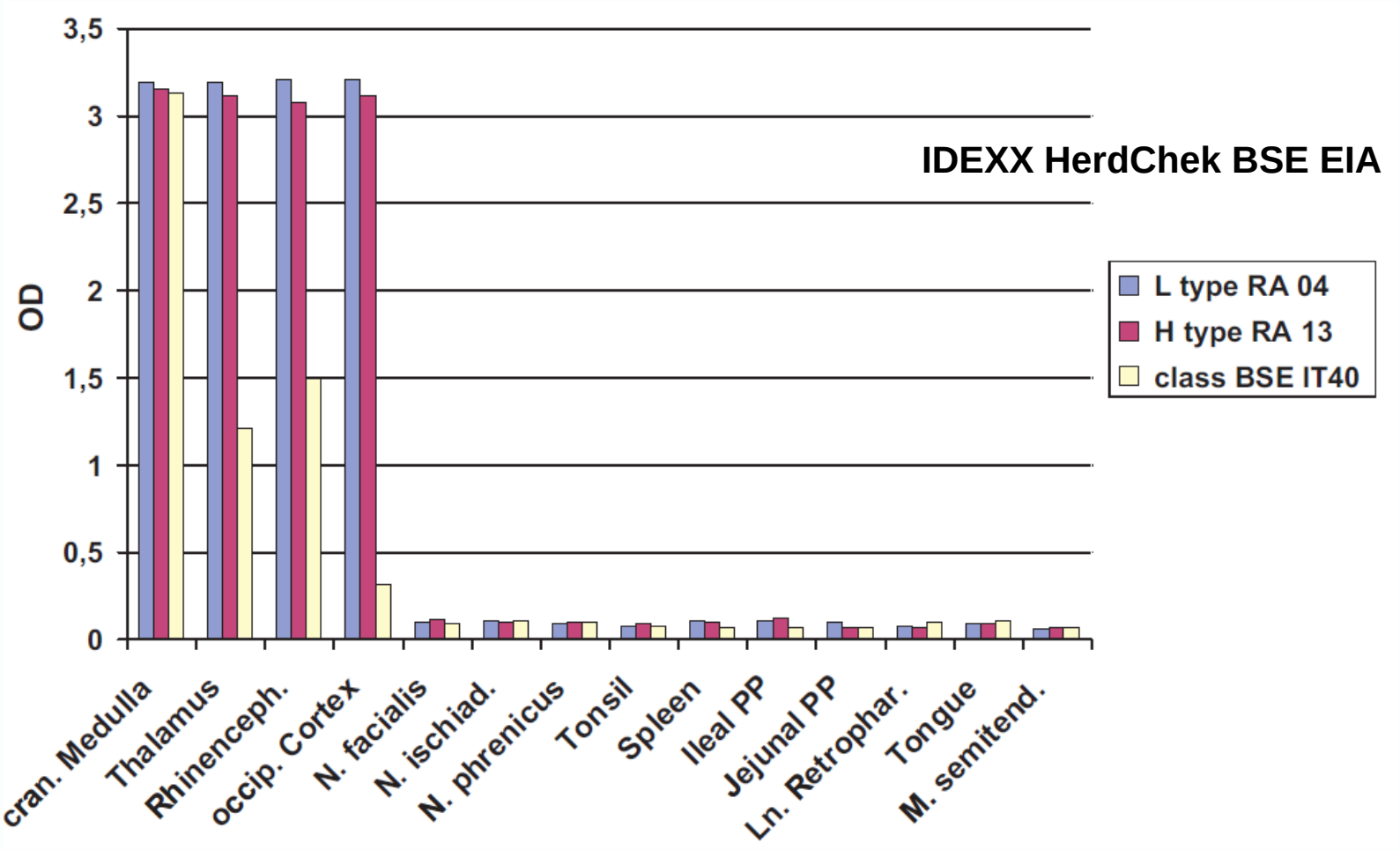
Transmission of L and H forms to cattle

mAb 145 can differentiate H- and L- types at the obex



Konold *et al* 2012

Zoonotic risk of atypical BSE

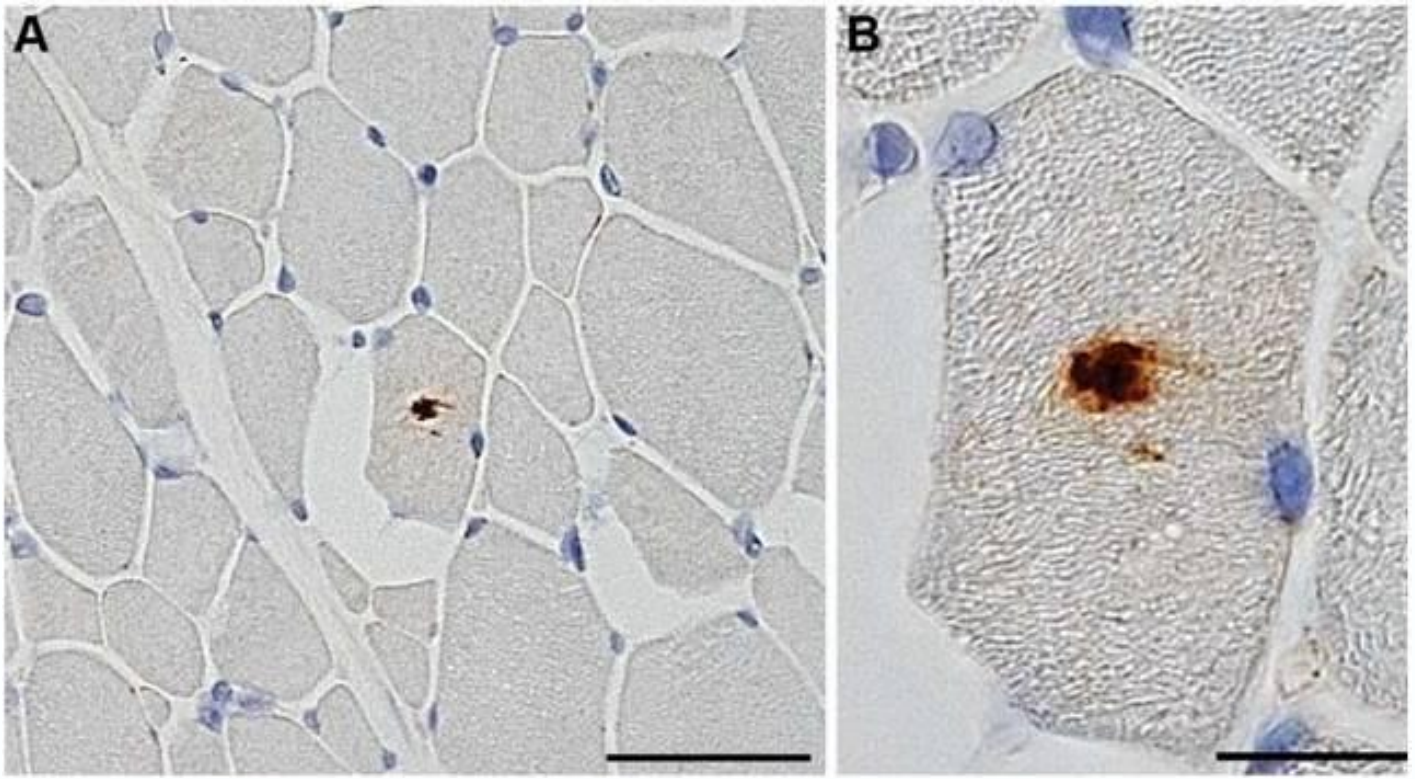


Balkema-Buschmann *et al* 2011

Zoonotic risk of atypical BSE – L type

IPX in 4 /16 muscles

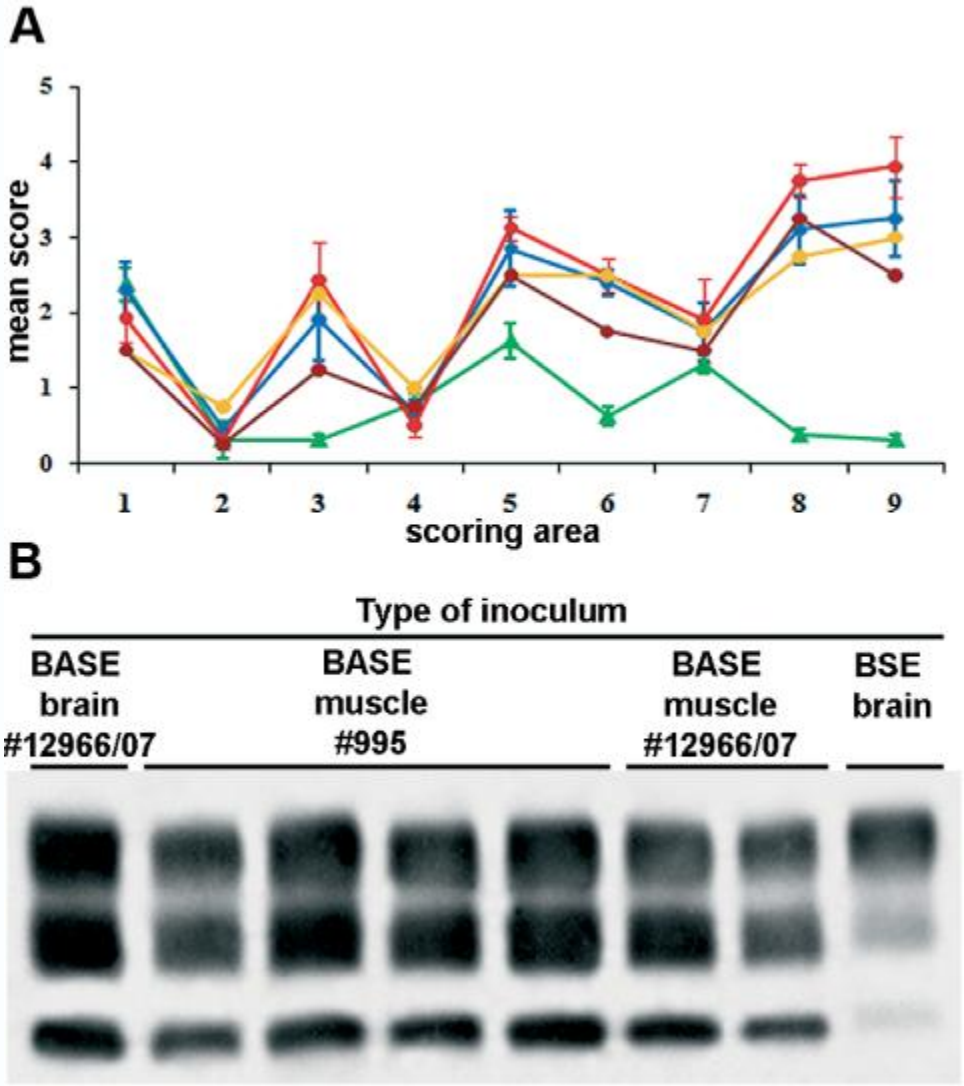
- *M. trapezius, M. biceps femoris, M. peroneus, M. semitendinosus*
- WB –ve (sampling artefact)



Suardi *et al* 2012

Zoonotic risk of atypical BSE – L type

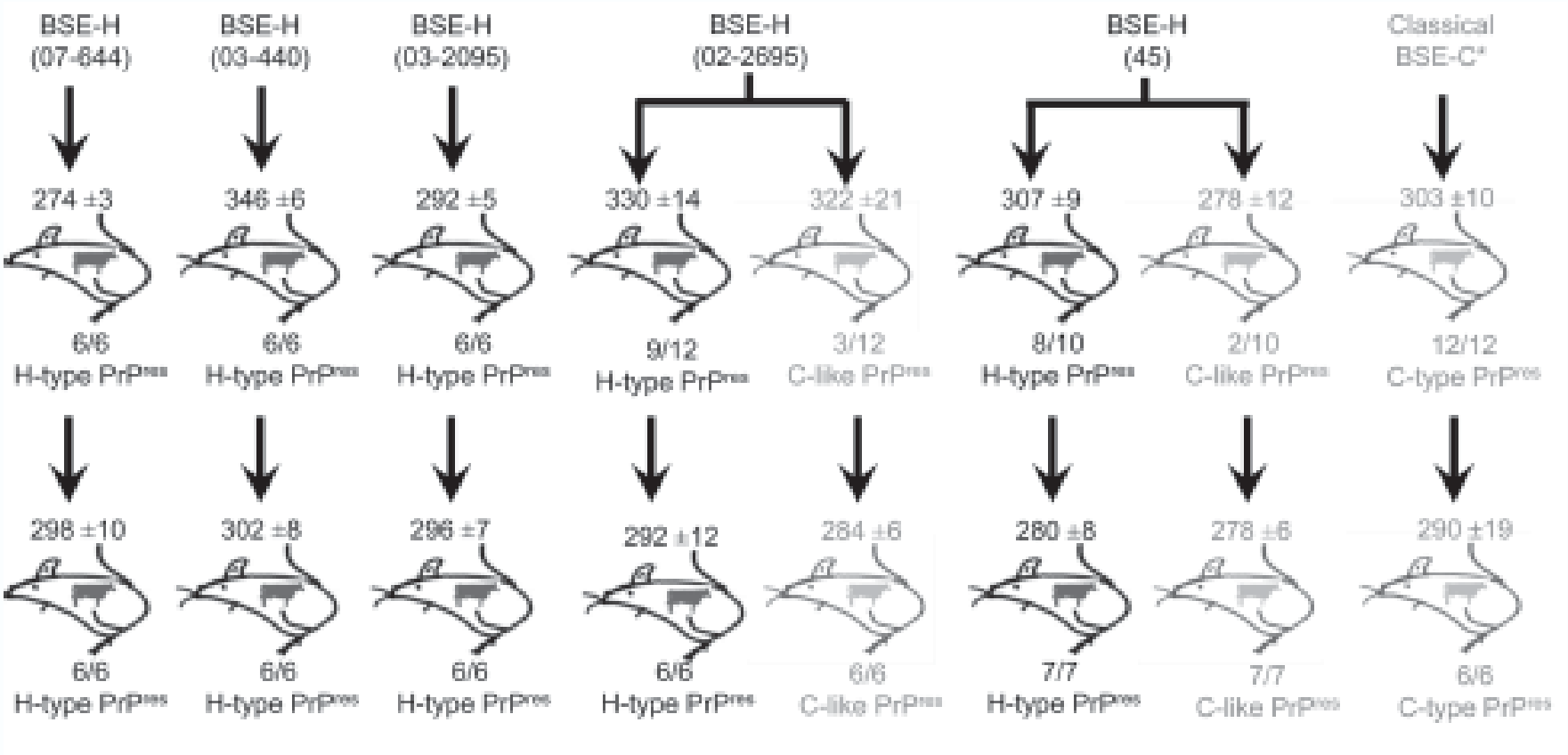
- TgBov XV mice
  - Muscle
    - 70% experimental inocula
    - 10% natural inocula
  - Kidney , spleen, cervical ln
    - No transmission



Suardi *et al* 2012

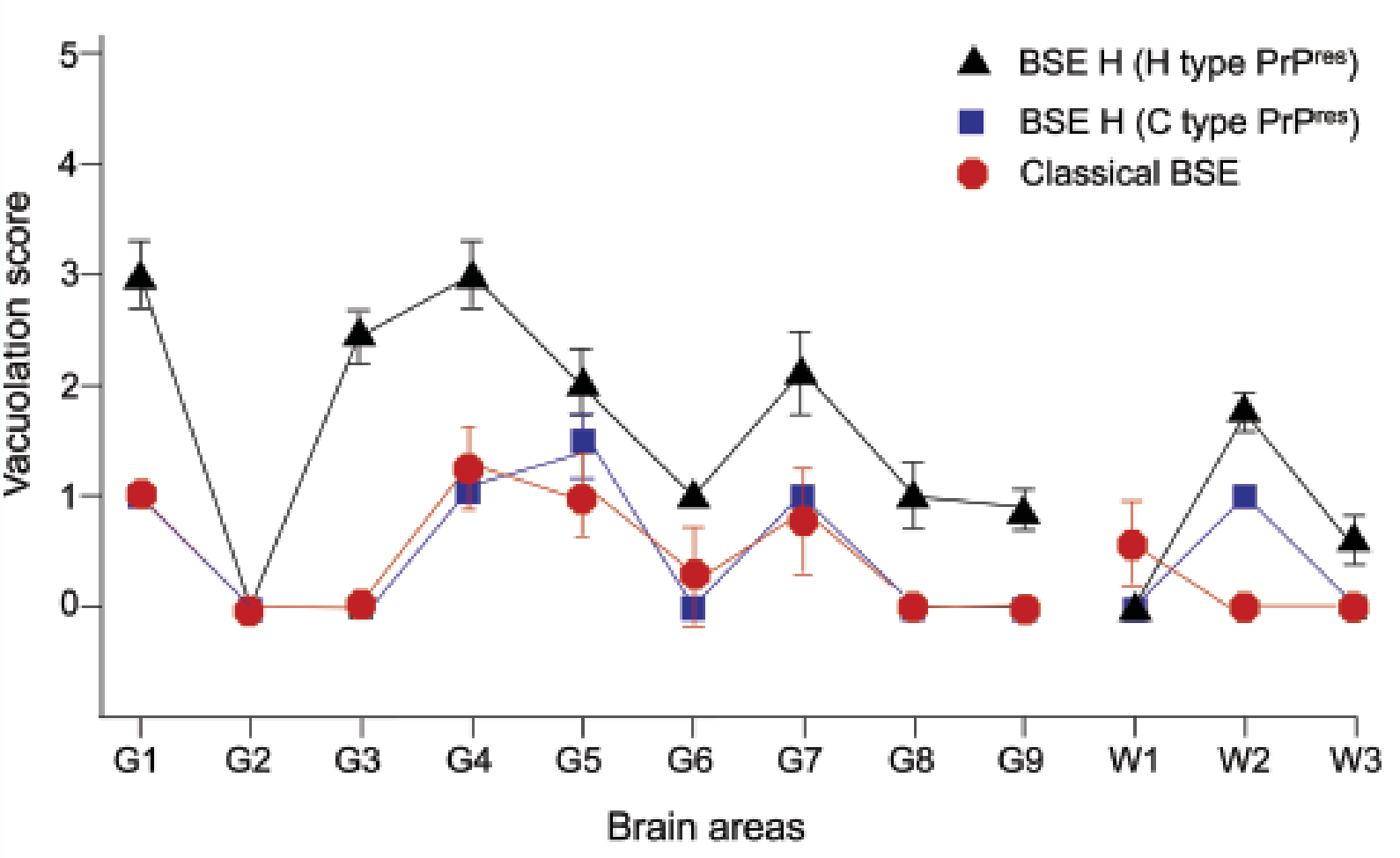
Serial passage of H type in mice

wt and bovinised mice



Torres et al 2011

Serial passage of H type in mice

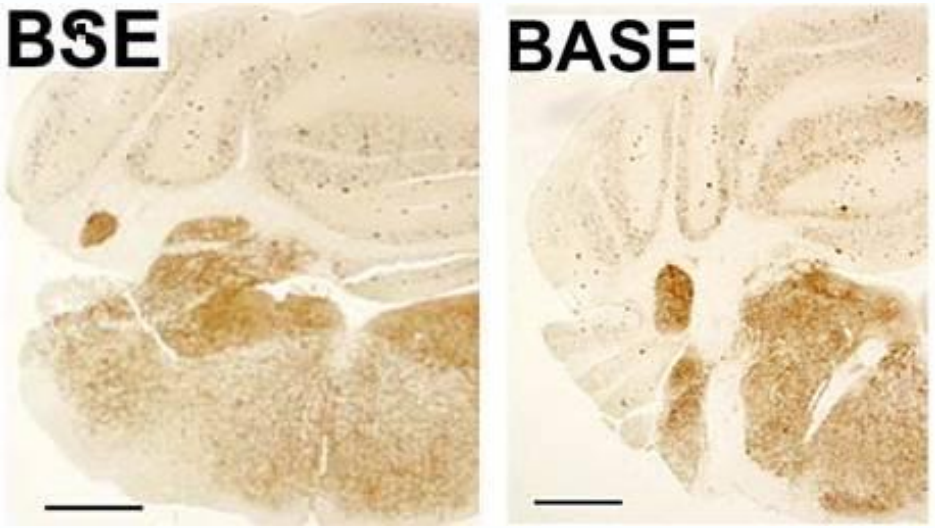


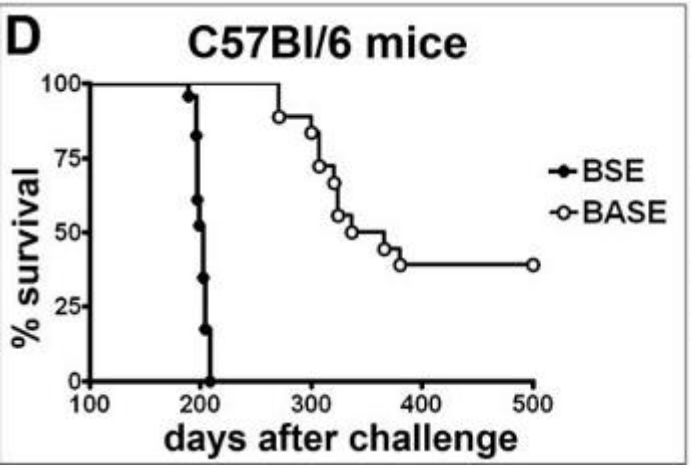
Torres et al 2011

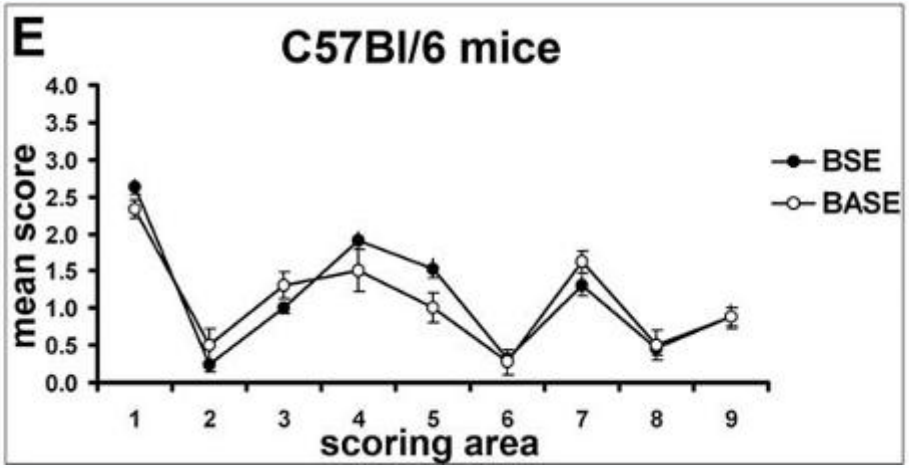
Serial passage of L type in mice

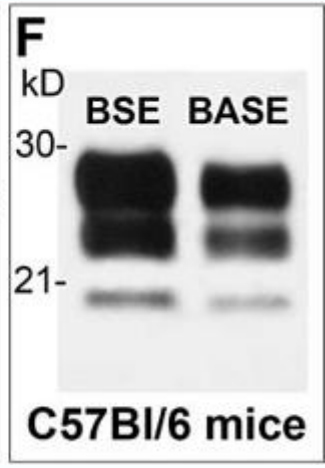
Inbred and Tg VRQ mice

L type could generate classical BSE









Capobianco et al 2007

Atypical BSE – Transmission

L type

- Bovinised mice
  - Short IP
- Ovinised mice
- Cattle
- Humanised mice (100%)
  - Over-expressing PrP
- Not in humanised mice
  - Normal PrP expression
- Macaque

H type

- Bovinised mice
  - Long IP
- Ovinised mice
- Inbred C57Bl6 mice
- Cattle
- Not in humanised mice*
  - Over-expressing PrP

Atypical BSE – current implications

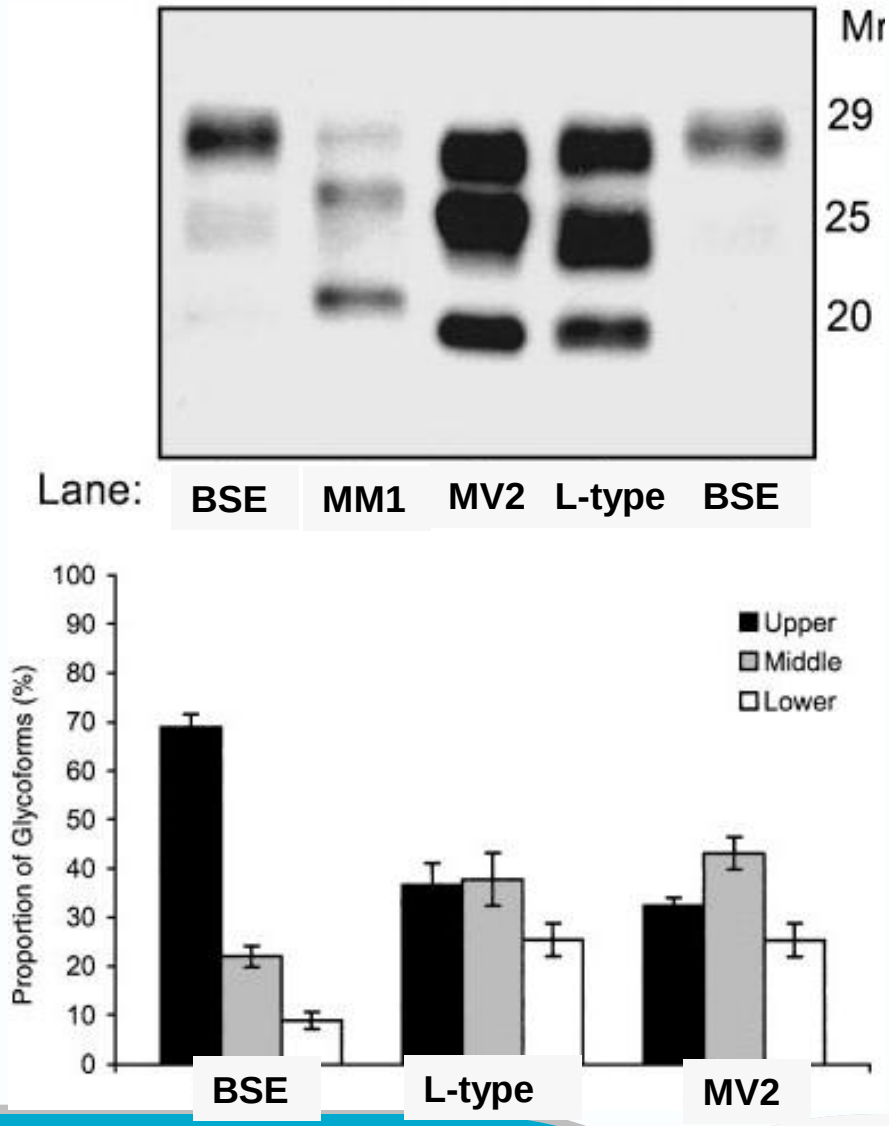
OIE Code - all cattle which, during their first year of life, were reared with the BSE *cases* during their first year of life, and which investigation showed consumed the same potentially contaminated feed during that period, or if the results of the investigation are inconclusive, all cattle born in the same *herd* as, and within 12 months of the birth of, the BSE *cases*, if alive in the country, *zone* or *compartment*, are permanently identified, and their movements controlled, and, when slaughtered or at death, are completely destroyed.

BSE is BSE

Atypical BSE and parallels with spCJD – L type

- Neuropathological phenotype
- PrP^{Sc} distribution
- Glycotype

MV at *PRNP* codon 129 and type 2 PrPSc



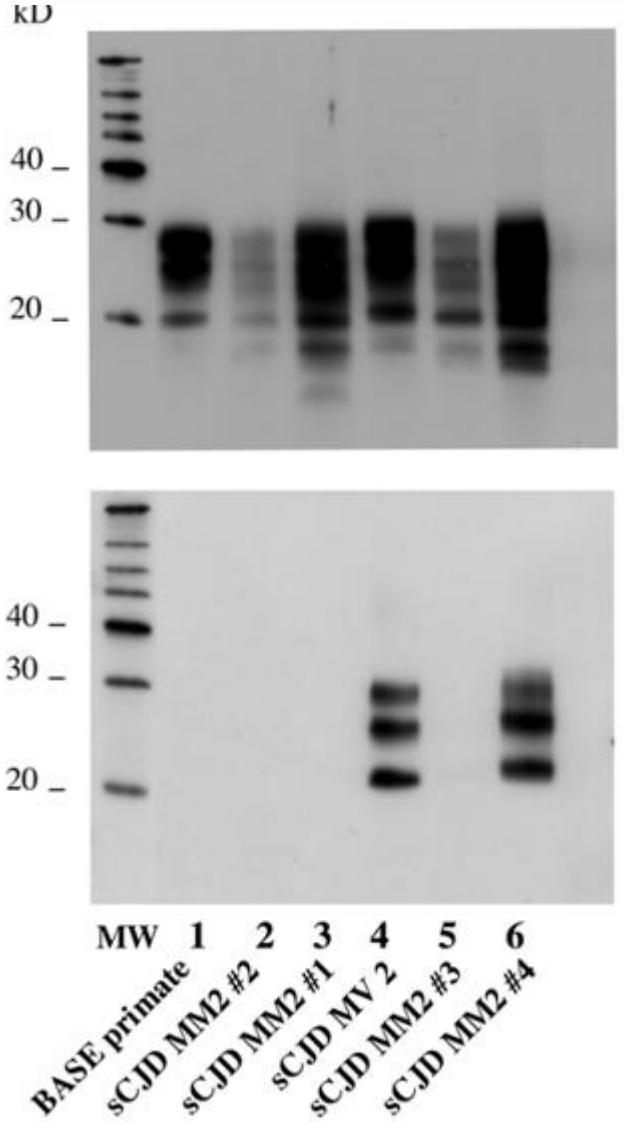
Casalone *et al* 2004

Atypical BSE and parallels with spCJD – L type

- Proteinase K sensitivity in region of octapeptide repeats

MM at *PRNP* codon 129 and type 2 PrPSc

- Lived in same country as affected bovine



Comoy et al 2008

New York Times, Friday, October 27, 2000

British Wrongly Lulled People on 'Mad Cow,' Report Finds

LONDON, Oct. 26 - For 10 years, British officials consistently misled the public by deliberately playing down the possibility that mad-cow disease could be transmitted to humans…….the government sought to insulate (the public) from unpleasant information, using "an approach whose object was sedation.“

Phillips *et al (2000)*

Therapeutics

- None exist
  - Inhibiting formation of PrP^{Sc}
    - Prolongs IP
    - Downstream pathogenesis not well understood
      - Difficult therapeutic access to CNS

The future

- PrP^{C}-deficient farm animals
  - Cytokines, growth factors, therapeutic antibodies
    - PRNP knock-out cattle and goats
- Clarifying the structure of PrP^{Sc}
  - Prediction of PrP^{c} conversion
  - Drug development
- Prion-like mechanisms in other protein-misfolding disorders
  - Alzheimer's Disease
  - Parkinson's Disease
  - Type 2 diabetes

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Short Communications

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R. M. Barlow, D. J. Middleton

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