# ATYPICAL BSE CASES IN IRELAND: NEUROLOGICAL SIGNS, BRAIN HISTOPATHOLOGY AND TISSUE DISTRIBUTION OF $PrP^{res}$

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## INTRODUCTION

Atypical bovine spongiform encephalopathy (BSE) is a prion disease, generally of older cattle with a low and relatively constant prevalence.[1]

Two types have been described, L-type and H-type, that differ from each other and from classical BSE on the basis of the molecular characteristics of the prion protein,[2] the distribution and type of disease-associated prion protein in the brain.[3]

In Ireland, BSE was first diagnosed in 1989 and up to May 2023 a total of 1663 cases have been diagnosed, of which, five H-type (H-1 to -5), and one L-type.

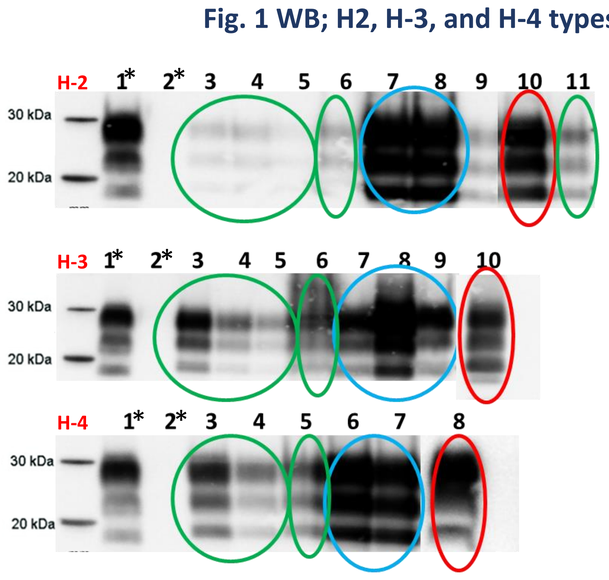
## RESULTS: CLINICAL HISTORY

All animals were beef-breed females, and had vague clinical histories of depression, inappetence, incoordination, and recumbency, lasting different time (Tab. 1).

**Tab. 1: Anamnesis and clinical history**

| Case | Age | Year of conf | Clinical signs | Category |
|---|---|---|---|---|
| **H-1** | 11y | 2002 | 'Tetany like illness' – recovered for 1 day; suspected injured back; recumbency. | Fallen stock |
| **H-2** | 16y 6m | 2010 | None. Culled due to old age. | Healthy slaughter |
| **H-3** | 14y 3m | 2011 | Staring; grinding teeth; incoordination; eventual recumbency. Duration not recorded. | Fallen stock |
| **H-4** | 14y 9m | 2013 | 'Moping and inappetant' 3-4 days prior to euthanasia. Recumbent in last 24 hours. | Fallen stock |
| **H-5** | 14y 2m | 2020 | 'Getting stiff' 6 weeks prior to death. Incoordination. Intermittent recumbency. | Fallen stock |
| **L-type** | 18y 10m | 2015 | 'Getting stiff' 2 weeks prior to euthanasia. Intermittent recumbency in the 2 days prior to death. | Fallen stock |

**Fig. 1 WB; H2, H-3, and H-4 types (above), and L-type (below)**

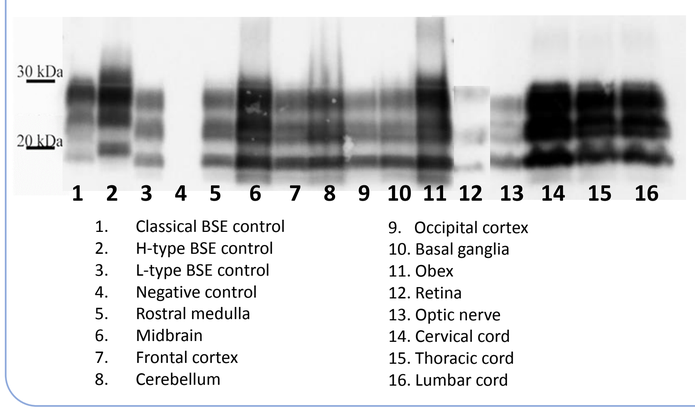



1. *Classical BSE control
2. *Negative control
3. Frontal cortex
4. Parietal cortex
5. Occipital cortex
6. Cerebellum
7. Thalamus
8. Midbrain
9. Hippocampus
10. Medulla - obex
11. Cerebellum

3. Frontal cortex
4. Parietal cortex
5. Occipital cortex
6. Cerebellum
7. Basal ganglia
8. Midbrain
9. Thalamus
10. Medulla - obex

3. Frontal cortex
4. Occipital cortex
5. Cerebellum
6. Basal ganglia
7. Thalamus
8. Medulla - obex

- $PrP^{res}$ distributed through all levels of brain
- Medulla consistently strong signal
- Thalamus, midbrain, basal ganglia consistently strong
- Cerebral cortex, cerebellum with lower signal in the healthy slaughter




1. Classical BSE control
2. H-type BSE control
3. L-type BSE control
4. Negative control
5. Rostral medulla
6. Midbrain
7. Frontal cortex
8. Cerebellum
9. Occipital cortex
10. Basal ganglia
11. Obex
12. Retina
13. Optic nerve
14. Cervical cord
15. Thoracic cord
16. Lumbar cord

- Strong signal at all levels of neuraxis
- Moderate to weak signal in retina and optic nerve
- No signal detected in other tissues

(Blots with Sha31 only shown)

**Tab. 2: Idexx EIA; H-2, -3, and -4 types, and C-BSE (comparison)**

| Case | Frontal Cortex | Parietal Cortex | Occipital Cortex | Basal Ganglia | Thalamus | Midbrain | C'bellum | Rostral Medulla | Obex |
|---|---|---|---|---|---|---|---|---|---|
| **C-BSE** | **1.15** | **1.24** | **nd** | **nd** | **3.83** | **3.66** | **3.72** | **nd** | **3.5** |
| **H-2** | 1.15 | .622 | .613 | nd | 4.02 | 4.192 | 1.372 | 3.9 | 3.8 |
| **H-3** | 3.75 | 3.12 | nd | 4.04 | 4.07 | 4.3 | 4.03 | nd | 3.77 |
| **H-4** | 3.6 | nd | 2.99 | 2.75 | 3.91 | 4.18 | 3.50 | 4.06 | 3.5 |

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## OBJECTIVES AND MATERIALS AND METHODS

To describe the neurological characteristics, brain histopathology, topographical distribution, and signal intensity of $PrP^{res}$.

All cases were identified through active and passive surveillance using commercial kit (Idexx HerdChek BSE/Scrapie Ultrashort Protocol).

**Clinical history** was retrieved from the DAFM archives.

Whole brains/brainstems of H-type animals, and the L-type, and selected peripheral tissues of L-type were further studied by:

**Histopathology** (suitable obices of H-1, -2 and -5, and the whole brain of H-5);

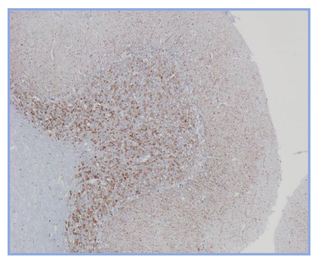
**Immunohistochemistry** (IHC - MAb F89);

**Immunoblotting** (APHA BioRad TeSeE Hybrid). Investigations on $PrP^{res}$ distribution on the H-5 ongoing.

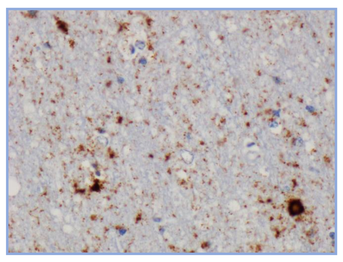
## RESULTS: HISTOPATHOLOGY

H-type: Among the suitable obices for histopathology (H-1, -2 and -5), and the whole brain of H-5, vacuolation was only detected in H-5 only.

L-type: Inconclusive changes at level of obex. Neuropil vacuolation was most marked in thalamus and midbrain.



L-type. Cerebellum - diffuse granular staining in molecular and granular layers (4x).



L-type. Cerebrum - subcortical coarse granular immunostaining and plaques (40x)

## RESULTS: IHC

H-type: Positive immunostaining at the obex (H-1), in medulla, thalamus, cerebellum (H-2), and at all levels of the brain (H-3, H-5).

L-type: Positive staining at all levels of neuraxis and in optic nerve.

## RESULTS: IMMUNOBLOT AND IDEXX EIA

H-type: In the fallen cases, WB and Idexx EIA were consistently strong in all brain levels. In the healthy slaughtered (H-2), $PrP^{res}$ levels were lower in cerebellum and cerebral cortex (Fig. 1; Tab. 2). Much of the brain tissue available for 3 of the cases.

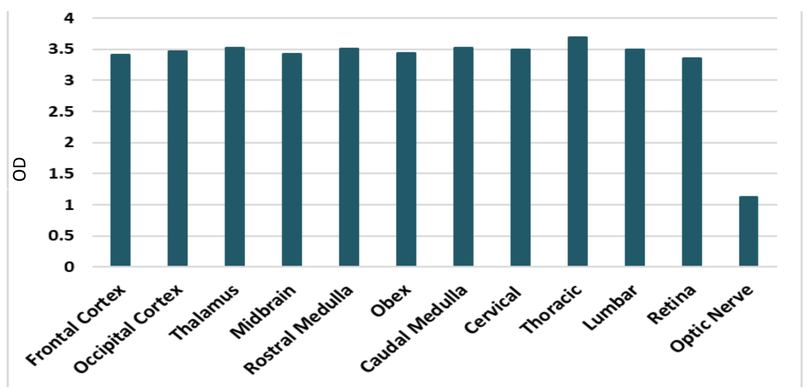
L-type (47 different neuronal and extraneuronal tissues tested): $PrP^{res}$ was detected by immunoblotting, Idexx EIA and IHC at all levels of the neuraxis, and immunoblotting only in the optic nerve and retina (Fig. 1; Tab. 3; Chart 2).

**Tab. 3: $PrP^{res}$ tissue distribution in L-type using Idexx EIA, immunoblot and IHC**

| Tissue | Idexx EIA | Immunoblot | IHC |
|---|---|---|---|
| ***Central Nervous System*** | | | |
| **Brain (all levels)** | **Positive** | **Positive** | **Positive** |
| **Spinal cord (all levels)** | **Positive** | **Positive** | **Positive** |
| **Optic nerve** | **Positive** | **Positive** | **Positive** |
| **Retina** | **Positive** | **Positive** | nd |
| ***Gastrointestinal Tissues*** | | | |
| **Rumen** | Negative | nd | nd |
| **Abomasum** | Negative | nd | nd |
| **Duodenum** | Negative | Negative | Negative |
| **Caecum** | Negative | Negative | Negative |
| **Colon** | Negative | Negative | nd |
| **Rectum** | Negative | nd | nd |
| ***Peripheral Nervous System*** | | | |
| **Multiple** | Negative | nd | nd |
| ***Lymphoid Tissue*** | | | |
| **Multiple** | Negative | nd | Negative |
| ***Skeletal Muscle*** | | | |
| **Multiple** | Negative | nd | Negative |
| **Tongue** | Negative | nd | nd |
| ***Other Tissues*** | | | |
| **Kidney** | Negative | nd | nd |
| **Liver** | Negative | nd | nd |
| **Mammary gland** | Negative | nd | nd |
| **Mesenteric fat** | Negative | nd | nd |
| **Nasal mucosa** | Negative | nd | nd |

nd = not done

**Chart 1: IDEXX EIA OD values; L-type case**




## CONCLUSIONS

Clinical courses were generally short and non-specific (similar to experimental atypical cases).

$PrP^{res}$ intensity in all cases was generally high at all levels of the brain tested including the obex, the official target area for BSE surveillance.

**REFERENCES**

1. EU Commission Report on the monitoring and testing of ruminants for the presence of TSE in the EU in 2014
2. Jacobs JG *et al.* 2007.J Clin Microbiol, 25, 1821-1829.
3. Priemer G *et al.* 2013. PLoS ONE 8(6): e67599.doi:10.1371/journal.pone.0067599

**ACKNOWLEDGEMENTS**

Colleagues in Regional Veterinary Laboratories for collecting the brain material. Colleagues in TSE Division and RVOs for clinical information on cases. Identigen, and Enfer Labs for performing some of the Idexx tests.