



German NRL TSE

- Cow R4/21-



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Institute for Novel and Emerging Infectious Diseases

Isle of Riems, 13.10.2021

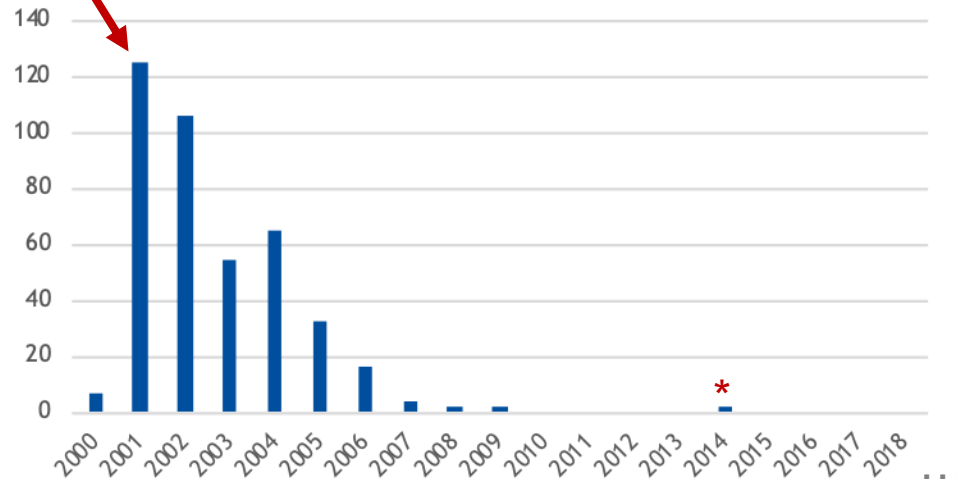


BSE-Cases in Germany



2001: Start active surveillance

BSE Cases in Germany



* Atypical BSE
- 1x L-Type
- 1x H-type

2000-2021: in total 415 BSE Fälle

- 411 Classical BSE
- 4 Atypical BSE (two each type)

Only two clinical suspects!

Status Quo ... Now



FRIEDRICH-LOEFFLER-INSTITUT

seit 1910

FLI

Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

Cow R4/21



Anamnestic data known so far:

Breed: Pinzgauer cattle (endangered breed)

Born: 23.03.2007

Emergency slaughter: 27.09.2021(14 years)

Clinical signs: injury



Rapid Test (IDEXX HerdChek)



1st Run

Benutzername BSE-Labor produktiv

Reihenfolge des Ausdrucks:
1: Proben-ID 1
2: Overflow
3: Befundungsergebnisse

	1	2	3	4	5	6	7	8	9	10	11	12
A	NEG1 0.0300 neg	2109280005 0.0470 neg	2109280013 0.0440 neg	2109280021 0.0410 neg	2109280029 0.0520 neg	2109280037 3.7620 REA	2109280045 0.0530 neg	2109280053 0.0370 neg	2109280061 0.0250 neg	2109280069 0.0500 neg	2109280077 0.0330 neg	2109280085 0.0360 neg
B	NEG2 0.0410 neg	2109280006 0.0440 neg	2109280014 0.0490 neg	2109280022 0.0430 neg	2109280030 0.0520 neg	2109280038 0.0600 neg	2109280046 0.0400 neg	2109280054 0.0360 neg	2109280062 0.0330 neg	2109280070 0.0370 neg	2109280078 0.0360 neg	2109280086 0.0380 neg
C	POS1 5.3050 REA	2109280007 0.0500 neg	2109280015 0.0500 neg	2109280023 0.0370 neg	2109280031 0.0500 neg	2109280039 0.0450 neg	2109280047 0.0340 neg	2109280055 0.0450 neg	2109280063 0.0330 neg	2109280071 0.0400 neg	2109280079 0.0350 neg	2109280087 0.0360 neg
D	POS2 6.0030 REA	2109280008 0.0450 neg	2109280016 0.0540 neg	2109280024 0.0400 neg	2109280032 0.0530 neg	2109280040 0.0400 neg	2109280048 0.0420 neg	2109280056 0.0330 neg	2109280064 0.0250 neg	2109280072 0.0370 neg	2109280080 0.0360 neg	2109280088 0.0310 neg
E	2109280001 0.0450 neg	2109280009 0.0450 neg	2109280017 0.0630 neg	2109280025 0.0340 neg	2109280033 0.0610 neg	2109280041 0.0450 neg	2109280049 0.0400 neg	2109280057 0.0410 neg	2109280065 0.0410 neg	2109280073 0.0370 neg	2109280081 0.0350 neg	2109280089 0.0340 neg
F	2109280002 0.0430 neg	2109280010 0.0370 neg	2109280018 0.0590 neg	2109280026 0.0570 neg	2109280034 0.0480 neg	2109280042 0.0380 neg	2109280050 0.0370 neg	2109280058 0.0370 neg	2109280066 0.0400 neg	2109280074 0.0370 neg	2109280082 0.0290 neg	2109280090 0.0300 neg
G	2109280003 0.0650 neg	2109280011 0.0470 neg	2109280019 0.0380 neg	2109280027 0.0380 neg	2109280035 0.0370 neg	2109280043 0.0330 neg	2109280051 0.0340 neg	2109280059 0.0330 neg	2109280067 0.0330 neg	2109280075 0.0500 neg	2109280083 0.0310 neg	2109280091 0.0290 neg
H	2109280004 0.0540 neg	2109280012 0.0420 neg	2109280020 0.0590 neg	2109280028 0.0420 neg	2109280036 0.0730 neg	2109280044 0.0550 neg	2109280052 0.0430 neg	2109280060 0.0330 neg	2109280068 0.0370 neg	2109280076 0.0450 neg	2109280084 0.0330 neg	2109280092 0.0450 neg

Benutzer Prompts
Charge Nr.: GT044

Befundungsdefinition
Messgruppe Nr. 1:
Eingangsdaten: Overflow
REA: values >= (NC1+0.120 = 0.1555)
neg: (NC1+0.120 = 0.1555) > values >= (0.0066 = 0.0066)
I: values < (0.0066 = 0.0066)

Messparameter
SUNRISE
Seriennummer des Instruments: 910004160
Messmodus: Absorption
Messwellenlänge: 450 nm
Referenzwellenlänge: 620 nm
Messmodus: Normal
Einheit: OD
Datum: 2021-09-28, Zeit: 16:40:44

2nd Run:

Benutzername BSE-Labor produktiv

Reihenfolge des Ausdrucks:
1: Proben-ID 1
2: Overflow
3: Befundungsergebnisse

	1	2	3	4	5	6	7	8	9	10	11	12
A	NEU1 0.0440 neg											
B	NEG2 0.0370 neg											
C	POS1 3.9230 REA											
D	POS2 4.0540 REA											
E	2109280005 4.0540 REA											
F	2109280037 3.9870 REA											
G												
H												

Benutzer Prompts
Charge Nr.: GT044

Befundungsdefinition
Messgruppe Nr. 1:
Eingangsdaten: Overflow
REA: values >= (NC1+0.120 = 0.1605)
neg: (NC1+0.120 = 0.1605) > values >= (0.0066 = 0.0066)
I: values < (0.0066 = 0.0066)

Messparameter
SUNRISE
Seriennummer des Instruments: 910004160
Messmodus: Absorption
Messwellenlänge: 450 nm
Referenzwellenlänge: 620 nm
Messmodus: Normal
Einheit: OD
Datum: 2021-09-28, Zeit: 18:54:00

29.09.2021:
Sent to FLI for confirmation

Confirmatory Western Blot

*In-house OIE-Immunoblot,
2g brain stem material*

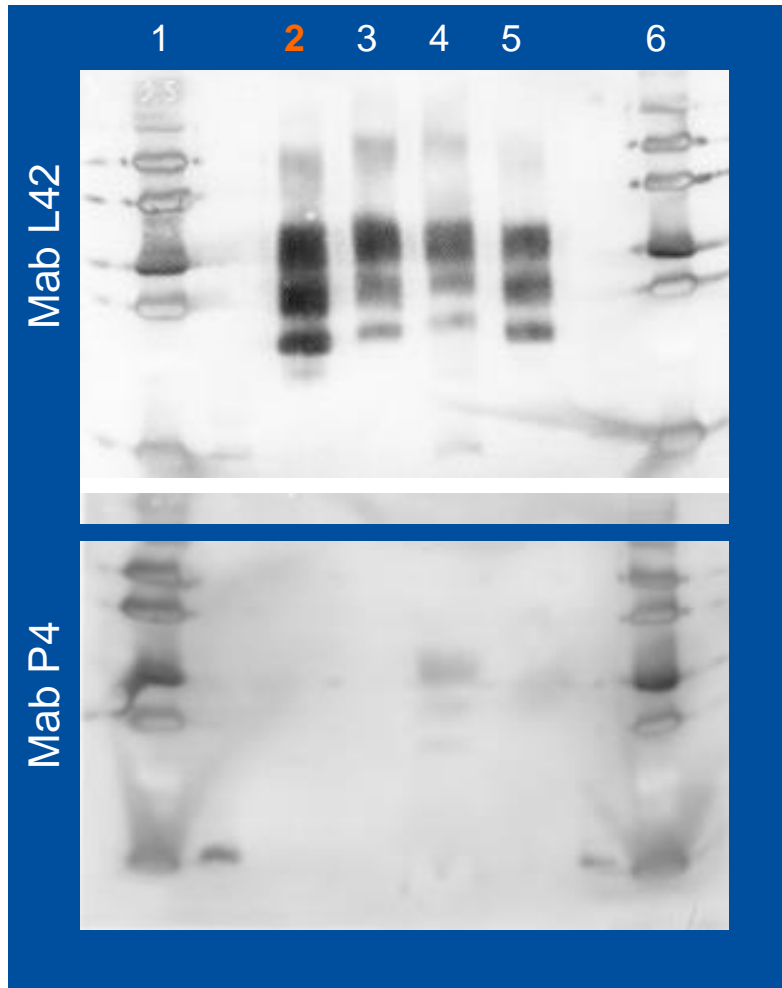


- 1 Loading buffer
- 2 Marker
- 3 Pos Ctrl +PK
- 4 Pos Ctrl -PK
- 5 Loading buffer
- 6 Neg Ctrl +PL
- 7 Neg Ctrl -PK
- 8 R4/21 +PK**
- 9 Marker
- 10 Loading buffer



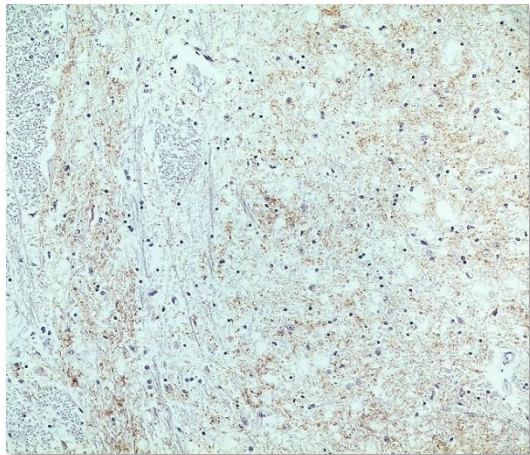
Discriminatory Immunoblot

*In-house FLI-test**



- 1 Marker
- 2 R4/21**
- 3 Classical BSE Ctrl
- 4 H-Type BSE Ctrl
- 5 L-Type BSE Ctrl
- 6 Marker

*Data analysed with Image Lab Software



Obex / Spinal tract of trigeminal nerve, mab L42



Discrimination of classical and atypical BSE by a distinct PrP^{Sc} profile

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Introduction

To date, classical BSE (C-type) and two atypical BSE forms (L- and H-type) are known. The challenge to surveillance programs is to differentiate classical from atypical BSE cases, since C-type BSE is feed borne and atypical BSE still of unknown origin. However, until now a clear discrimination between the BSE forms is based on biochemical characteristics only.

The goal of our study was to identify type-specific PrP^{Sc} profiles by using immunohistochemistry as an additional method.

Results:

The histopathological (H.&E.) analysis showed clear differences among the individual cattle, but the IHC data points towards the existence of a distinct PrP^{Sc} profile between the BSE-types (Table 2). This profile involved both the specific brain areas affected and the cellular pattern of the PrP^{Sc} deposition (Fig.2-6).

Animals, Material and Methods

In total 21 cattle (Table 1), intracerebrally inoculated with C-type, H-type and L-type BSE, were used. All cattle showed clear clinical signs and revealed at least a moderate PrP^{Sc} accumulation in the brain. Six well-defined brain areas were examined by H&E staining and by immunohistochemistry (IHC, Fig. 1), using two different anti PrP^{Sc} antibodies: mab F99 (C-terminal) and mab 6C2 (Core-region).

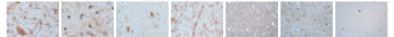


Fig. 1: IHC reaction pattern: (A) diffus intraneuronal (ITNR-D); (B) intracerebellar (ITMG); (C) stellate (STEL); (D) linear (LIN); (E) fine particular (PART-F); (F) coarse particular (PART-C); (G) Plaque-like (PL); (H) Intraastroglial (ITAS), submeningeal (SMENG), perineuronal (PNER) not shown, Bar 20 µm

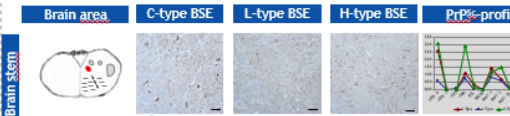


Fig. 2: A distinct ITNR PrP^{Sc} accumulation is seen in the Hypoglossal nuclei in C- and H-type BSE, but never in the (rarely affected) L-type, mab F99, Bar 50 µm

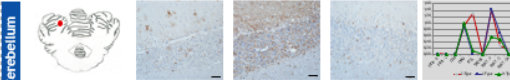


Fig. 3: In the Molecular layer a distinct STEL pattern is seen in C-type BSE, an evenly distributed PART accumulation in L-type BSE and a predominance of an ITMG PrP^{Sc} profile in H-type BSE, mab F99, Bar 50µm



Fig. 4: Distinct PrP^{Sc} distribution in the Hilus: evenly distributed in L-type BSE, a preference for the central parts in C-type BSE and a restriction to the peripheral parts in H-type BSE, mab F99, Bar 100µm

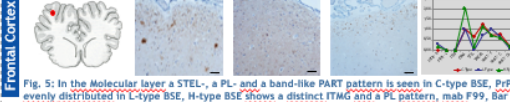


Fig. 5: In the Molecular layer a STEL-, a PL- and a band-like PART pattern is seen in C-type BSE, PrP^{Sc} is evenly distributed in L-type BSE, H-type BSE shows a distinct ITMG and a PL pattern, mab F99, Bar 50µm

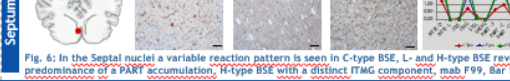


Fig. 6: In the Septal nuclei a variable reaction pattern is seen in C-type BSE, L- and H-type BSE reveal a predominance of a PART accumulation; H-type BSE with a distinct ITMG component, mab F99, Bar 50µm

Table 1: Anamnestic data of the cattle involved

	C-type BSE	L-type BSE	H-type BSE
FLI	n/a	n=5	n=5
Months p.i.	n/a	12-17	13-17
CFIA	n=7	n=2	n=2
Months p.i.	19-26	17-18	17-18
Breed	Holstein-Frisian (FLI)	Angus-Hereford Cross (CFIA)	

Table 2: Most important differences seen between the BSE-types

	C-type BSE	L-type BSE	H-type BSE
Hypoglossal Nucleus	Variable pattern	Rarely affected, no ITNR	ITMG
Cerebellum/ Mol. layer	STEL	PART evenly distributed, LIN	Distinct ITMG
Hippocampus/ Hilus	Central region, variable pattern including PL	All regions, variable pattern	Perih. region, variable pattern
Frontal Cortex/ Mol. layer	Perih. band-like PART, STEL, PL	PART evenly distributed	PL, ITMG
Septal Nucleus	Variable pattern	PART evenly distributed	PART, ITMG

Additional useful area: Red Nc., Cerebellar/Cerebral White Matter
Characteristic PrP^{Sc} profiles are already seen in mildly affected areas

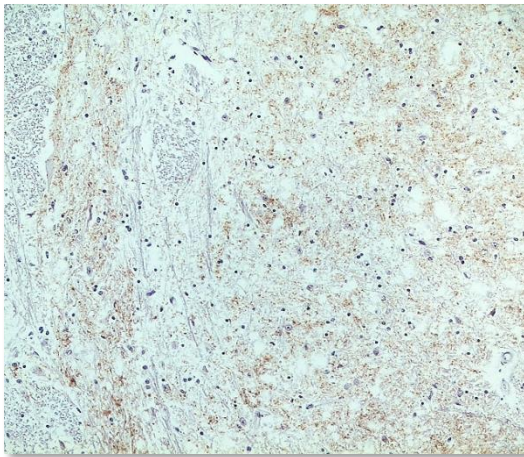
Conclusion:

Discrimination of classical, L- and H-type BSE by IHC is clearly possible in advanced stages of the disease requiring experienced reader.

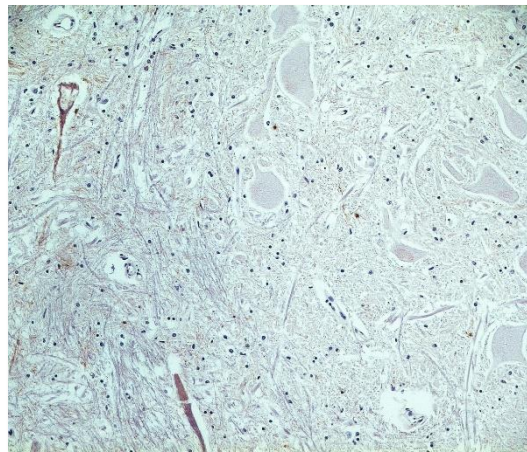
- C-type BSE:**
Most variable PrP^{Sc} profile with a prominent STEL reaction pattern
- L-type BSE:**
A predominance for an evenly distributed PART PrP^{Sc} accumulation
- H-type BSE:**
Extensive ITMG (up to 100%) PrP^{Sc} deposition in almost all regions examined



Immunohistochemistry



Obex / Spinal tract of trigeminal nerve, mab L42



Obex / Nc. hypoglossus, mab L42





Diagnosis L-Type BSE

Diagnosis:
Atypical BSE / L-type



Thanks!

**Cathleen Klement
Dan Balkema**

**... and for your
attention!**