The Biology, Genetics and Surveillance of BSE

John Williams

Roslin Institute (Edinburgh)
## TSEs Across Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans</td>
<td>CJD, GSS, FFI, Kuru</td>
</tr>
<tr>
<td>Sheep</td>
<td>Scrapie</td>
</tr>
<tr>
<td>Goats</td>
<td>Scrapie</td>
</tr>
<tr>
<td>Cattle</td>
<td>BSE</td>
</tr>
<tr>
<td>Deer</td>
<td>CWD</td>
</tr>
<tr>
<td>Mink</td>
<td>TME</td>
</tr>
<tr>
<td>Cats</td>
<td>FSE</td>
</tr>
</tbody>
</table>
Hallmarks of TSE Disease

Features:
- Invariably fatal
- Long incubation period
- Short clinical course
- No immune response

Symptoms:
- Progressive dementia & ataxia
- Behavioral abnormalities
- Memory loss and disorientation

CNS Pathology
- Vacuolation/Spongiosis
- Gliosis
- Neuronal apoptosis
- Accumulation of PrPSc
Pathology

- **PrPC**
  - Soluble
  - Protinase digestable
  - $\alpha$ helix

- **PrP^{BSE}**
  - Protein aggregates
  - Highly resistant to protinase
  - $\beta$ sheet
Infection via the Oral Route

Infectious material → Gastro-Intestinal tract → Gut epithelium → Peyer’s Patch

M-Cells

Peripheral Nervous System

Central Nervous System

Lymphohoreticular system (FDCs)

Spleen
BSE in the UK

- Nov 1986: Disease Identified
- Dec 1987: Feed identified as source
- June 1988: Ruminant feed ban
- 1993-1994: Peak 1000 suspects / week
- August 1996: Total ban of MBM
- July 2001: EU MBM ban

- 183,496 cases in UK
- 1.6M cattle may have been infected
- ~75 BARB cases
- 147 cases in 2003 (To October)
Transmission Between Cattle

- **Horizontal**
  - within herd incidence of BSE <2.7%
  - no transmission between cows (?)

- **Vertical**
  - enhanced risk in offspring of clinical cases
  - no maternal effect two years before clinical disease
BSE Cases in Europe

BSE in Cattle Destined for the Food Chain (data 2001)

<table>
<thead>
<tr>
<th>Country</th>
<th>&gt;30 mo. Tests (some &gt;24 mo.)</th>
<th>No. +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ireland</td>
<td>636,930</td>
<td>34</td>
</tr>
<tr>
<td>Portugal</td>
<td>28,384</td>
<td>19</td>
</tr>
<tr>
<td>France</td>
<td>2,382,225</td>
<td>83</td>
</tr>
<tr>
<td>Belgium</td>
<td>359,435</td>
<td>28</td>
</tr>
<tr>
<td>Netherlands</td>
<td>454,649</td>
<td>11</td>
</tr>
<tr>
<td>Germany</td>
<td>2,565,341</td>
<td>36</td>
</tr>
<tr>
<td>Spain</td>
<td>328,517</td>
<td>35</td>
</tr>
<tr>
<td>Denmark</td>
<td>250,414</td>
<td>3</td>
</tr>
<tr>
<td>Italy</td>
<td>377,201</td>
<td>27</td>
</tr>
<tr>
<td>TOTAL (EU)</td>
<td>7,670,176</td>
<td>279</td>
</tr>
</tbody>
</table>
TSE Strains

Surveillance in Italy

- >1.6 M cattle screened
- 103 sub-clinical BSE cases
- 2 atypical: sCJD-like

Identification of a second bovine amyloidotic spongiform encephalopathy: Molecular similarities with sporadic Creutzfeldt–Jakob disease

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Edited by Stanley B. Prusiner, University of California, San Francisco, CA, and approved December 23, 2003 (received for review September 9, 2003)

PrP^Sc distribution in brain

Larger glyco-form
**vCJD**

- Identified March 1996
- Early onset
- Pathology of brain different from CJD
- Evidence of BSE origin
  - Incubation period in inbred strains of mice
  - Distribution of lesions

## TSE Incidence in Man

<table>
<thead>
<tr>
<th>Year</th>
<th>Referrals</th>
<th>Year</th>
<th>Sporadic</th>
<th>Iatrogenic</th>
<th>Familial</th>
<th>GSS</th>
<th>vCJD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>53</td>
<td>1990</td>
<td>28</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>33</td>
</tr>
<tr>
<td>1991</td>
<td>75</td>
<td>1991</td>
<td>32</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>1992</td>
<td>96</td>
<td>1992</td>
<td>45</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>53</td>
</tr>
<tr>
<td>1993</td>
<td>78</td>
<td>1993</td>
<td>37</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>46</td>
</tr>
<tr>
<td>1994</td>
<td>118</td>
<td>1994</td>
<td>53</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>-</td>
<td>61</td>
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<tr>
<td>1995</td>
<td>87</td>
<td>1995</td>
<td>35</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>47</td>
</tr>
<tr>
<td>1996</td>
<td>134</td>
<td>1996</td>
<td>40</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>1997</td>
<td>161</td>
<td>1997</td>
<td>59</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>1998</td>
<td>154</td>
<td>1998</td>
<td>63</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>18</td>
<td>89</td>
</tr>
<tr>
<td>1999</td>
<td>170</td>
<td>1999</td>
<td>62</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>2000</td>
<td>178</td>
<td>2000</td>
<td>49</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>28</td>
<td>81</td>
</tr>
<tr>
<td>2001</td>
<td>174</td>
<td>2001</td>
<td>55</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>20</td>
<td>82</td>
</tr>
<tr>
<td>2002</td>
<td>152</td>
<td>2002</td>
<td>62</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>2003</td>
<td>134</td>
<td>2003*</td>
<td>53</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>1780</td>
<td>Deaths</td>
<td>697</td>
<td>44</td>
<td>39</td>
<td>20</td>
<td>137</td>
<td>927</td>
</tr>
</tbody>
</table>

*At Dec 2003

Source: DoH Statistics
Options for Control of BSE

- Surveillance
- Identification of infected animals
  - To remove from food chain
  - Prevent infection of other stock
- Breeding for resistance
### Risk of Scrapie

- **Polymorphisms at 3 sites in *Ovine PrP***
  - codons 136, 154, 171

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Suffolk type</th>
<th>Cheviot type</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR/ARR</td>
<td>Highly resistant</td>
<td>Highly resistant</td>
</tr>
<tr>
<td>ARQ/ARR</td>
<td>Occasional</td>
<td>Highly resistant</td>
</tr>
<tr>
<td>VRQ/ARR</td>
<td>Increasing risk</td>
<td></td>
</tr>
<tr>
<td>VRQ/ARQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VRQ/VRQ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **High risk of scrapie**
  - ARQ/ARQ

---

Polymorphisms at 3 sites in *Ovine PrP* - codons 136, 154, 171.
vCJD and Genotype

• To date 137 confirmed cases (Dec 2003)
  • All codon129 Met/Met homozygous

• Codon 129 allele frequency in UK
  • Met/Val  50%
  • Met/Met  32%
  • Val/Val  17%
**Bovine PrP**

- PrP polymorphisms

Exons 1  2  3

1) Octa-peptide repeat
   Alleles with 5, 6 or 7 copies

2) SNP (Hind II RFLP)
Bovine PrP and BSE

Octapeptide Polymorphism
- 5 repeats
- 6 repeats
- 7 repeats

Bovine PrP and BSE

Silent Polymorphism

A allele
B allele

Up to Now No Evidence for Influence of Variations in PrP on BSE Incidence

PrP gene sequenced
  • Many polymorphisms
    – mostly silent
    – some in promoter

Hills et al. (2003) Animal Genetics 34 183-190

• Additional case/control samples
• BARB cases.....

Exons 1 2 3

23 bp indel
Suggestive association with disease in 30 BSE cases in Germany

Sander et al Mammalian Genome (Submitted)
Are There Other Loci Involved in TSE Susceptibility?

Genome Scan for BSE Susceptibility Loci in Cattle

• Families 4 major half-sib sire families
  • 358 BSE affected
  • 172 unaffected half-sibs (age, sex, farm matched)

• Markers 166 tested in sires
  • 20 homozygous in all sires
  • 146 tested across progeny

Mouse Studies

Chromosome 9

Chromosome 2

Chromosome 11

Chromosome 12


Lloyd et al (2001) PNAS 98 6279-6283
Comparative Information

Hexosaminidase A (Hex A)

Mouse

BTA 10

Implementation of Genetic Information

- UK National Scrapie Plan for sheep
  - Polymorphisms at 136, 154 and 171 tested by PCR amplification and sequencing
  - Males with resistant genotypes used for breeding
- Currently data on genetics of BSE susceptibility insufficient
Current Diagnosis / Surveillance

Central Nervous System

Histopathology

Presence of PrP^{BSE}
# UK BSE Surveillance (2003)

<table>
<thead>
<tr>
<th>Year</th>
<th>Ongoing Surveys (Cattle)</th>
<th>Tested</th>
<th>Results pending</th>
<th>BSE not confirmed</th>
<th>BSE confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Fallen Stock Survey: Compulsory</td>
<td>89487</td>
<td>0</td>
<td>69402</td>
<td>85</td>
</tr>
<tr>
<td>2003</td>
<td>Casualties - on Farm/In transit</td>
<td>108995</td>
<td>0</td>
<td>108730</td>
<td>265</td>
</tr>
<tr>
<td>2003</td>
<td>Casualties sourced at OTMS Abattoirs</td>
<td>11176</td>
<td>0</td>
<td>11169</td>
<td>7</td>
</tr>
<tr>
<td>2003</td>
<td>24-30 month casualty cattle sourced at fresh meat abattoirs</td>
<td>1421</td>
<td>0</td>
<td>1421</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>Over Thirty Months Scheme - Random Animals (Before feed ban)</td>
<td>10847</td>
<td>0</td>
<td>10838</td>
<td>9</td>
</tr>
<tr>
<td>2003</td>
<td>Over Thirty Months Scheme - Animals born after July 1997 (12)</td>
<td>141337</td>
<td>0</td>
<td>141331</td>
<td>6</td>
</tr>
<tr>
<td>2003</td>
<td>Animals sampled as 96/97 cohort (10) (excluding fallen stock, casualties etc)</td>
<td>50943</td>
<td>0</td>
<td>50941</td>
<td>2</td>
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<tr>
<td>2003</td>
<td>Birth cohorts of BSE cases</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>BSE offspring (12)</td>
<td>365</td>
<td>0</td>
<td>365</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>Animals slaughtered for human consumption: Over thirty months (Beef Assurance Scheme)</td>
<td>108</td>
<td>0</td>
<td>108</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Animals born in 96/97 cohort (11) (including fallen stock, casualties etc)*</td>
<td>70418</td>
<td>0</td>
<td>70408</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Total for other cohort test categories as at 31 December 2003 (14)</td>
<td>324281</td>
<td>0</td>
<td>323917</td>
<td>364</td>
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<tr>
<td></td>
<td>All cattle tested between 01 January 2003 - 31 December 2003</td>
<td>394699</td>
<td>0</td>
<td>394325</td>
<td>374</td>
</tr>
</tbody>
</table>

= 1 in 1054

Source DEFRA Statistics

<table>
<thead>
<tr>
<th>Year</th>
<th>Ongoing Surveys (Cattle)</th>
<th>Tested</th>
<th>Results pending</th>
<th>BSE not confirmed</th>
<th>BSE confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Fallen Stock Survey: Compulsory</td>
<td>30911</td>
<td>8</td>
<td>30878</td>
<td>25</td>
</tr>
<tr>
<td>2004</td>
<td>Casualties - on Farm/In transit</td>
<td>49116</td>
<td>8</td>
<td>49048</td>
<td>60</td>
</tr>
<tr>
<td>2004</td>
<td>Casualties sourced at OTMS Abattoirs</td>
<td>4530</td>
<td>0</td>
<td>4527</td>
<td>3</td>
</tr>
<tr>
<td>2004</td>
<td>24-30 month casualty cattle sourced at fresh meat abattoirs</td>
<td>437</td>
<td>0</td>
<td>437</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>Over Thirty Months Scheme - Random Animals (Before feed ban)</td>
<td>3391</td>
<td>0</td>
<td>3390</td>
<td>1</td>
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<tr>
<td>2004</td>
<td>Over Thirty Months Scheme - Animals born after July 1997</td>
<td>44726</td>
<td>0</td>
<td>44726</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>Animals sampled as 96/97 cohort (excluding fallen stock, casualties etc)</td>
<td>39903</td>
<td>0</td>
<td>39903</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>Birth cohorts of BSE cases</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>BSE offspring</td>
<td>110</td>
<td>0</td>
<td>110</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>Animals slaughtered for human consumption: Over thirty months (Beef Assurance Scheme)</td>
<td>40</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Animals born in 96/97 cohort (including fallen stock, casualties etc)</td>
<td>47995</td>
<td>0</td>
<td>47995</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total for other cohort test categories as at 23 January 2004</td>
<td>125169</td>
<td>16</td>
<td>125064</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>All cattle tested between 01 January 2004 - 23 January 2004</td>
<td>173164</td>
<td>16</td>
<td>173059</td>
<td>89</td>
</tr>
</tbody>
</table>

Source: DEFRA Statistics

= 1 in 1944
**Erythroid Related Differentiation Factor**

**Differential Display of Gene Expression in Spleens of Healthy and Scrapie-Infected Mice**

- EDRF
- 18S rRNA
- Hemoglobin β chain
- Control
- TSE

**Mouse-Scrapie**

- Bone marrow
- Whole blood
- Control
- TSE

- EDRF
- Hemoglobin β chain
- 18S rRNA

Control

BSE

Macro/Microarrays

• Simultaneous comparison of expression of 23,000 genes
Gene Expression During BSE Infection

Comparison:

Uninfected vs 30 month pre-clinical BSE

From 23K probes
342 with varied expression
50 most convincing being followed
Summary

- Genetics
  - TSEs in man, sheep and mice are under genetic control
  - Evidence of genetic control of BSE in cattle is limited
    - PrP? Other Loci?

- Diagnosis
  - PrP$^{sc}$ is central to disease but additional markers, for early diagnosis are required:
  - Other genes seem to be regulated in brain
  - Live test requires markers in blood
    - EDRF – down regulated in erythroid lineage
    - Potential for addition markers in blood
Acknowledgements

- David Hills
- Pam Wiener
- Jules Hernandez-Sanchez
- Dave Waddington
- Mike Clinton
- Gino Miele
- Richard Moore
- Iain Maclean

- MAFF / DEFRA
- European Commission
- BBSRC

- Steve Hawkins
- Danny Matthews
- Judy Ryan
- John Wilesmith
  - VLA Weybridge
- Luca Ferretti
- Sergio Comencini
- Maria Forti
  - U Pavia, Italy
- Hans Lerach
- Michal Janitz
- Ralf Herwig
  - MPI BERLIN