Pesky Prions: Disease, Disinfection and Diagnosis

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CSIRO ANIMAL, FOOD AND HEALTH SCIENCES
www.csiro.au

GEELONG-VICTORIA
Geelong - Victoria

- 75 km south west of Melbourne
- Victoria's largest provincial city
- Approx 300,000 people
- Victoria's fastest growing region
- Extensive transport links
- Nestled on shores of Corio Bay
- Close to world renowned surf beaches

Enviable lifestyle
The Mighty CATS

CSIRO Australian Animal Health Laboratory (AAHL)
Australian Animal Health Laboratory (Geelong)

The world’s most advanced high containment laboratory

Five storeys of technology

BIOCONTAINMENT AT AAHL

Level 5
Level 4
Level 3
Level 2
Level 1

Plant room (clean)
Air distribution and treatment
Work floor

Pesky Prions: Disease, Disinfection, and Diagnosis | Jean Payne: AAHL-CSIRO
Access restrictions

AAHL – its organisation

Diagnosis, surveillance and response

to meet the needs of those trading in livestock and livestock products both nationally and internationally

AAHL
a national facility managed by CSIRO

Research

to manage the risks from exotic, emerging and new livestock disease
Zoonotic capacity

Ability to work on a range of diseases affecting both humans and animals including Hendra virus, Nipah virus, Menangle virus, Australia bat lyssavirus, SARS and AI with both small and large animals at PC4 level

Biosafety level 4 – Hendra virus, Nipah virus and SARS
Biosafety level 4 – Hendra virus, Nipah virus and SARS

AAHL - its people

International staff with unique skills and training
Animal facilities

**Full range of animal holding facilities from Physical Containment Level 2 (PC2) – PC 4 level**

Costs

- Cost $185m (in 1985) to build but today’s $650M
- Annual Operation Overall $61m (2010-2011)
  - $40.6 m from CSIRO
    - $24.5 m Business Unit and Corporate O/H
    - $14.4 m building depreciation
    - $1.9 m appropriation for science
  - $7.1 m from DAFF
  - $12.5 m from external sources (e.g. national RDCs, UN, pharmaceutical industry, NIH, CSL)
Achievements – Hendra virus

- Hendra virus outbreak in Australia in 1994
- Vic Rail, prominent Queensland horse trainer, and 14 horses died from the deadly virus
- AAHL’s diagnostic team isolated and identified the virus which had not been reported anywhere else in the world
- Scientists believe fruit bats are the natural ‘host’ of Hendra virus
- Research on Hendra virus and Nipah virus, a closely related virus first discovered in 1999 in Malaysia, continues at AAHL

Presentation Outline:

- What is a prion?
- Abnormal prions
- Prion diseases - animal - human
- TSE surveillance - Australia
- Detection
- Decontamination
What is a prion?

• Protein
  • Readily digested by Proteinase K
  • Function not certain
    – May play role in normal synaptic function, memory, maintenance of circadian sleep patterns

• Expressed in a wide range of tissues
  • high levels in neurons and astrocytes
  • lower levels – heart, lungs, reproductive tract, spleen

• Human PrP gene resides on chromosome 20
  • Mutations in gene may trigger transformation of PrP to pathological isoform

Normal prion protein - PrPc

• C-terminal end
  • Structural or folded domain
• N-terminal end
  • Flexibly disordered
• Post-translational processing
  • Removes peptide at either end
  • Residual protein 209 aa
Abnormal prion protein - PrP\textsuperscript{Sc}

- Unique pathogen
  - An agent specific nucleic acid has not been detected

- Conversion of PrP\textsuperscript{C} to PrP\textsuperscript{Sc} is post-translational
  - Therefore have same aa sequence
  - Likely occurs on either plasma membrane or along endocytic pathway
  - PrP\textsuperscript{Sc} accumulates on cell surface

- Conversion of PrP\textsuperscript{C} to PrP\textsuperscript{Sc} is key event in all TSE diseases
  - PrP\textsuperscript{Sc} is resistant to digestion by proteinase K
  - Altered structure is extremely stable and accumulates in infected tissue

Abnormal prion protein - PrP\textsuperscript{Sc}

- Conversion of PrP\textsuperscript{C} to PrP\textsuperscript{Sc} involves a conformational change
  - More beta sheets
  - Fewer alpha helices
  - “Amyloid” structures polymerise and stack to form aggregates or plaques

- Conversion is a self-perpetuating process – a chain reaction
The prion protein – abnormal isoform

- PrP\textsuperscript{sc} and PrP normal derived from same gene (in genetic disease)
- PrP\textsuperscript{sc} same primary structure as PrP
  - Protease digestion produces residual resistant molecule PrP\textsuperscript{sc} 27-30
  - Basis of diagnostic detection systems

Prion Theory

- PrP\textsuperscript{sc} according to prion theory is sole component of infectious particle. – being different to conventional disease pathogens
- Neurological dysfunction induced by changes in metabolism PrP can be
  - acquired – infective particle
  - inherited- defective gene
Prion diseases - TSEs

- Transmissible Spongiform Encephalopathy
  - Uniformly fatal
  - Affect both humans and animals
  - No detectable immune response
  - Still MUCH TO LEARN about epidemiology and pathogenesis of natural prion diseases

Prion diseases - TSEs

- Causes
  - transmission of infectious particle
    - iatrogenic CJD, BSE, sheep scrapie
  - inherited through a defective prion protein gene
    - GSS, Fatal familial insomnia, Classical CJD
  - sporadically by unknown mechanism
    - sCJD
  - In all cases PrP\(^{Sc}\) that accumulates is derived from host PrP\(^{C}\)

- May be zoonotic
  - BSE → variant CJD

- Species barrier
  - the resistance to TSE by one species after exposure to TSE agent from another species
Animal TSEs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrapie</td>
<td>Sheep, goats, moufflon</td>
</tr>
<tr>
<td>Bovine Spongiform Encephalopathy (BSE)</td>
<td>Bovine - cattle</td>
</tr>
<tr>
<td>Feline Spongiform Encephalopathy (BSE)</td>
<td>Felines – domestic and exotic</td>
</tr>
<tr>
<td>Exotic Ungulate Encephalopathy (BSE)</td>
<td>Greater kudu, nyala, eland, oryx</td>
</tr>
<tr>
<td>Transmissible Mink Encephalopathy</td>
<td>Mink</td>
</tr>
<tr>
<td>Chronic Wasting Disease</td>
<td>Mule deer, elk</td>
</tr>
</tbody>
</table>

Animal TSEs – clinical signs

- Prolonged incubation period
  - cattle 4-5 years

- Neurological signs
  - ataxia, abnormal gait, weakness, hyper-reactivity

- Many varied differential diagnoses
  - Trauma
  - Metabolic diseases (e.g. hypocalcaemia)
  - Infection (e.g. brain or spinal abscess)
  - Poisoning (e.g. plant, lead)
Animal TSEs – diagnosis

- AAHL
  - BSE and Scrapie
  - Other prion diseases as requested if antibodies available
- Referred due to
  - clinical history of nervous disease
  - vacuolation in brain on H+E
- We may receive
  - Paraffin blocks
  - Slides
  - Tissue (preferably brainstem at the obex)
    - Fixed tissue is cut in, 1 hour formic acid, washed, processed on routine brain schedule
- H+E and immunohistochemistry (IHC)

Diagnostic Methods at AAHL

- ELISA
- Western Blot
- Electron Microscopy - Scrapie Associated Fibrils (SAF)
- Immunohistochemistry (IHC)
Prion IHC

- Dewaxed brain sections
- Pre-treat with 99-100% formic acid 30 minutes
- DAKO PT Link 97°C – 30 minutes
- DAKO Autostainer-
  - H$_2$O$_2$ - 10 minutes
  - TRIS Wash
  - Proteinase K – 5 minutes
  - TRIS Wash
  - Antibody (F99, R145) – 60 minutes
  - TRIS Wash
  - Envision Link – 15 minutes
  - TRIS Wash
  - Envision Flex HRP – 20 minutes
  - TRIS Wash
  - AEC Chromagen – 10 minutes
  - Water Wash
  - Heamatoxylin – 30 seconds
  - Scotts tap water
  - Mount with aqueous mountant

What do we look for?

- H+E
- Vacuolation of neurons and neuropil

IHC

PrP$^\text{Sc}$ deposition
Scrapie

• Known about for 250 years
• Transmits from sheep to sheep
• Genetic makeup affects sheep susceptibility
• Neurological disease
  • erratic involuntary movements
  • ataxia
  • excessive scratching
• Australia and NZ scrapie-free

• No demonstrable connection with CJD

Classical Scrapie - IHC
Scrapie – natural transmission sheep to sheep

- Horizontal transmission
  - oral cavity
  - saliva - salivary gland
  - renal papillae
  - faeces
  - skin
  - milk - mammary lymphoid tissue
  - placenta (by ingestion)

- Environmental reservoirs
  - soil

Animal TSEs – Atypical scrapie

- First seen in Norway-1998
  - Reported in 2003
- Tends to affect older sheep of the more resistant PrP genotypes to typical scrapie
- Just one in the flock
- No indication of vertical or lateral transmission.
- Experimental transmission successful
- Sporadic form like that of sCJD?
- Identified by distribution of PrP\textsuperscript{Sc} in brain tissue
Atypical scrapie: Australian case studies

- Blocks containing ovine brain
  - Western Australia
  - Victoria
    - 8 months later
- Clinical signs - ‘circling’
- Mild vacuolation on H+E.
- TSE exclusion requested

- H+E and IHC using antibody F99 was performed

Case Study- H+E
Comparison of Classical Scrapie and Atypical Scrapie

Classical Scrapie. St. Site 1  Atypical Scrapie St. Site 1
Atypical Scrapie

<table>
<thead>
<tr>
<th>SCRAPIE</th>
<th>ATYPICAL SCRAPIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulla oblongata (Site 1)</td>
<td>+</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>-</td>
</tr>
<tr>
<td>White Matter</td>
<td>-</td>
</tr>
</tbody>
</table>

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BSE – Bovine Spongiform Encephalopathy

- Mad Cow Disease
Animal TSEs – Bovine spongiform encephalopathy

- 1986 – BSE described in UK cattle
  - Nervous tissue, offal infected
  - Entered human food chain
  - vCJD
- At height of epidemic >1000 cattle per week diagnosed (Jan 1993)
- Rapid diagnostic tests developed
  - ELISA
  - WB
- Slaughtered >280,000 cattle
- Not transmitted by embryo, semen now widely accepted

Control of animal disease – BARB cases

BSE Epidemic in the UK

[Graph showing the BSE epidemic in the UK with key events and timelines.]
Worldwide Geographic Distribution of BSE

![Worldwide Geographic Distribution of BSE](image)


BSE diagnostic methods at AAHL

- Blocks, slides or fixed tissue is sent to AAHL on suspect cases
- Confirmatory test is IHC at AAHL
  - No positive cases found!
- Rapid testing of brains for abnormal PrP by ELISA at RVL Toowoomba
TSE surveillance - Australia

- Began 1990, revised 1998
- Meets OIE requirements to support trade
  - Passive surveillance
    - sheep and cattle cases meet age and clinical criteria
- Collect brain including brain stem
  - fixed in 10%NB Formalin
  - plus piece fresh spinal cord
- Histopathologic examination

TSE Surveillance - Australia

- Histology
  - Focus on medulla at level of obex
......and in Australia

- 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)

Or we could just........................
Australia is BSE and SCRAPIE FREE

HUMAN PRION DISEASES

- Human prion diseases can be due to
  - PrP gene mutation
  - Infected with infective particle
  - Incubation can be months to years
  - No effective vaccines, specific treatment
  - No reliable methods of detection in asymptomatic patients
- Include
  - depression
  - psychiatric problems
  - severe mental impairment
  - loss of co-ordination
  - myoclonus – involuntary twitching
  - insomnia
  - memory problems
  - personality changes
Diagnosis

• Problems just like detection of animal prion diseases
• Long incubation periods
• Even though conversion of normal PrP to abnormal form may have commenced
• Often mis-diagnosed
• No methods to detect levels of PrP^{Sc} in accessible body fluids (urine, blood) but research continuing ..........

• Clinical symptoms and one of
  ❏ EEG (electroencephalogram) – periodic sharp wave complexes
  ❏ Positive 14-3-3 CSF assay in patients with <2 years duration (protein assay)
  ❏ Abnormalities in caudate nucleus by MRI

AND WITHOUT ROUTINE INVESTIGATION INDICATING AN ALTERNATIVE DIAGNOSIS

Diagnosis and Treatment

• Post mortem Proteinase K digestion and measurement of molecular weight and glycoform profiles of Prot K resistant PrP^{Sc} core
• Post mortem neuropathological methods including immunohistochemistry (rare invasive brain Bx)
• Generally accepted no treatment
• Experimental drugs
• Treat symptoms
HUMAN PRION DISEASES

<table>
<thead>
<tr>
<th>Human Prion Disease</th>
<th>Genetic Predisposition/Infective Particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creutzfeldt-Jacob Disease (familial)</td>
<td>Genetic</td>
</tr>
<tr>
<td>Variant CJD</td>
<td>Infective Particle – maybe genetic</td>
</tr>
<tr>
<td>Iatrogenic CJD</td>
<td>predisposition</td>
</tr>
<tr>
<td>Sporadic CJD</td>
<td>Infective particle</td>
</tr>
<tr>
<td>Gerstmann-Straussler-Scheinker</td>
<td>Unknown</td>
</tr>
<tr>
<td>Syndrome</td>
<td></td>
</tr>
<tr>
<td>Fatal Familial Insomnia</td>
<td>Genetic</td>
</tr>
<tr>
<td></td>
<td>– Autosomal dominant gene</td>
</tr>
<tr>
<td>Kuru</td>
<td>Infective particle</td>
</tr>
</tbody>
</table>

Human Prion Diseases

- **KURU**
  - Rare fatal brain disorder
  - **Fore** people of New Guinea highlands
  - Epidemic numbers 1950-1960’s
  - Due to ritualistic cannibalism – consuming of deceased relatives, including brain
  - More women affected – consumed more of the brain
  - Symptoms - unsteady gait, tremors, slurred speech, comatose
  - Death 6-12 months after first symptoms
  - Controlled by discouraging cannibalism
CJD

• Four forms of CJD -

<table>
<thead>
<tr>
<th>Type of CJD</th>
<th>% Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial</td>
<td>Genetic – 10-15%</td>
</tr>
<tr>
<td>Variant</td>
<td>Infective particle - ????</td>
</tr>
<tr>
<td>Iatrogenic</td>
<td>Infective particle - 1%</td>
</tr>
<tr>
<td>Sporadic</td>
<td>Unknown – 80-85%</td>
</tr>
</tbody>
</table>

CJD

• First described - German neurologist Hans Gerhard Creutzfeldt in 1920, and shortly after – Alfons Maria Jakob
• Affects both men and women
• CJD mortality rate 1-2 people in every 1,000,000/year worldwide
• Incubation period can be months to years
• Fatal within 1 year after onset of symptoms
• No effective vaccines, no reliable methods to detect infection in asymptomatic people, no specific therapy for treatment
• In humans homozygosity at codon 129 is associated with higher susceptibility to CJD
IATROGENIC CJD (iCJD)

• Type of ACQUIRED CJD
• First reported, paper by Duffy et al 1974

• Deaths have been recorded from iCJD after
  o Corneal Grafts
  o multi use of neurosurgical equipment
  o treatment with human growth hormone
  o post Dura Mater grafts

Sporadic CJD

• Most common form 1/1,000,000
• Most have no risk factors for the disease
• Has not been linked to Scrapie or BSE as is worldwide
• “Out of the Blue”
• Early symptoms - depression
• Progresses quickly
• Death within weeks to months (usually within 6 months)
Sporadic CJD vs variant CJD

- Different clinical signs
  - psychiatric, unsteadiness, involuntary movements, immobile and mute
- Different pathology
  - illness duration, MRI findings, neuropathologic lesions
- vCJD distinguished by presence of amyloid plaque surrounded by halo of spongiform changes
vCJD

• 9 years after identification of BSE in the UK - 1996
• 5 suspects still alive
• All vCJD patients tested homozygous for methionine at the polymorphic codon 129 (except for 1 bloodborne transmission case heterozygous-MV at codon 129)

Country vCJD Human Cases (October 2009)

<table>
<thead>
<tr>
<th>Country</th>
<th>vCJD Human Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td>170</td>
</tr>
<tr>
<td>France</td>
<td>25</td>
</tr>
<tr>
<td>Spain</td>
<td>5</td>
</tr>
<tr>
<td>Ireland</td>
<td>4</td>
</tr>
<tr>
<td>United States</td>
<td>3</td>
</tr>
<tr>
<td>Netherlands</td>
<td>3</td>
</tr>
<tr>
<td>Portugal</td>
<td>2</td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
</tr>
<tr>
<td>Canada</td>
<td>1</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>1</td>
</tr>
</tbody>
</table>

• All consumed prion infected meat products (BSE) EXCEPT 3 from UK – infected from blood products from asymptomatic, infected donor
• All cases are from or have history of exposure within a country where BSE was occurring
• Total of 217 people from 11 countries
Control of human exposure

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

Biosecurity and biocontainment

- PPE
- Resistant to most normal disinfection techniques
  - chemical, physical, gaseous, formaldehyde, standard autoclaving (121°C/15m)
- Appears only a fraction are resistant to autoclaving
  - special “scrapie” cycle – 121°C-132°C – 60 mins (gravity) or 132°C >20 minutes for pre vac
- Decontamination of surgical instruments
  - concerning as they are delicate making disinfection difficult
  - single use surgical instruments
- Alcohol can bind proteins to surfaces
- Tissue after fixation immersion in formic acid (99-100%)
Disinfection

### Chemical ineffective in inactivation of PRIONS (<3-log reduction in 1 hr)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Disinfectants decrease PRIONS by &gt;3logs in 1 hr / infectious strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia 1.0M</td>
<td>Chlorine 5000 ppm - Scrapie / CJD</td>
</tr>
<tr>
<td>Hydrogen peroxide 3%</td>
<td>Chlorine 8250 ppm - BSE</td>
</tr>
<tr>
<td>Iodine 2%</td>
<td>Na OH 1N - CJD/BSE</td>
</tr>
<tr>
<td>Formaldehyde 3.7%</td>
<td>Na OH 2N - Scrapie</td>
</tr>
<tr>
<td>Alcohol 50%, 100%</td>
<td></td>
</tr>
<tr>
<td>Hydrochloric acid 1.0N</td>
<td></td>
</tr>
<tr>
<td>Glutaraldehyde 5%</td>
<td></td>
</tr>
<tr>
<td>Phenol/Phenolics – 10% lysol, 0.6% Phenols</td>
<td></td>
</tr>
</tbody>
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**Prion diseases....in a nutshell**

- **Neurodegeneration and lethality**
  - TSEs – animal and human disease
  - Sporadic, infectious, genetic
  - Prolonged silent asymptomatic period
  - Vacuolation, gliosis, PrP<sup>sc</sup> 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<sup>C</sup>) and conversion into the disease-causing isoform (PrP<sup>Sc</sup>)
  - Circumvents innate and adaptive immunity

- **No exogenous genetic material involved in transmission**
Acknowledgements – AAHL “Team Histo”

- Dr Deb Middleton
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