



# Discrimination of Classical and Atypical BSE by a Distinct Immunohistochemical PrP<sup>Sc</sup> Profile

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> Vet Rec. 1987 Oct 31;121(18):419-20. doi: 10.1136/vr.121.18.419.

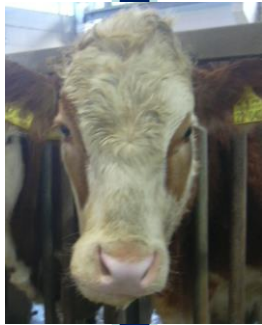
## A novel progressive spongiform encephalopathy in cattle

G A Wells<sup>1</sup>, A C Scott, C T Johnson, R F Gunning, R D Hancock, M Jeffrey, M Dawson, R Bradley

Affiliations + expand

PMID: 3424605 DOI: 10.1136/vr.121.18.419

1987



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

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2004

EMBO  
reports

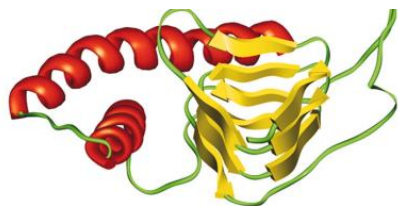
scientific report

## Distinct molecular phenotypes in bovine prion diseases

Anne-Gaëlle Biacabe<sup>1</sup>, Jean-Louis Laplanche<sup>2</sup>, Stephen Ryder<sup>3</sup> & Thierry Baron<sup>1\*</sup>

<sup>1</sup>AFSSA-Lyon, Unité "Virologie-ATNC", Lyon, France, <sup>2</sup>UPRES EA 3621, Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris, Paris, France, and <sup>3</sup>Neuropathology Unit, Department of Pathology, Veterinary Laboratory

2004





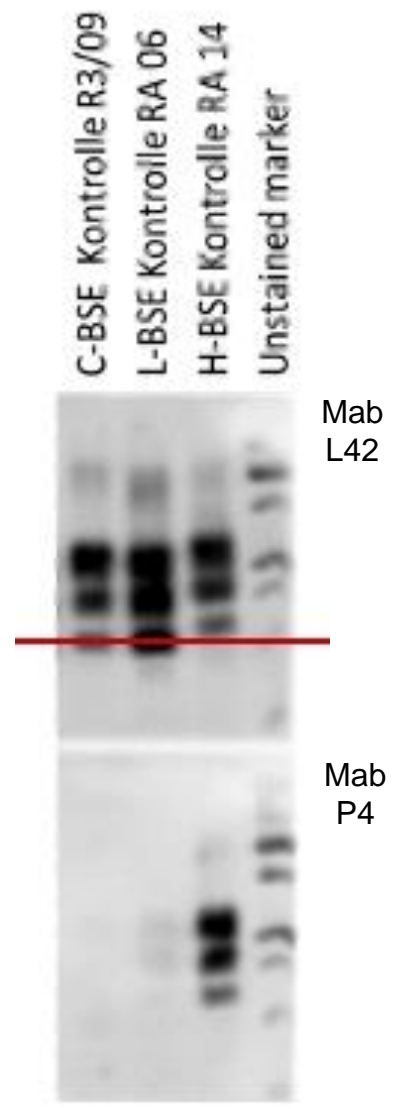
# Discrimination of BSE-types

PrP<sup>Sc</sup> molecular signature of H- and L-type BSE differed from C-BSE in terms of protease-resistant fragment size and glycoform pattern



## Discriminatory Immunoblot

	C-Type	L-Type	H-Type
2x glycos band	Most distinct band	<40%, comparable with unglycosylated band	Most distinct band
P4/L42	L42 >>> P4	L42 >>> P4	P4 > L42
MW unglyco-sylated band	Medium	Low	High





## **Aim of the study:**

**Establishing immunohistochemistry as an additional tool to discriminate BSE types**



Table S2: PrP<sup>Sc</sup> profiles in brainstem described in literature for C-, H- and L-type BSE infected cattle (literature review)

BSE	DMNV	Nc. Sol. Trac.	Nc. Trigon.	Olive	Ret. Form	Brainstem in	Others/Remarks	
C-type (field)	PrP <sup>Sc</sup> ++[1] FAKTH[1] F-C-PART++[4] C-PART-CoL[1] CoL[1] ITMG[2][14] STEL++[4] ITNR[10][14] ITNR[10][14] PNER[10][14] LIN++[1]	PrP <sup>Sc</sup> ++[1] FAKTH[1] Agg.[1] F-C-PART++[4] CoL[1] CoL[1] ITMG[2][14] STEL++[4] ITNR[10][14] ITNR[10][14] PNER[10][14] LIN++[1]	PrP <sup>Sc</sup> ++[1]					
	PrP <sup>Sc</sup> ++[1]	PrP <sup>Sc</sup> ++(+)[1] or ++[1]						
	No ITNR[1] ITMG[1]	ITMG[1]						

Table S3: PrP<sup>Sc</sup> profiles in different brain regions described in literature for C-, H- and L-type BSE infected cattle (literature review)

BSE	Cerebellum	Midbrain	Thalamus	Hypothalamus and Hippocampus	Cerebrum	Other brain regions	Remarks
C-type (field)	Centr. F-C-PART[1] ITNR[1] PNER[1] STEL++[1] MLay-STE[1] Molau:	PrP <sup>Sc</sup> ++[1] FAKTH[1] FAKTH[1] FAKTH[1] STEL++[1] ITNR[1] Molau: PNER[1] F-C-PART[1] ITNR[1] STEL[1] LIN[1] Ventr-STE[1] F-C-PART[1]	PrP <sup>Sc</sup> ++[1] FAKTH[1] FAKTH[1] FAKTH[1] STEL++[1] ITNR[1] Molau: PNER[1] FL[1]	Hyp-PrP <sup>Sc</sup> ++[1] ITNR[1] FAKTH[1] Agg.[1]	Centr. PrP <sup>Sc</sup> ++[1] FAKTH[1] FAKTH[1] STEL[1] ITNR[1] Molau: FL[1]	CS-F-C-PART[1] FAKTH[1] STEL[1] LIN[1] OB[1] LIN Caud-Put: ITNR[1] STEL[1] LIN[1] Sept.: ITNR[1] LIN[1] Str. Pyr.[1] PNER[1] Str. Oriens /	Cerebral Cortex the least affected[1]
	WB PrP <sup>Sc</sup> ++[1] GLay: (+)[1] in general: FAKTH[1] ITNR[1]	PrP <sup>Sc</sup> ++[1] FAKTH[1] ITMG[1]	PrP <sup>Sc</sup> ++[1] FAKTH[1] ITNR[1]	Hyp-PrP <sup>Sc</sup> negative[1]	Centr. PrP <sup>Sc</sup> ++[1] FAKTH[1] ITNR[1]	SE PrP <sup>Sc</sup> negative[1]	NA
	H-type (field)	Centr. STEL[1] MLay PrP <sup>Sc</sup> (+)[1] or (+), FAKTH[1] multifocal[1]	PrP <sup>Sc</sup> PrP <sup>Sc</sup> ++[1] F-C-PART[1] ITMG[1] WM: ITNR[1] in different Nc.	PrP <sup>Sc</sup> PrP <sup>Sc</sup> ++[1] STE[1] ITNR[1] PNER[1]	Hyp-PrP <sup>Sc</sup> ++[1] F-C-PART[1] STEL[1] Hyp-PrP <sup>Sc</sup> ++[1] CoL[1] STEL[1]	Centr. PrP <sup>Sc</sup> ++[1] FAKTH[1] CoL[1] ITMG[1] ITNR[1]	Extensive FAKTH but distinct ITMG/STEL[1] No ITNR with SAF2 and B103 (N-terminal)[1]

L-type (field)	PrP <sup>Sc</sup> (+)[1] Negative[1] Agg.[1]	Agg.[1]					
L-type (IC)	ITNR[1]	ITNR[1]					
L-type (oral)	PrP <sup>Sc</sup> ++[1]	NA	NA				

Legend: DMNV = Dorsal motor nucleus of the vagus nerve; Nucleus hypoglossus; Vest. Compl. = Vestibular nucleus; nuclei/nuclei; PrP<sup>Sc</sup> = quantity of PrP<sup>Sc</sup>: (+) = weak, ++ = moderate, +++ = severe appearance; F-C-PART-Col. = fine (F) and/or coarse (C); I: intraneuronal; PNER = perineuronal; LIN = linear; Agg. = aggregate; FL = plaque; NA = not available; anti-PrP antibodies used recognize the core region of the protein; F199[1], F239[1], R142[1], R142[1], H42[1], T12[1], T12[1], T12[1], T12[1]

Fast et al. 2023

# Literature Review

## Numerous, in parts contrasting results

### *BUT*

## Indications for discriminatory potential in certain areas



## Systematic examination necessary!

Legend: Nc./Nc. = nucleus/nuclei; MLay = Molecular layer; GLay = Granular layer; WM = white matter; Subst. = Substantia; Centr. Gey = Central grey matter; Hyp. = Hypothalamus; Hip. = Hippocampus; Str. = Stratum; Pyr. = Corpus striatum; Caud.-Put. = Nc. caudatus-Putamen; Nucleus; OB = Olfactory bulb; LoP = Lobus piriformis; Claustr. = Claustrum; PrP<sup>Sc</sup> = quantity of PrP<sup>Sc</sup>: (+) = weak, ++ = moderate, +++ = severe accumulation of PrP<sup>Sc</sup>; FAKTH = F-amyloid; F-C-PART-Col. = fine (F) and/or coarse (C); PART = plaque; LIN = linear; Agg. = aggregate; FL = plaque; NA = not available; anti-PrP antibodies used recognize the core region of the protein; F199[1], F239[1], R142[1], R142[1], H42[1], T12[1], T12[1], T12[1], T12[1], T12[1]



BSE Type	Animal ID	Incubation Time (mpi)	Lesions (H&E)	Obex/PrP <sup>Sc</sup> Deposition
C-Type Canada	25015	26	++	+++
	25022	27	++	+++
	25023	27	++	+++
	25032	26	+	+++
	25034	24	++	+++
	29024	20	+	++
	29026	18	(+)	++
H-Type Canada	29018	18	+	++
	29033	17	+	++
L-Type Canada	29012	18	++	++
	29030	17	++	+
H-Type Germany	RA10	12	(+)	+
	RA13	15	++	+++
	RA14	14	+	++
	RA15	16	+	+++
	RA16	16	++	+++
L-Type Germany	RA02	17	+++	+++
	RA03	16	++	++
	RA04	16	+++	+++
	RA05	11	(+)	(+)
	RA06	14	++	+

## Material available

i.c. inoculation

Inocula:  
1ml of 10% brain homogenate

Inoculation Site:  
(Rostral) Midbrain



BSE Type	Animal ID	Incubation Time (mpi)	Lesions (H&E)	Obex/PrP <sup>Sc</sup> Deposition
C-Type Canada	25015	26	++	+++
	25022	27	++	+++
	25023	27	++	+++
	25032	26	+	+++
	25034	24	++	+++
	29024	20	+	++
	29026	18	(+)	++
H-Type Canada	29018	18	+	++
	29033	17	+	++
L-Type Canada	29012	18	++	++
	29030	17	++	+
H-Type Germany	RA10	12	(+)	+
	RA13	15	++	+++
	RA14	14	+	++
	RA15	16	+	+++
	RA16	16	++	+++
L-Type Germany	RA02	17	+++	+++
	RA03	16	++	++
	RA04	16	+++	+++
	RA05	11	(+)	(+)
	RA06	14	++	+
Oral C-Type Germany	IT18	50	+++	+++
	IT23	36	++	+++
	IT49	36	+++	+++

## Material available

i.c. inoculation

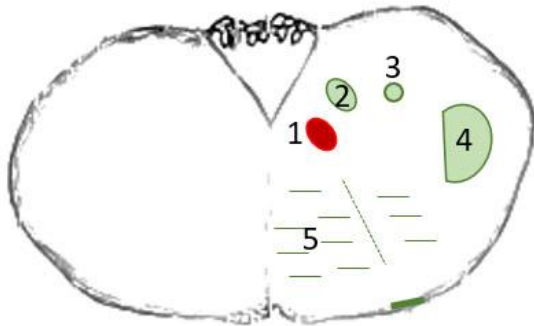
Inocula:  
1ml of 10% brain homogenate

Inoculation Site:  
(Rostral) Midbrain

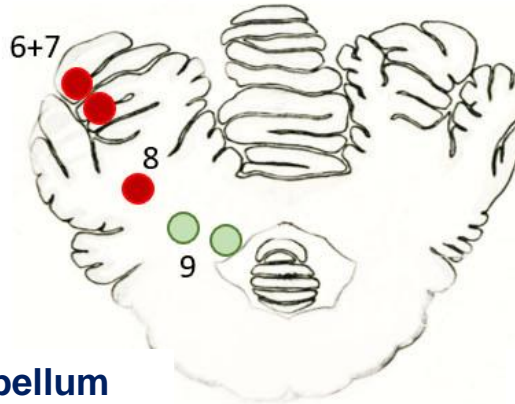
Oral inoculation  
100 ml 10% brain homogenate



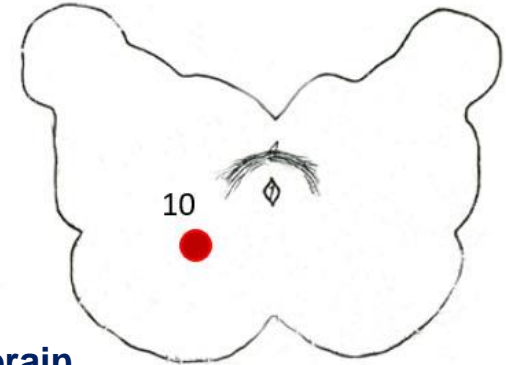
## 17 Regions examined:



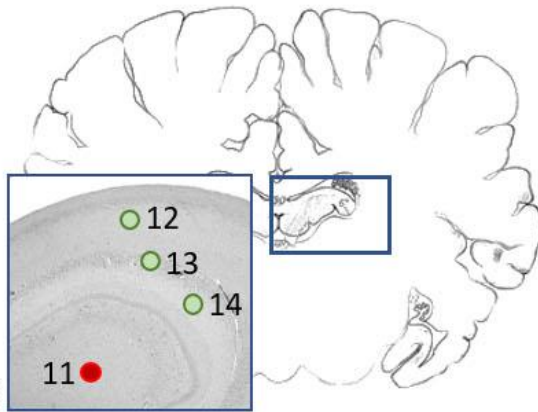
**Brainstem**



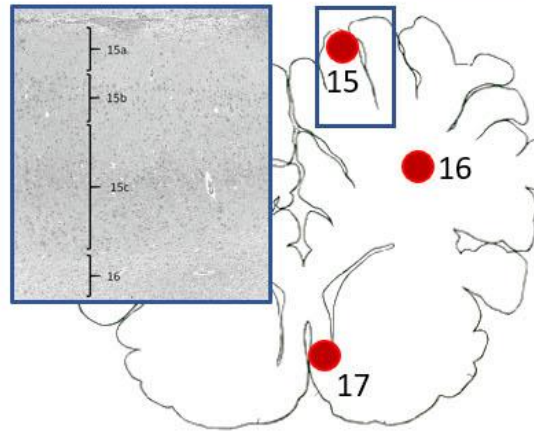
**Cerebellum**



**Mid brain**



**Hippocampus**



**Frontal Cortex and Septum**



**Lesion Profile**

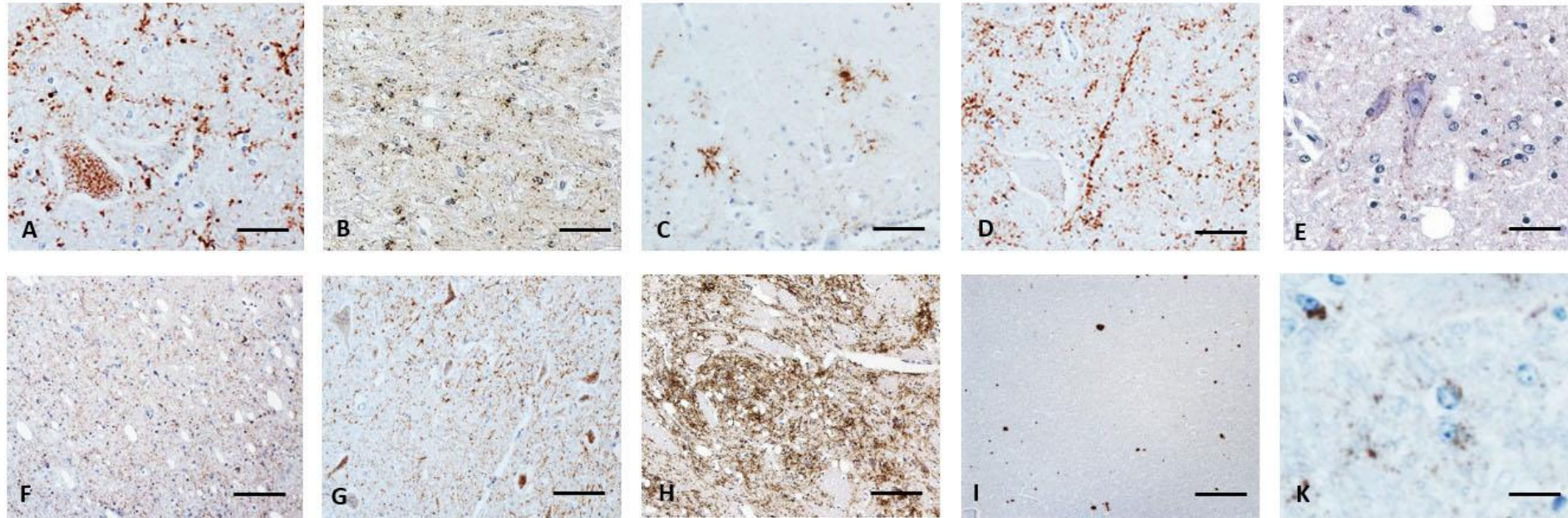
**PrP<sup>Sc</sup> Profile**





## 11 PrP<sup>Sc</sup> Reaction Pattern

➔ mab F99



- (A) intraneuronal (ITNR);
- (B) intramicroglial (ITMG);
- (C) stellate (STEL);
- (D) linear (LIN);
- (E) perineuronal (PNER);
- (F) fine particulate (f-PART);
- (G) coarse particulate (c-PART);
- (H) coalescing (COL);
- (I) plaque-like (PL)
- (J) Intra-astrocytic (ITAS);
- Submengeal (SMENG) is not shown

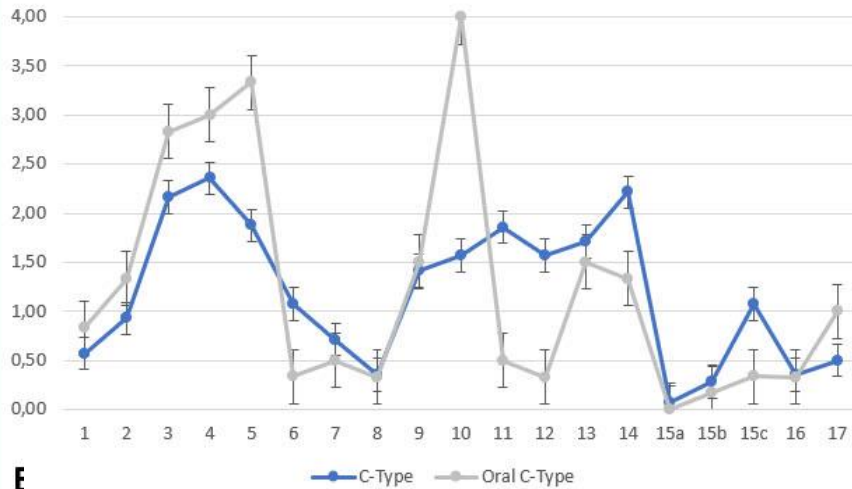


## Lesion Profile



## Lesion profile (H.&E.)

C-type BSE: oral vs. intracerebral Inoculation

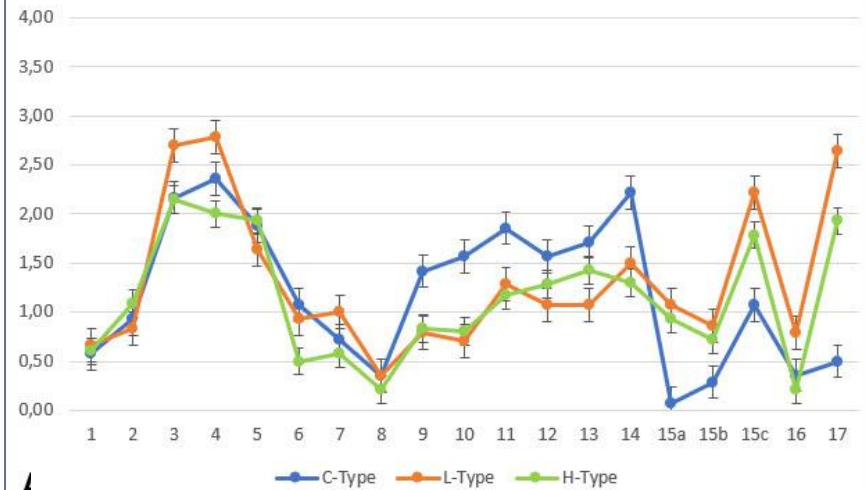


Highly accordant, but for Red nucleus in C-type

Frontal Cortex distinctly involved in Atypical BSE, but not in C-type



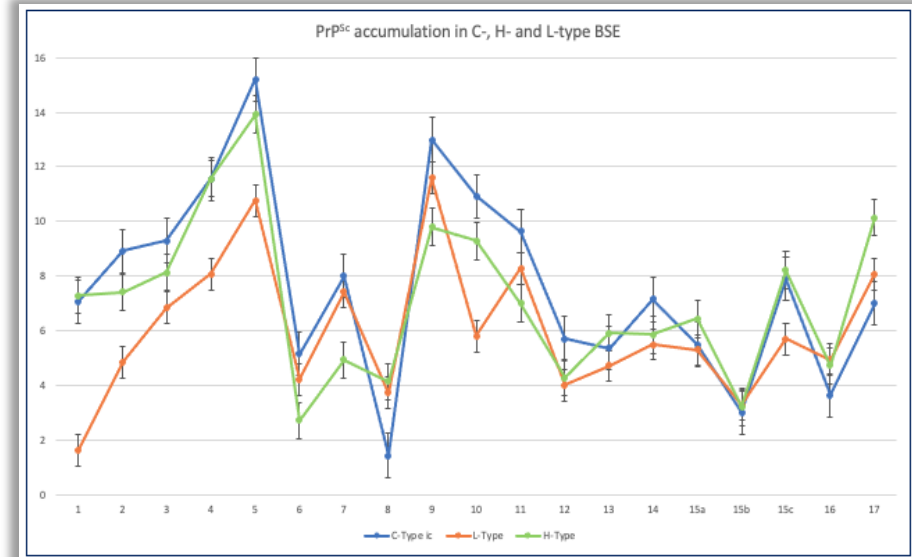
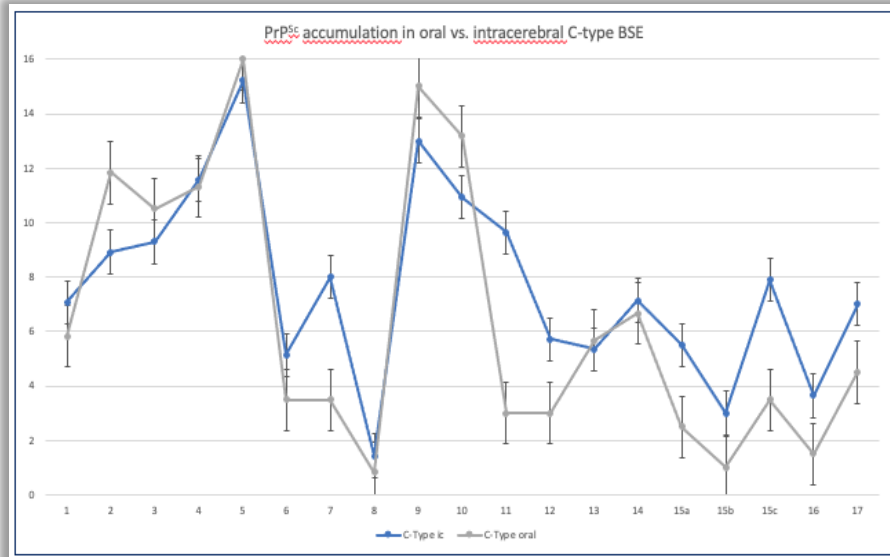
BSE Types / Lesion Profile







## PrP<sup>Sc</sup> profile in general



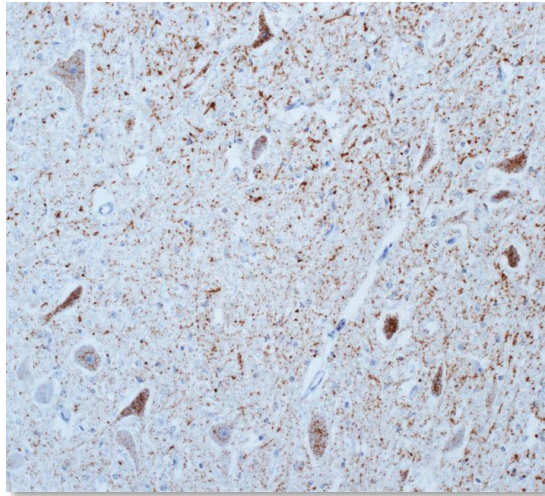
The extent of the different PrP<sup>Sc</sup> reaction patterns in each brain region was determined for each animal, and the following scores were used: weak (score 1.0), mild (2.0), moderate (3.0), and severe (4.0). These scores were then totalled to determine the total amount of PrP<sup>Sc</sup> accumulation for each brain region for each animal. For the graphs the average of all animals per group (C-, H-, and L-type and orally infected C-type) was determined for each brain region.



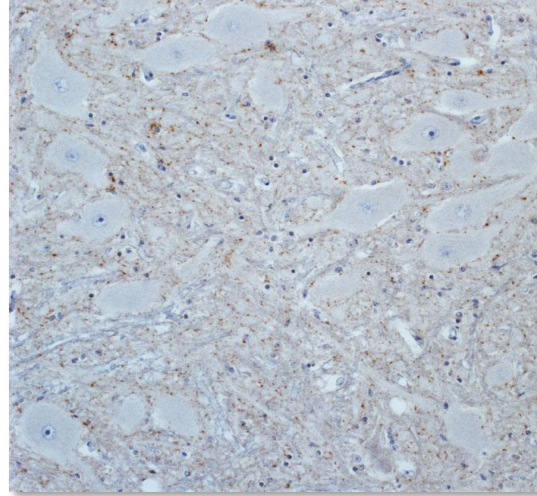
**Overall PrP<sup>Sc</sup> profiles showed a high similarity, independently of BSE type and route of infection**



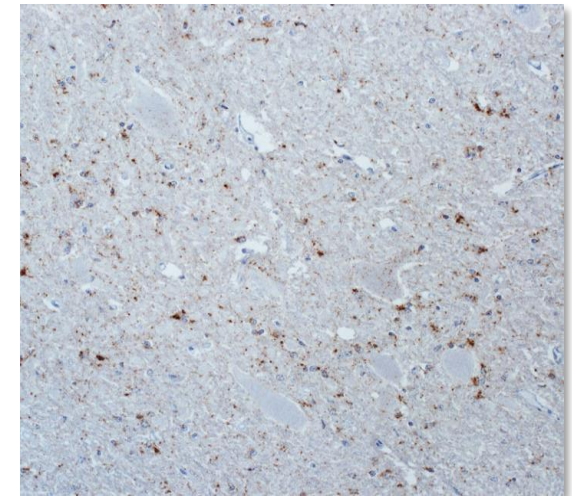
## PrP<sup>Sc</sup> profile / Nc. Hypoglossus (obex)



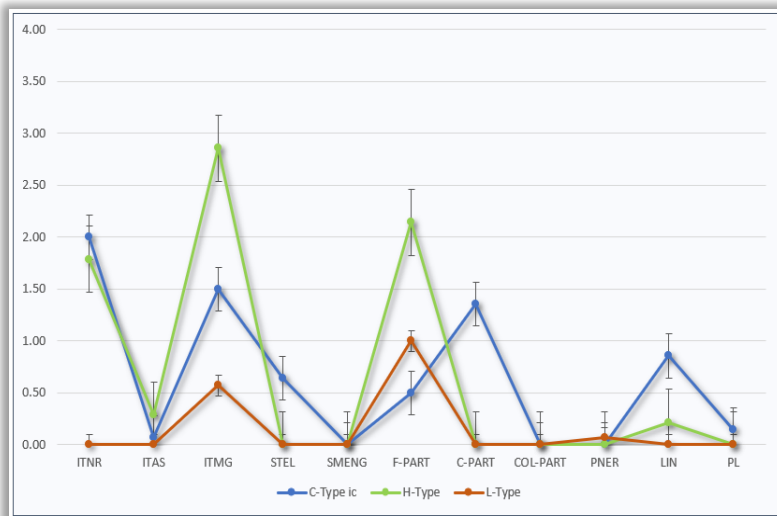
C-type



L-type



H-type



**L-type:**

No ITNR staining reaction

**H-type:**

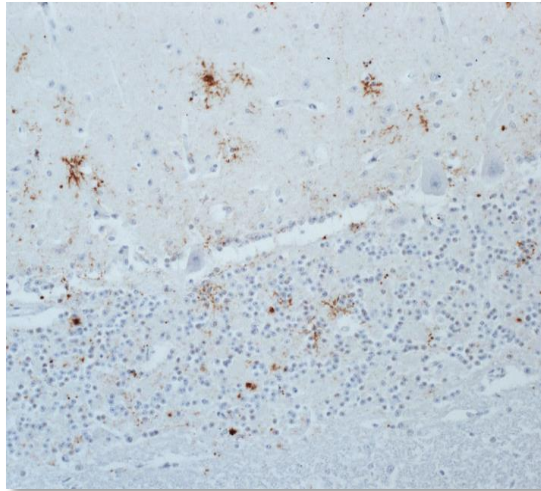
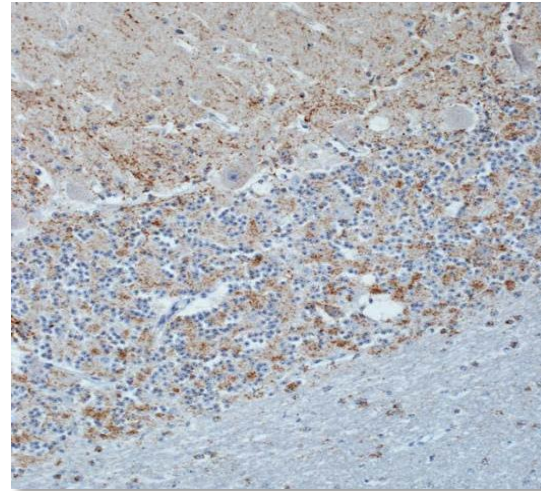
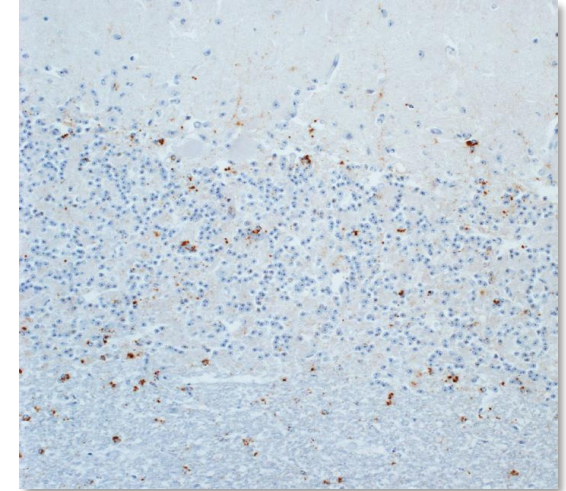
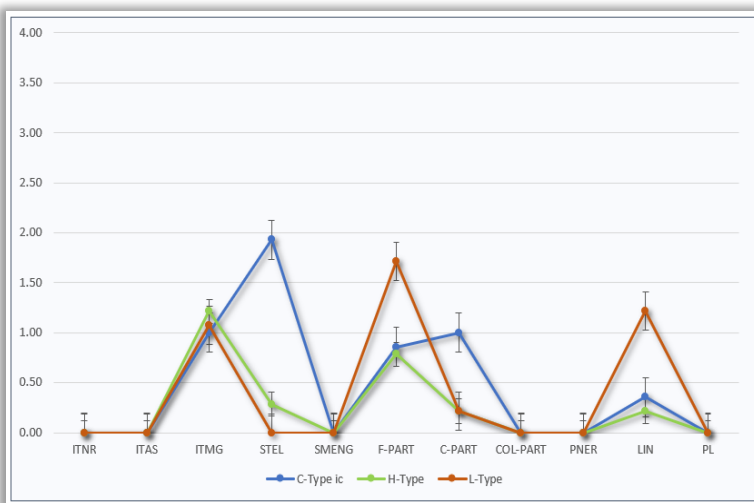
Distinct ITMG reaction pattern

**C-type**

Mixed pattern and distinct coarse-PART

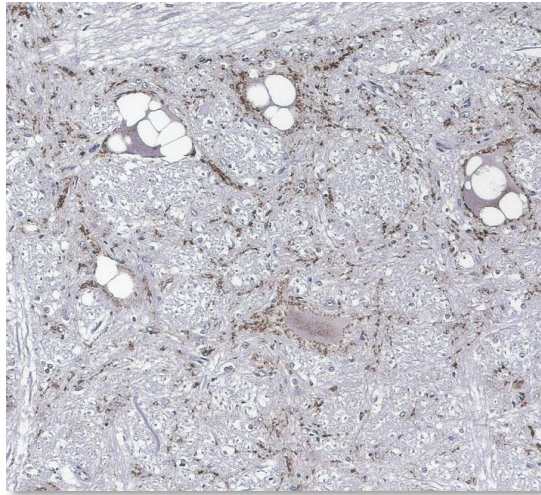


## PrP<sup>Sc</sup> profile / Cerebellum (Molecular + Granular Layer)

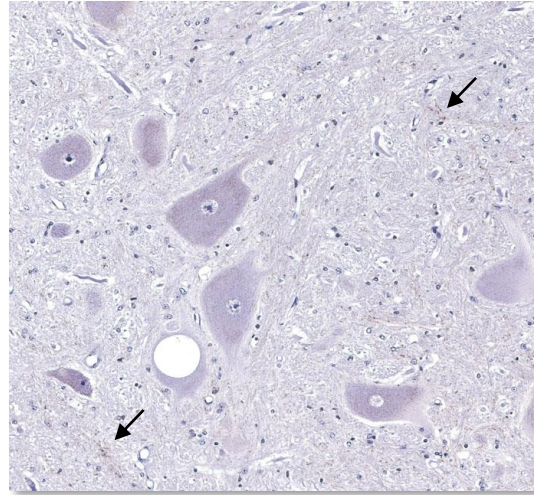
**C-type****L-type****H-type****L-type:****Diffuse fine PrP<sup>Sc</sup> deposition****H-type:****Distinct ITMG reaction pattern****C-type****Prominent stellate staining reaction**



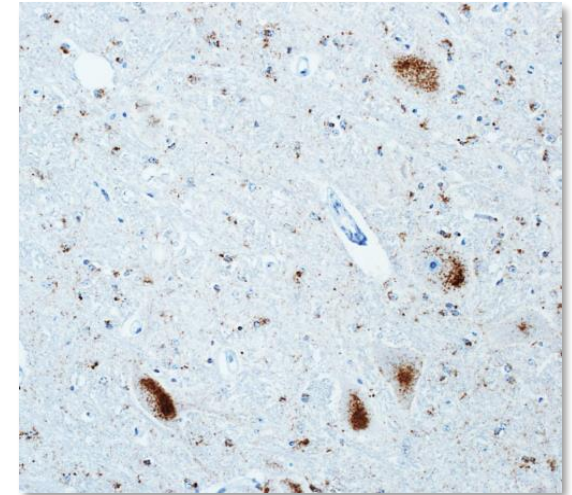
## PrP<sup>Sc</sup> profile / Red nucleus



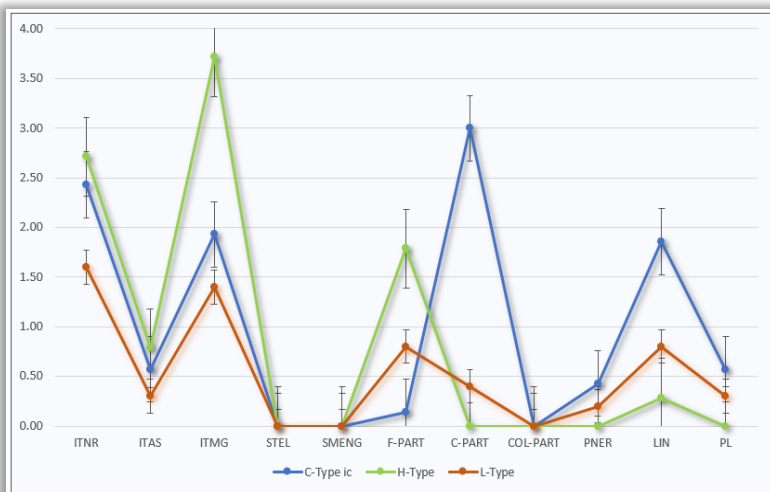
C-type



L-type



H-type



**L-type:**

**Multifocal fine PART**

**H-type:**

**Distinct ITMG reaction pattern**

**C-type**

**Highly variable staining reaction**

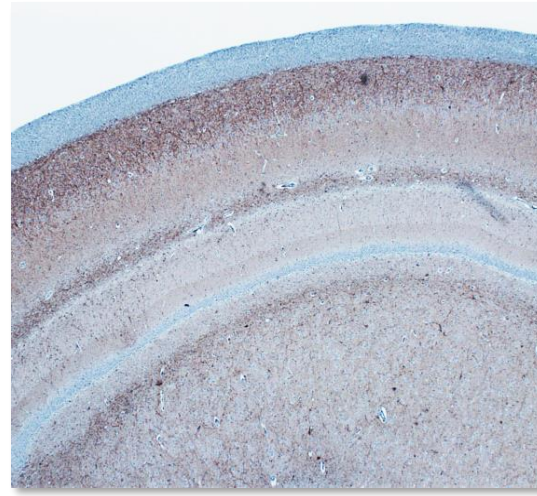




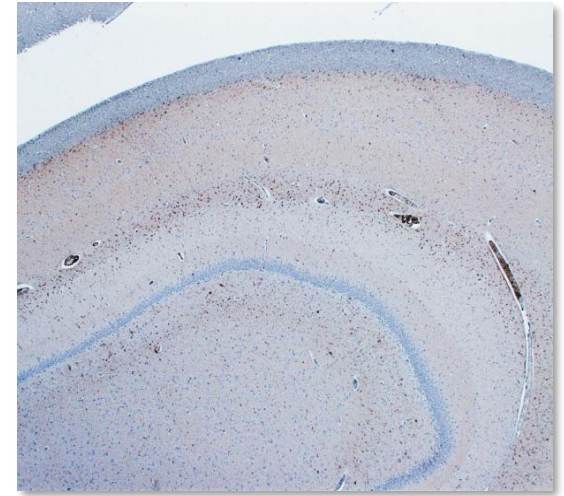
## PrP<sup>Sc</sup> profile / Hippocampus (Hilus)



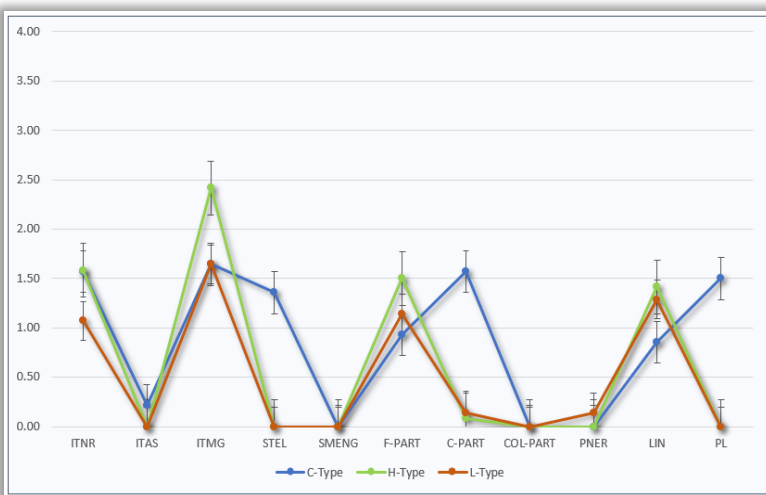
C-type



L-type



H-type

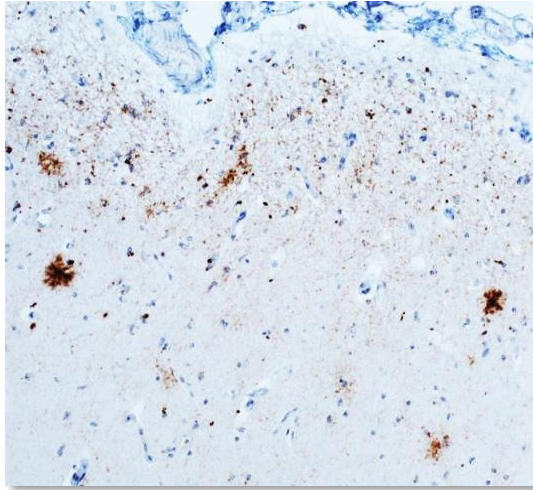


**L-type / H-type:**  
Mainly in peripheral parts of hilus

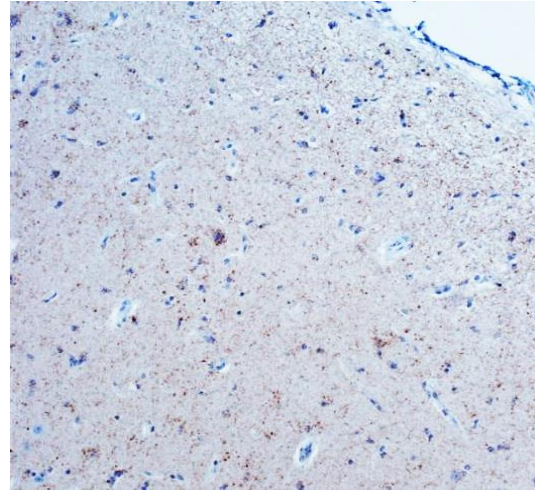
**C-type**  
Predominant in central parts of hilus



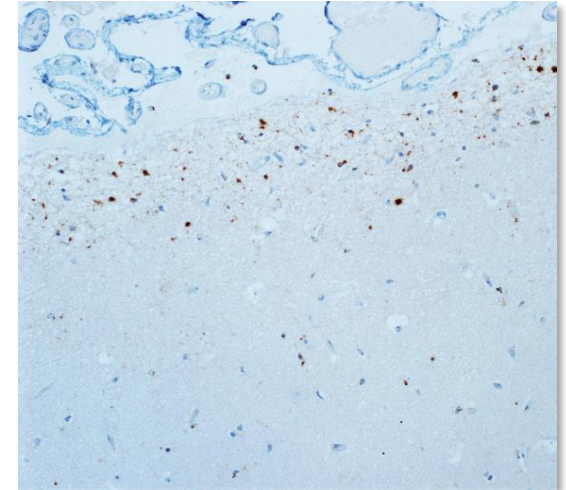
## PrP<sup>Sc</sup> profile / Cerebrum (Molecular Layer)



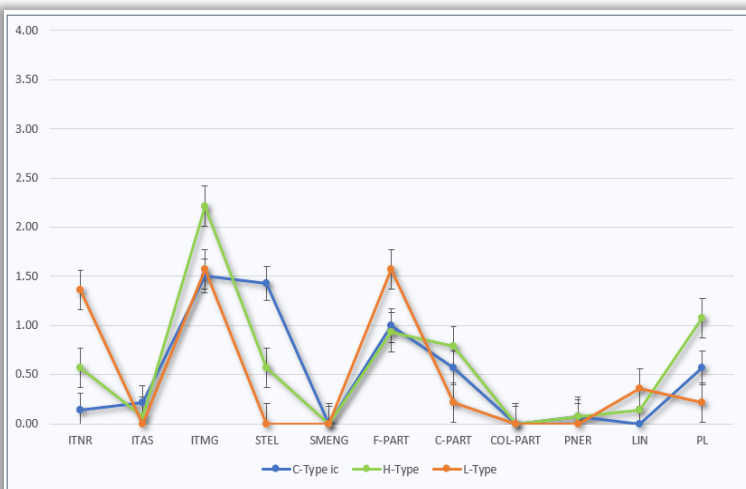
C-type



L-type



H-type



**L-type:**

Diffuse fine PART

**H-type:**

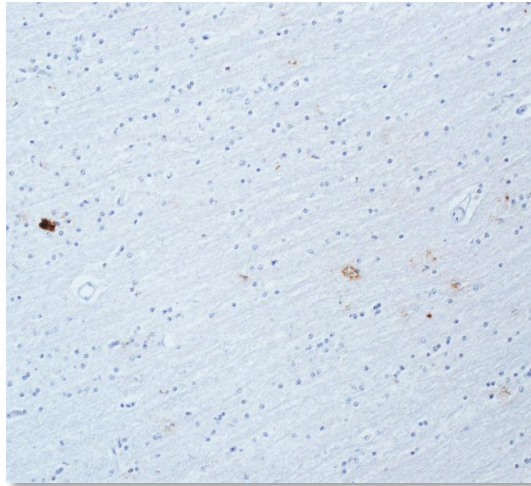
Distinct ITMG, mostly tape-like

**C-type**

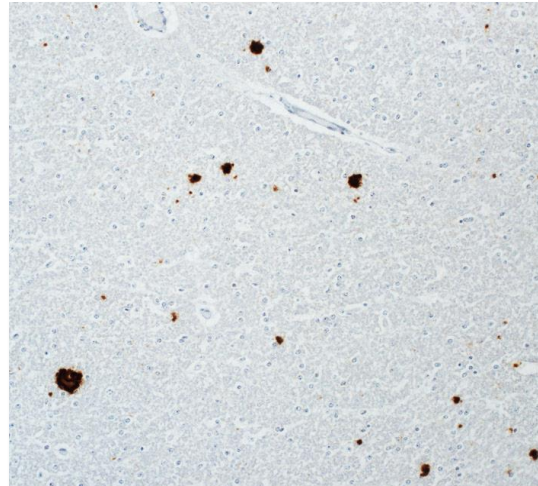
Tape-like pattern, multifocal STELL



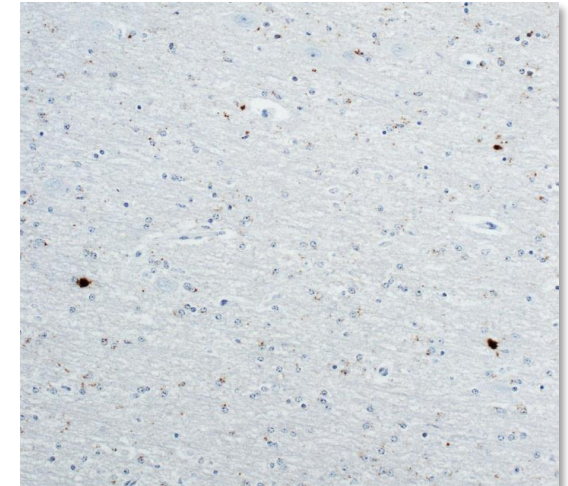
## PrP<sup>Sc</sup> profile / Cerebrum (White matter)



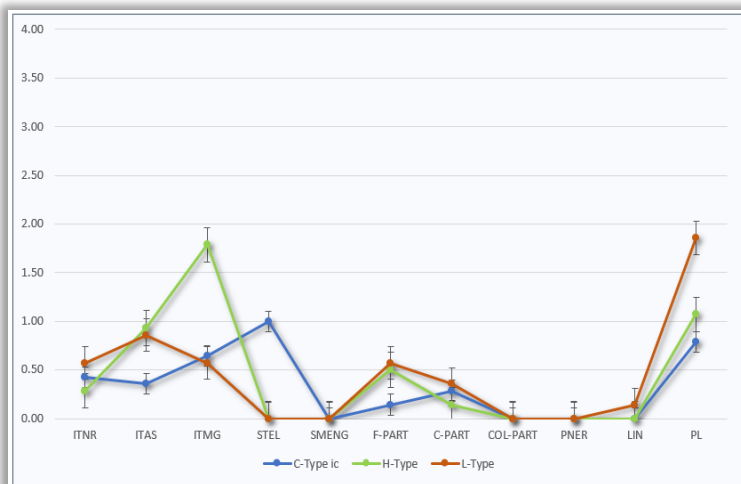
C-type



L-type



H-type



**L-type:**

**Predominant PL pattern**

**H-type:**

**Distinct ITMG**

**C-type**

**Multifocal STELL PrP<sup>Sc</sup> accumulation**



## Take Home Message

### Lesion and PrP<sup>Sc</sup> profile with slight differences between oral and i.c. C-type infected animals

➔ *Results of intracerebral studies are comparable to field cases*

### High similarity of the general PrP<sup>Sc</sup> profile independently of the BSE type and the route of infection

➔ *Depending on on consistent PrP<sup>C</sup> distribuion in cattle brain?*

### Qualitative differences of the cellular reaction pattern in a selection of neuro-anatomical structures

➔ *Nc. Hypoglossus; Cerebellum (Molecular-/Granular layer); Red nucleus; Hippocampus (Hilus); Septal nucleus (data no shown); Cerebrum (Molecular layer, White matter)*

### Characteristic cellular PrP<sup>Sc</sup> pattern for each BSE type throughout the brain

➔ *C-type highly variable; L-type diffuse fine PART; H-type distinct ITMG*



## However!

Field cases are usually autolytic

In most field cases only brain stem and (with some luck) cerebellum are available



It remains unclear if this approach works with routine submissions!



Do you have formalinfixated/paraffin embedded material available and, *if yes*, are you willing to share?