Atypical BSE prion progression in natural hosts - Progress report.

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Torino | 13th of May 2025

Atypical BSE in the natural host

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Study:
Infection study with H- and L-type BSE in the natural host

Method:
i.c. inoculation of 22 Frisian Holstein cattle (age ~ 7-8 months)

- 2 mock controls
- 10 H-type BSE
- 10 L-type BSE

Duration of the animal trial: 18 – 20 months

Study aim:
Propagation of reference material for active TSE surveillance according to EU RL 999/2001 for all European NRLs for TSE

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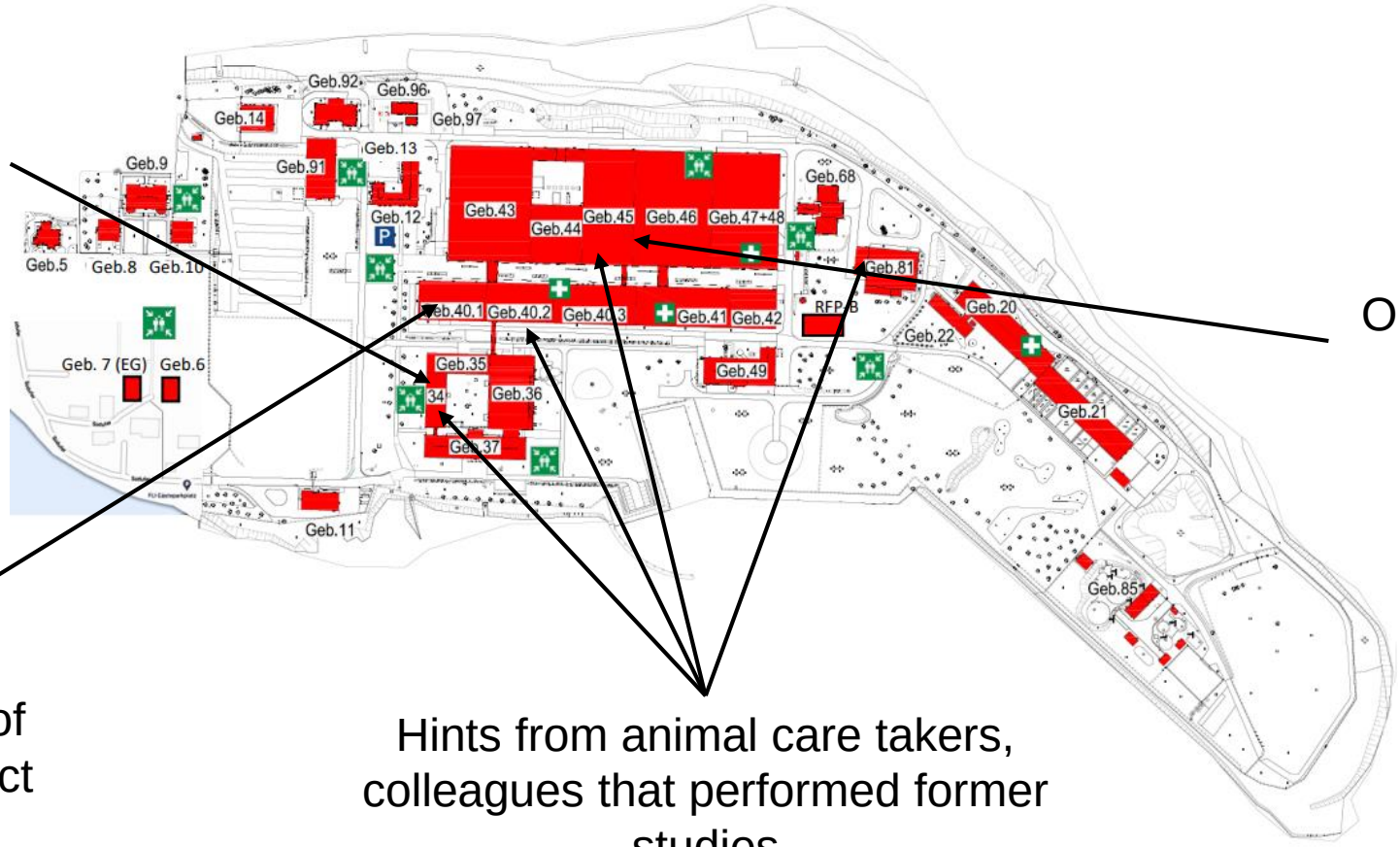
Treasure hunt at the FLI

August – October 2024

Documents from infection studies in the early 2000s



Old equipment of former infection studies

More documents of the goatBSE project

Hints from animal care takers, colleagues that performed former studies

Atypical BSE in the natural host - the building

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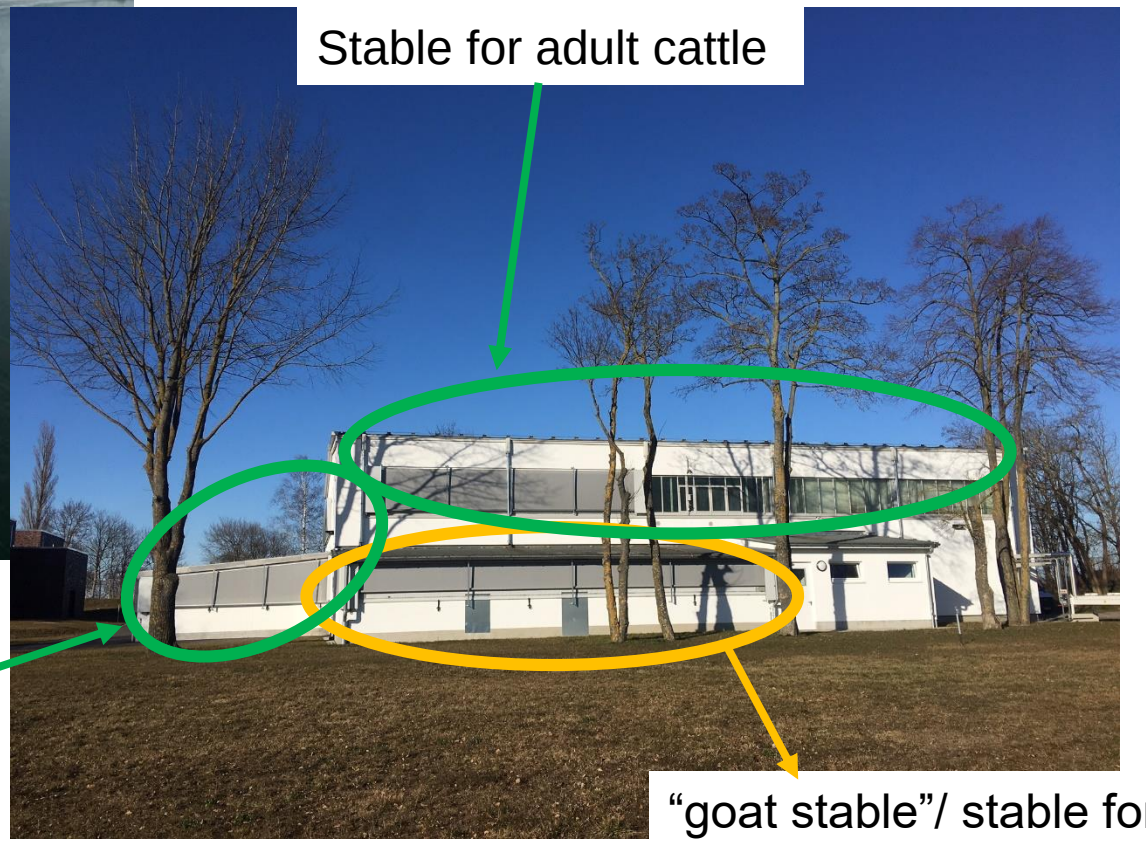

Paddock for adult cattle

“goat stable”/ stable for small ruminants (incl. calfs)

Atypical BSE in the natural host - the building

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The Stable/ Necropsy hall

November 2024 – March 2025

Complete renovation of the building from 2019 - 2021


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- Organisation of furniture, equipment for animal keeping/ work safety/ disection etc.

- Preparation/ Adjustment of the former goat stable for the calfs during the first cold winter months before i.c. inoculation


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- Organisation of technical adjustments in the big stable
  - Repairing the feeding grid
  - Repairs at the feeding table
  - Further adjustments at different ends

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The inoculation

February – March 2025

- Establishment of the anesthesia protocol (in cooperation with Svenja Mammerow)
  - propofol + detomidine + butorphanol

- Establishment of the surgery protocoll (in cooperation with Timm Konold, APHA, UK)


1
© FLI
Measurment of the head and identification of the drill position


2
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3
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Drilling the hole for the inoculation


4
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Insertion of the inouclation needle

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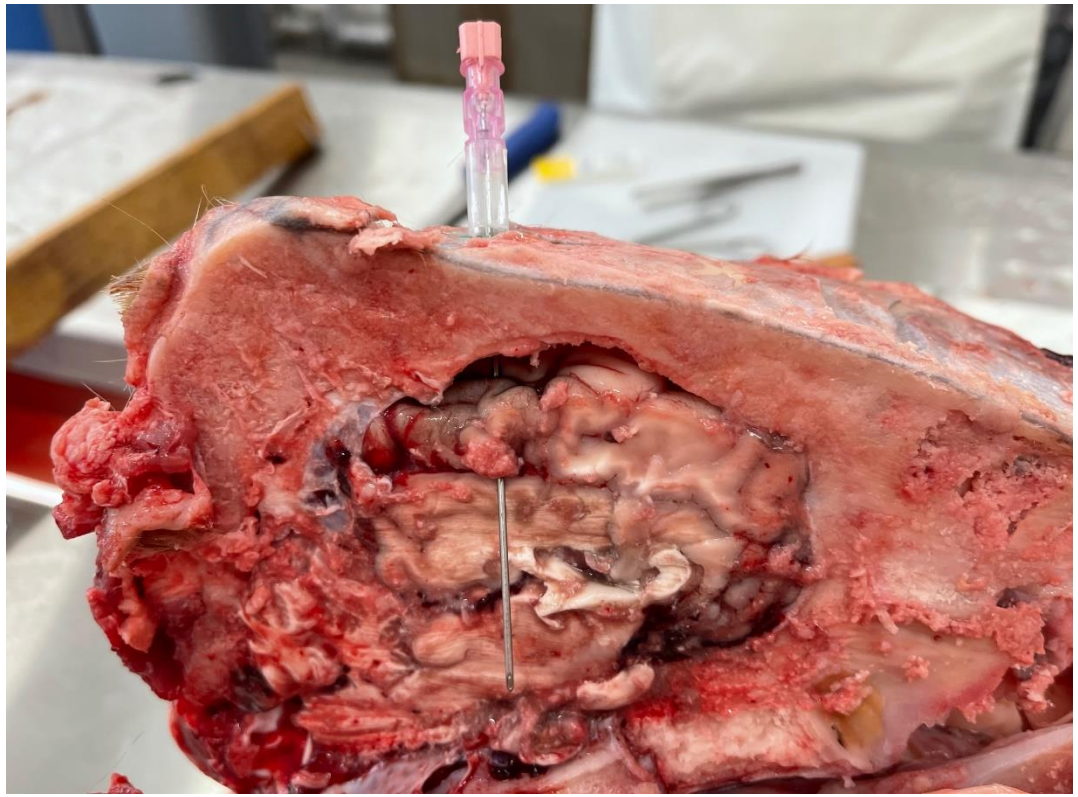
The inoculation

Did I hit the target?



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The animals

End of January 2025

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Arrival of the animals

- 22 Frisian Holstein calfs
- From two different farms
- 3 – 4 months old

February – end of March 2025

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Acclimatisation and growing up in the small ruminant stable

- Better conditions for calfs in this compartment
  - Better climate, less windy
  - straw bedding possible
- Parasitic treatment
- 1. Vaccination against BTV

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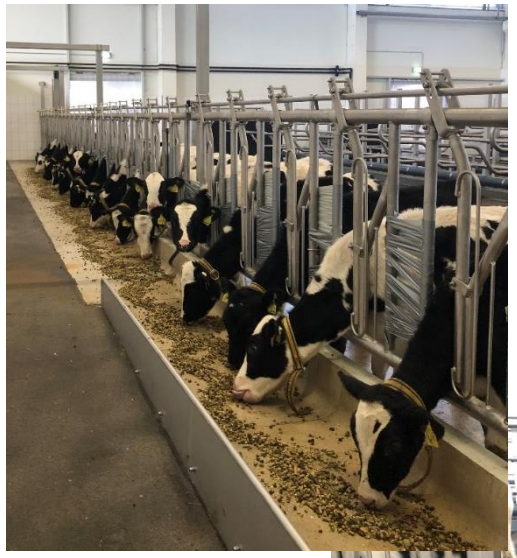
The animals

End of March 2025



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Moving to the „big“ cattle stable

- Blood analysis for anaesthesia
- Taking negative control samples
  - Blood (citrate)
  - Liquor cerebrospinalis

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April 2025

Final* approval of the stable by the authorities

Now we can start with the inoculations!

**This is a completely different story with many twists and turns …*

Atypical BSE in the natural host – The homogenates

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1x L-type BSE sample

ID	R4/21
Origin	Bavaria/ Germany emergency slaughter in 2021
Cattle breed	Pinzgauer
Age	14 years
Tissue	Brain stem

2x H-type BSE samples

ID	Italy	R152/04
Origin	Italy	Bavaria/ Germany Healthy slaughter
Cattle breed	Unknown	unknown
Age	Unknown	13 years
Tissue	Cerebrum and Septum	Mid brain

Atypical BSE in the natural host – The homogenates

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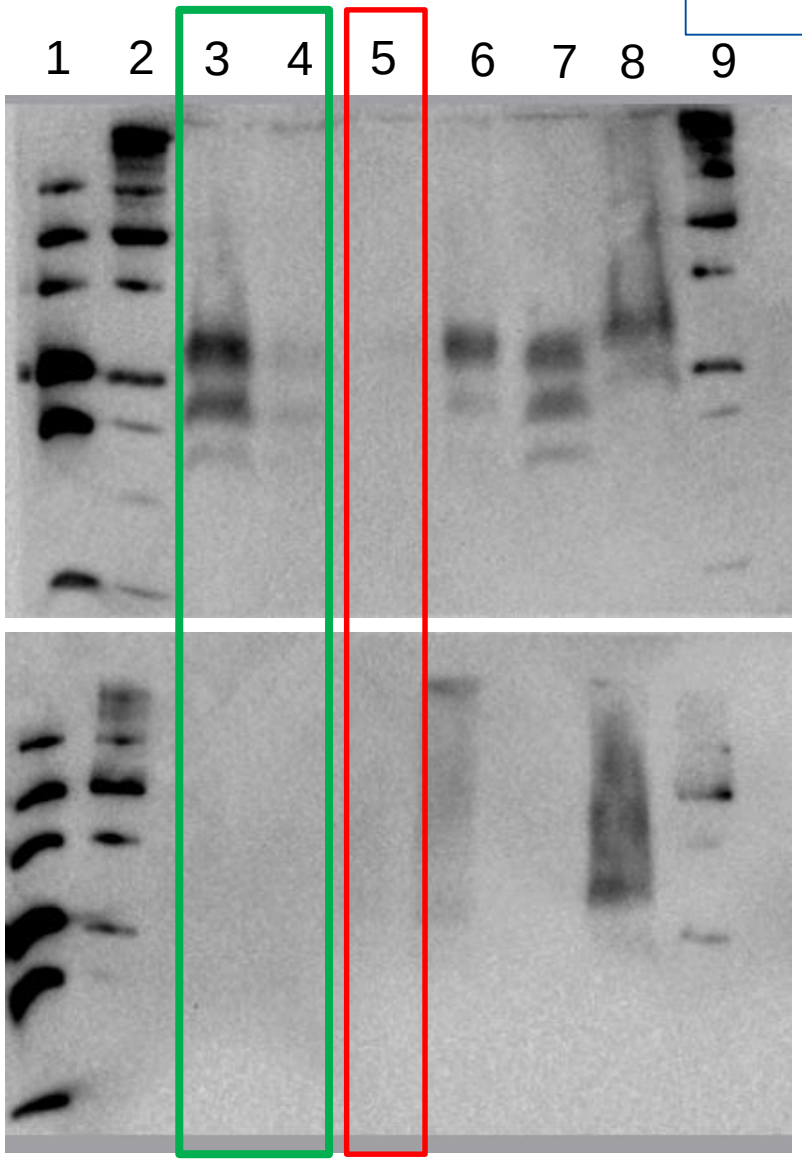
Western Blot



Lane	Sample
1	Dual Color Marker
2	Unstained Marker
3	**L-Typ R4/21 brain stem**
4	**L-Typ R4/21 cerebellum**
5	**H-Typ Italy**
6	C-type BSE Ctrl.
7	L-type BSE Ctrl.
8	H-type BSE Ctrl.
9	Unstained Marker

L-type R4/21 Germany

- Brain stem with strong signal with mab L42
- Cerebellum only week signal with mab L42
- No signal with mab P4

H-type Italy

- Only very week signal with both antibodies

Atypical BSE in the natural host - The homogenates

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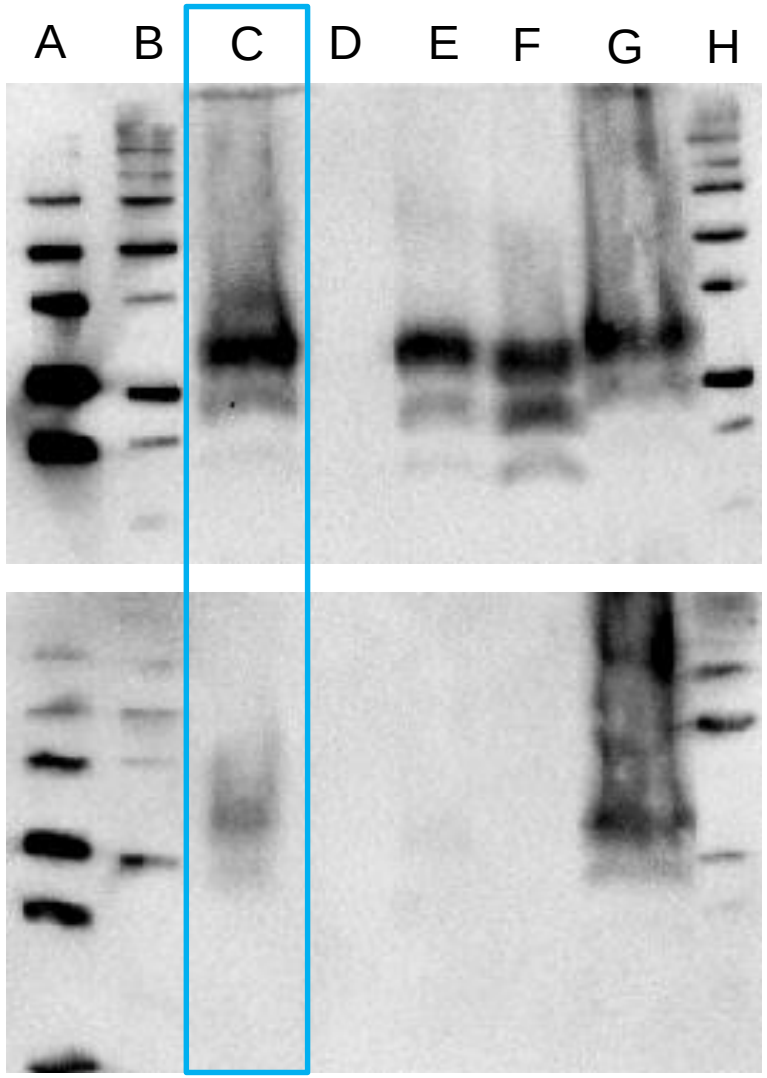
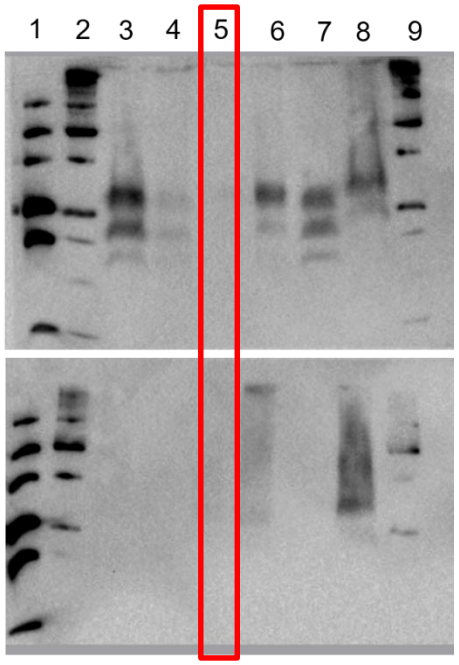
Western Blot



Lane	Sample
A	Dual Color Marker
B	Unstained Marker
C	**H-type BSE 152/04**
D	Sample buffer
E	C-type BSE Ctrl.
F	L-type BSE Ctrl.
G	H-type BSE Ctrl.
H	Unstained Marker

WB L-type Germany and H-type Italy:



H-type 152/04 Germany
- Strong signal with mab L42
- Clear signal with mab P4

→ stronger signals than H-Type Italy!

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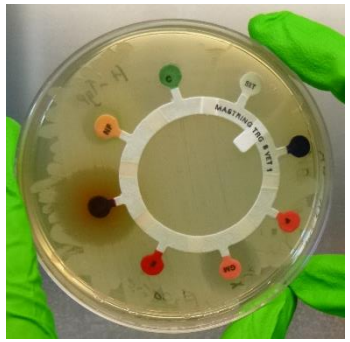
Microbiology results

	L-type R4/21	H-type Italy	H-type R152/04	Best antibiotic
Gram+	Several colonies (aerobic, anaerobic)	Several colonies (aerobic, anaerobic)	Several small colonies (aerobic, anaerobic)	Erythromycin
Gram-	E. coli	-	-	Streptomycin
Fungi	-	++	-	High resistance against antimycotics

Fungi with high resistance and weak WB signal

Decision to inoculate the H-type 152/04 Germany sample



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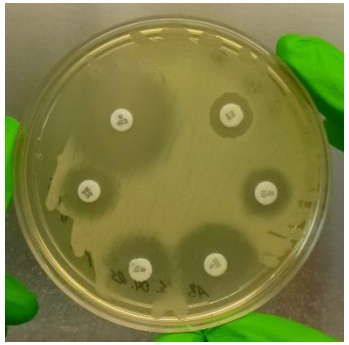

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i.c. Inoculation H-Typ BSE

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Anaesthesia and positioning of the calfs

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i.c. Inoculation H-Typ BSE

© Christine Fast Final positioning of the calfs

© Christine Fast Preparation of the drill

Inoculation of 10% homogenates in NaCl + 1% erythromycin

© Christine Fast Inoculation

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Outlook

1. i.c. inoculation of the L-type BSE inoculum end of May
2. Finalize *PRNP*-genotyping
3. Regular clinical examination and scoring during the incubation periods
4. Regular sampling of
   Blood (every 4-6 months)
   Liquor cerebrospinalis (every 8 month)

THANK YOU!

We would like to thank all colleagues of FLI that contributed to this work.

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