

Heterogeneity of CWD in Moose in the Nordic countries

**Benestad SL, Nonno R, Tran L, Våge J, Vuong TT, Pirisinu L,
Telling G, Sola D, Korpenfelt SL, Nøremark M, Gavier-Widen D**

20th Annual Meeting of the TSE EURL Roma, Italy –
2nd-3rd October 2023



Veterinærinstituttet
Norwegian Veterinary Institute

Reference Laboratory for
Chronic Wasting Disease

Reference Centre  World Organisation
for Animal Health
Founded as OIE

- Nordic CWD situation
- Moose heterogeneity and common points

- WB

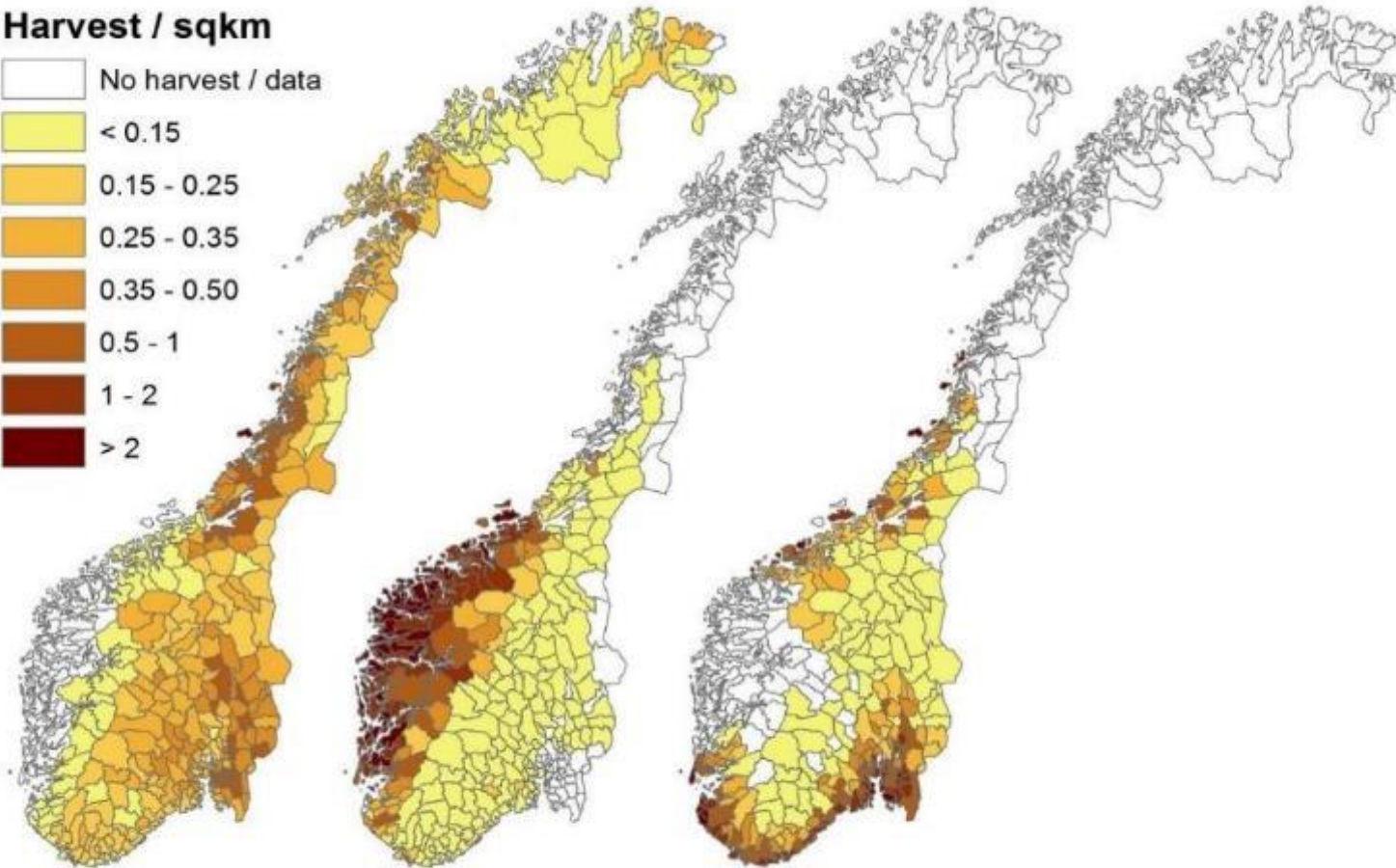
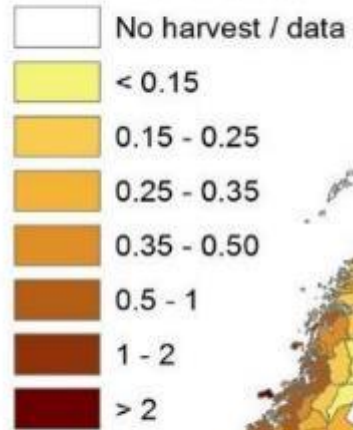
- IHC



Cervids in Norway

Christer Rolandsen

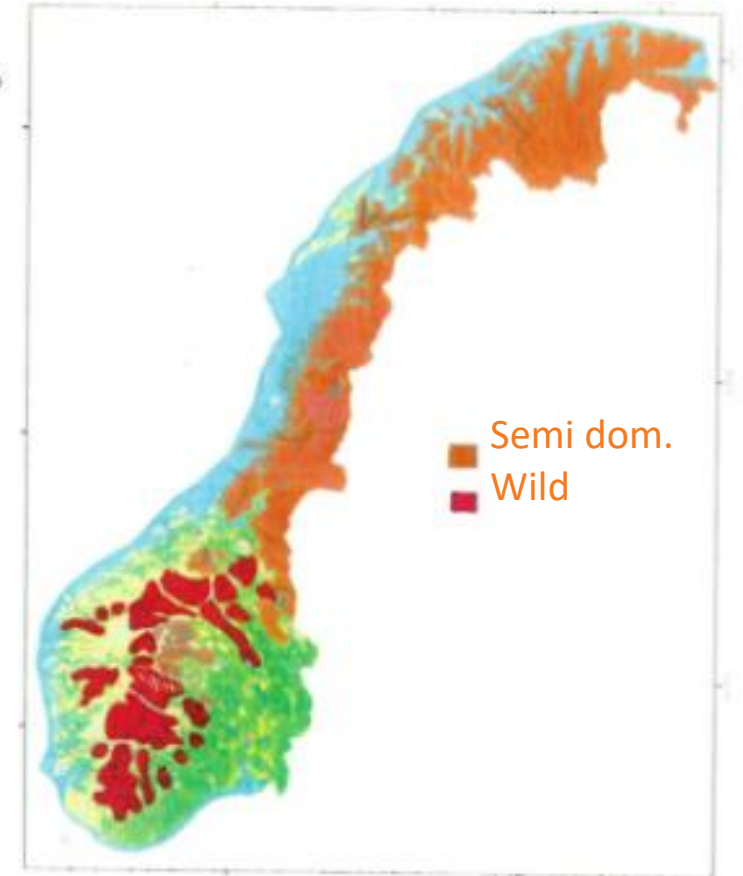
Harvest / sqkm



Moose

Red deer

Roe deer



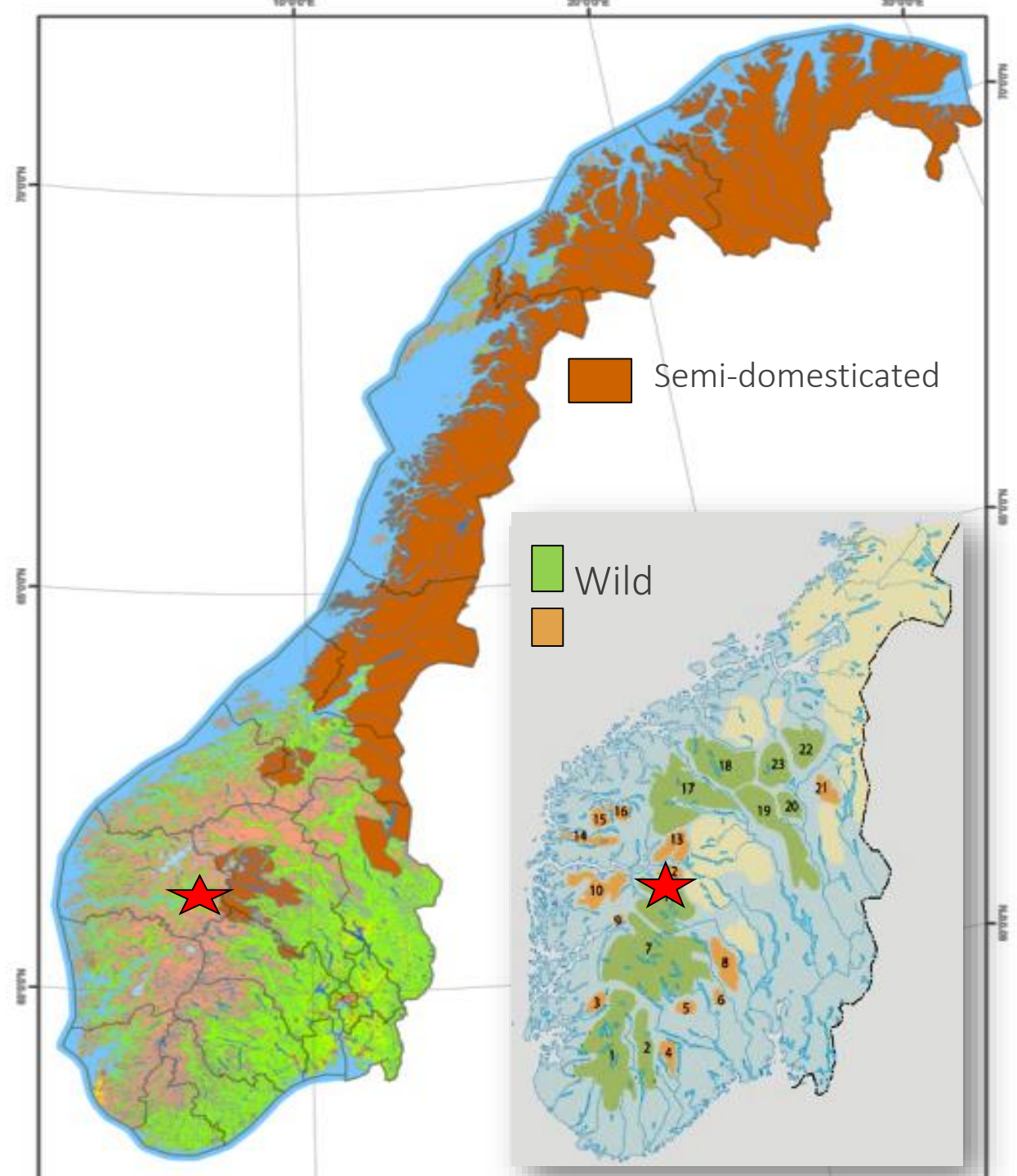
Semi dom.
Wild

Reindeer



Two populations of reindeer

«Semi domestic» reindeer:
no fence, free ranging most of
their life, few human interventions





Depopulation of Nordfjella reindeer
completed in May 2018

2471 reindeer tested
Both Brain and Ln analysed
19 positive
0,76 % frequency

September 2020:

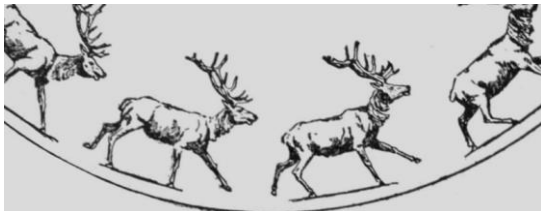
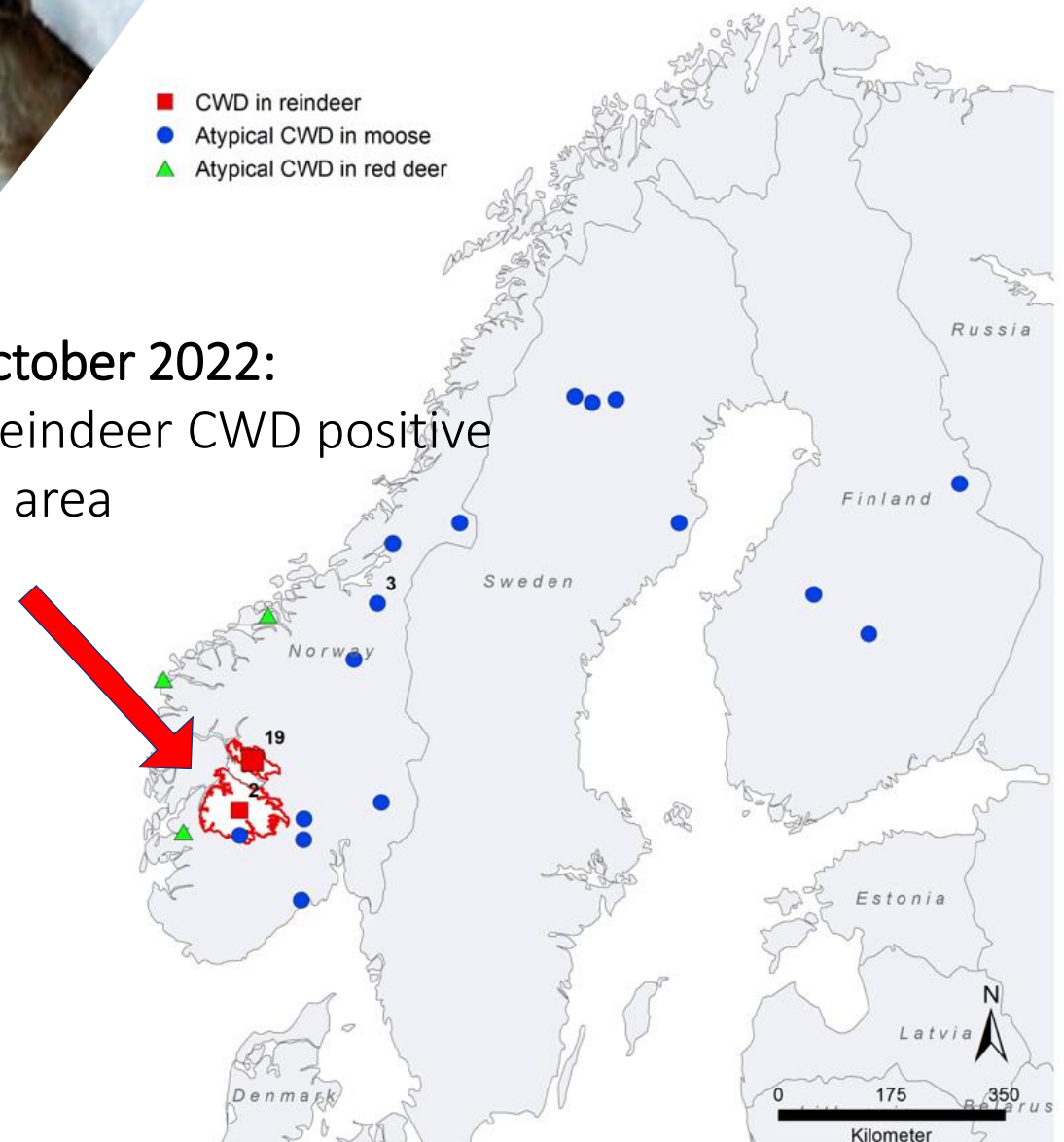
One reindeer CWD positive
(Hardangervidda) **outside**
the depopulated area



6th October 2022:

New reindeer CWD positive
in this area

- CWD in reindeer
- Atypical CWD in moose
- ▲ Atypical CWD in red deer



Surveillance program for CWD in Norway (from July 2016)

<http://apps.vetinst.no/skrantesykestatistikk/NO/#omrade>

Samples analysed the day they come to the lab.

Fallen stock: whole country, all cervids

Hunted: in and around CWD areas, all cervids

Slaughtered: semi domesticated reindeer, whole country

2016- today between 10 000 to 33 000 cervids per year

Total: over 170 000 cervids so far

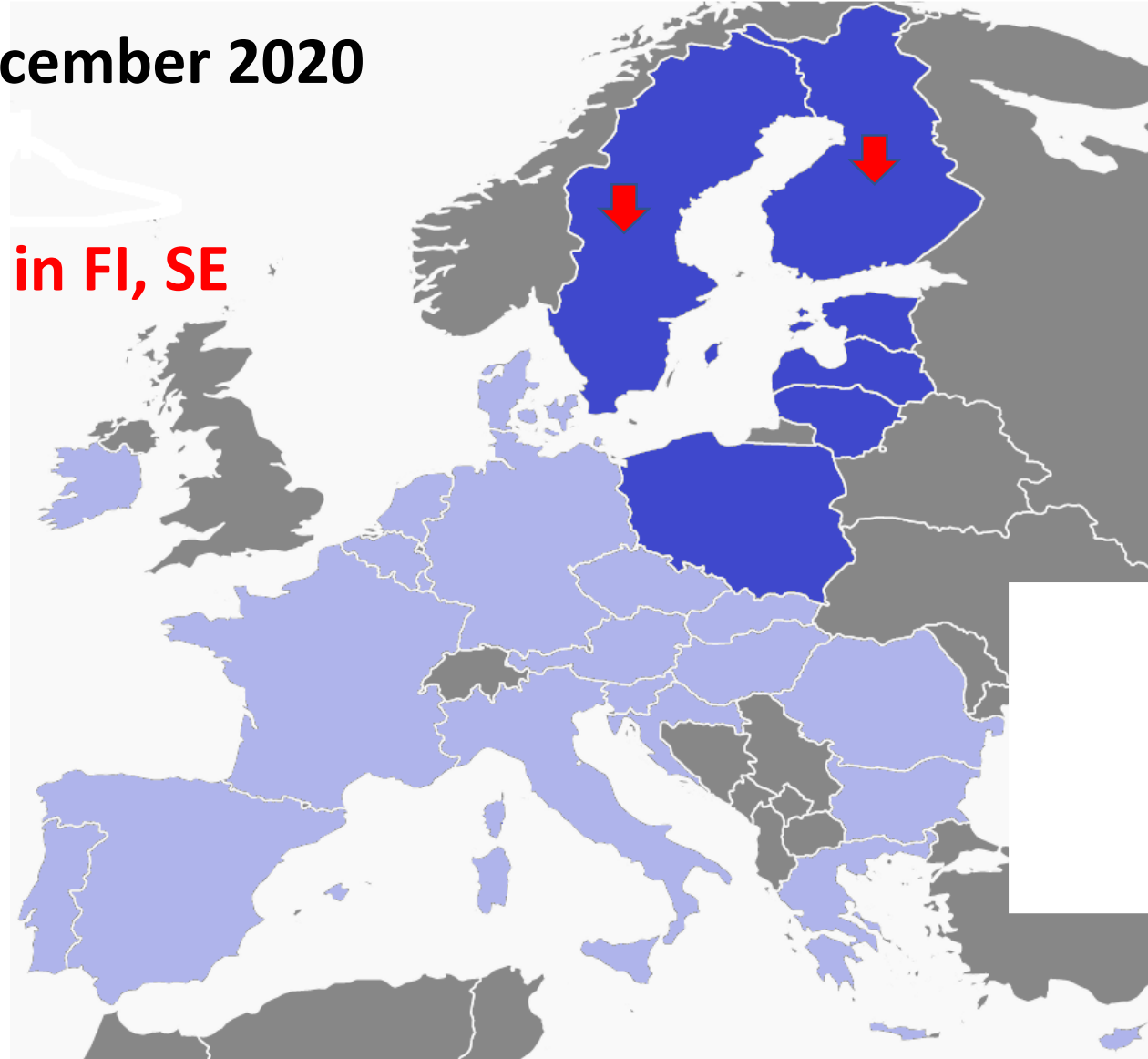
Photo: Jeger.no



3-year monitoring programme for CWD in 6 countries







Started 2018, ended in December 2020

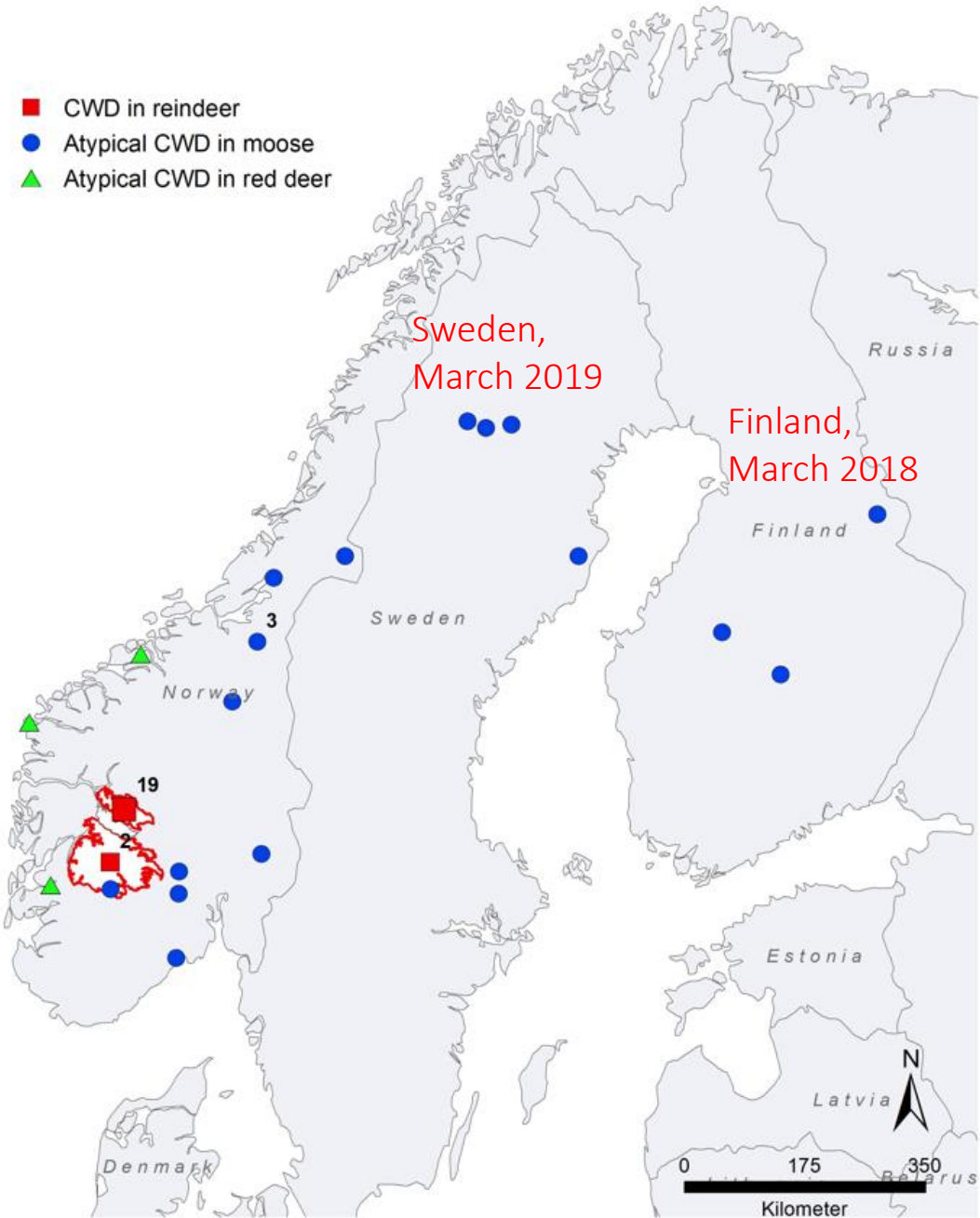
Showed CWD also present in FI, SE





Number of Nordic CWD cases

 Moose			
	= 11	= 4	= 3
 Reindeer	= 21		
 Red deer	= 3		



CWD in Nordic Moose ●

Norway
11 cases

Sweden
4 cases

Internal ID	Age	Sex	Genotype
M-NO1	13	F	KK109-MM209
M-NO2	14	F	KK109-MM209
M-NO3	13	F	KK109-MM209
M-NO4	15	F	QQ109-MM209
M-NO5	20	F	KK109-MM209
M-NO6	12	F	QQ109-MM209
M-NO7	17	F	KK109-MM209
M-NO8	13	M	KK109-MM209
M-NO9	17	F	KK109-MM209
M-NO10	20	F	QQ109-MM209
M-NO11	19	F	KK109-MM209

Internal ID	Age	Sex	Genotype
M-SW1	16	F	KK109-MM209
M-SW2	16	F	KK109-MM209
M-SW3	10	F	KK109-MM209
M-SW4	14	F	KK109-MM209

Finland
3 cases

Internal ID	Age	Sex	Genotype
M-FI1	15	F	KK109-MM209
M-FI2	18	F	KK109-MM209
M-FI3	15	F	KK109-MM209

Moose in Norway
(n=137)

Güere et al, Transbound Emerg Dis
2021

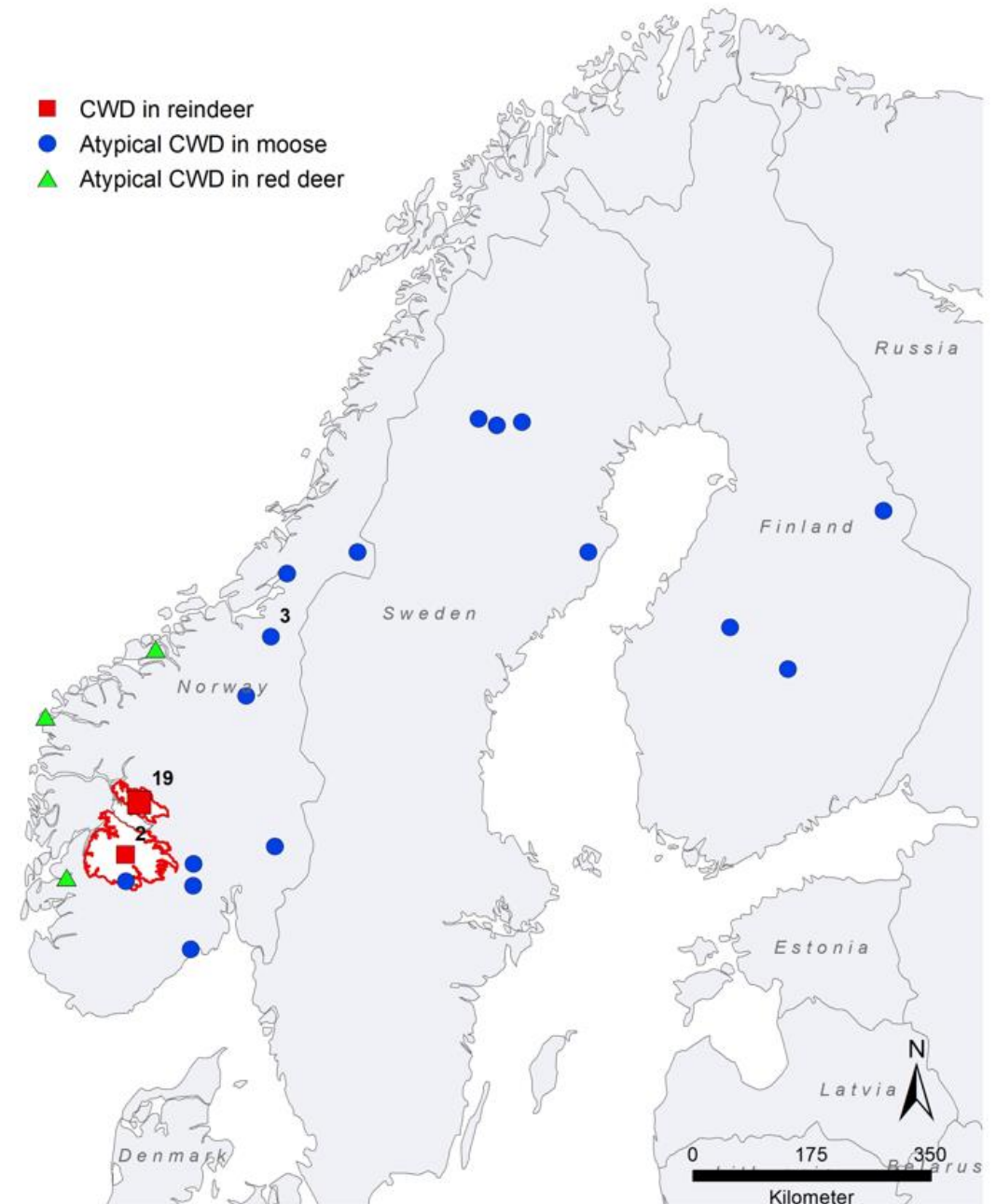
K109 77% (Wt)

Q109 23%

(No polymorphism at codon 209 in Europe)

All negative for PrP^{Sc}
in lymph nodes!
Called «Ly-»

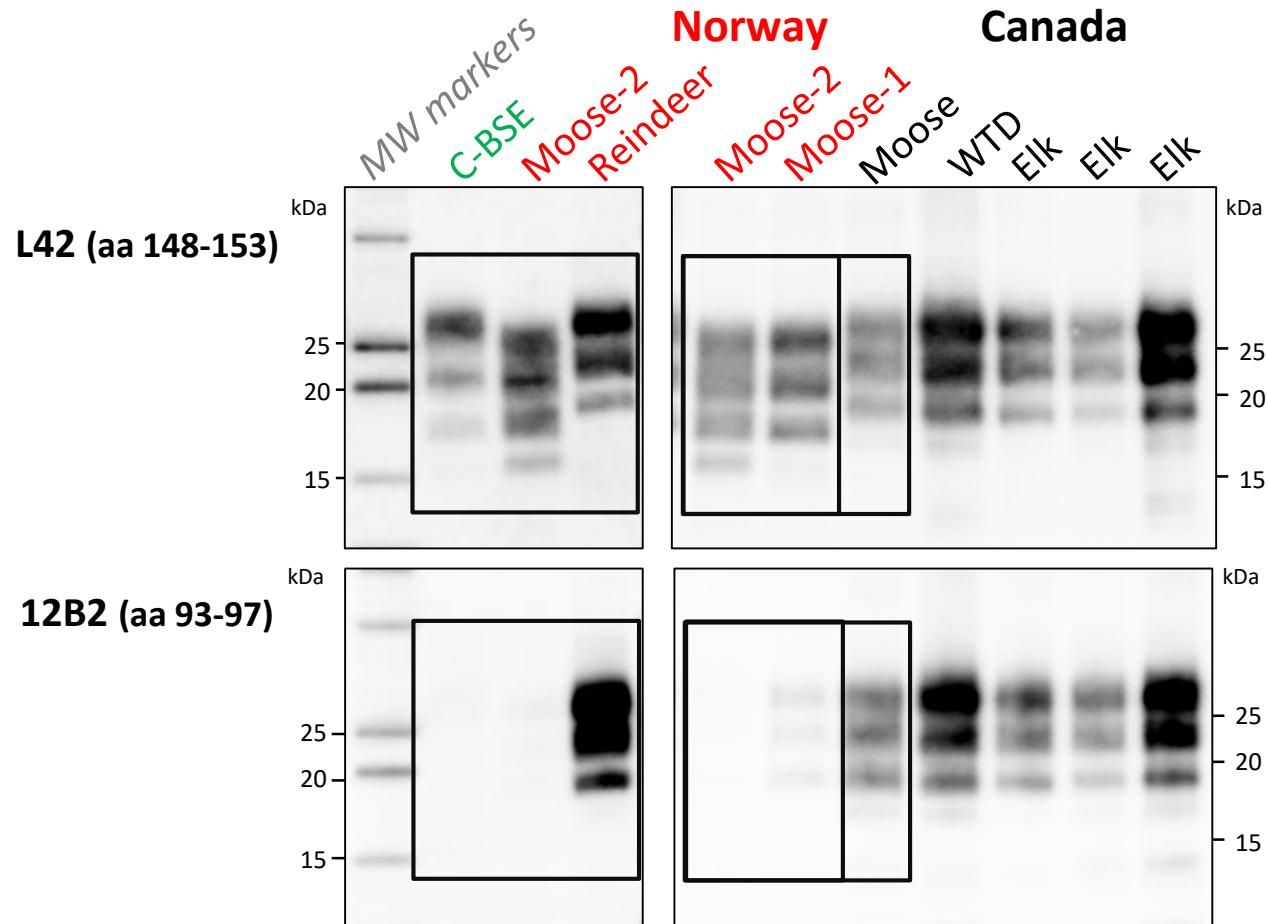
- CWD in reindeer
- Atypical CWD in moose
- ▲ Atypical CWD in red deer



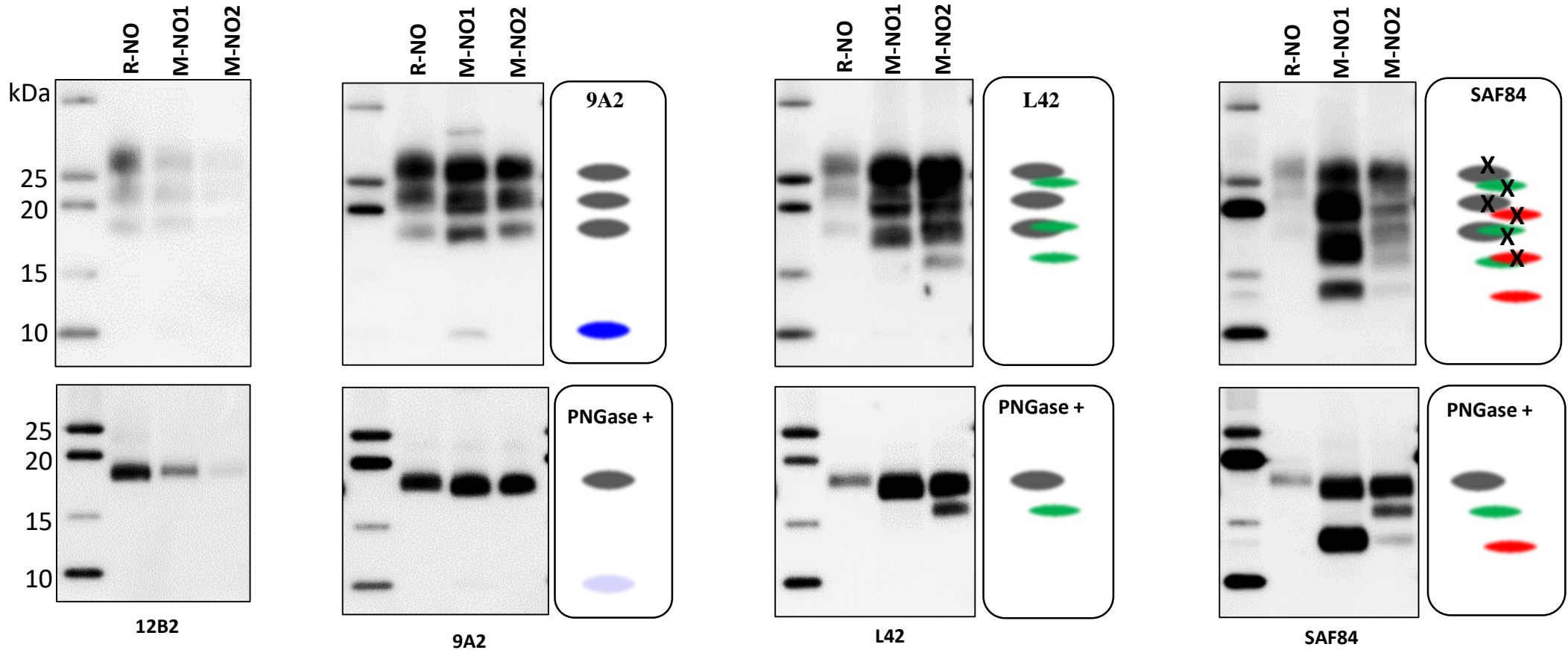
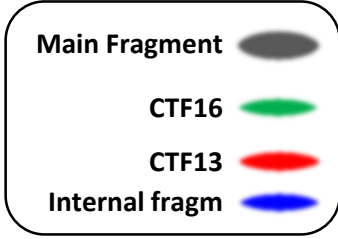
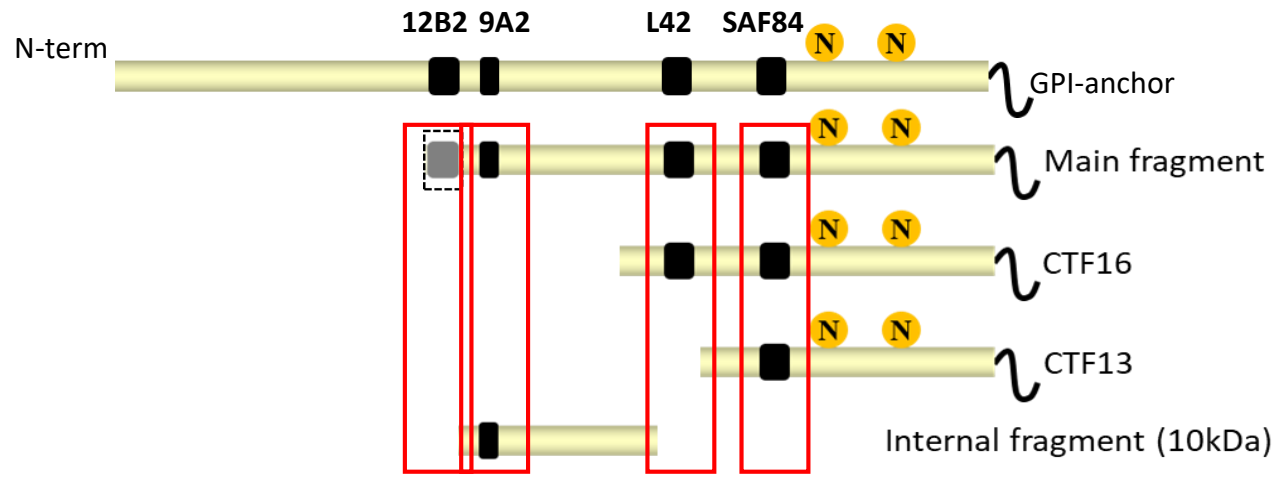
Moose characteristics using WB

ROMOLO LAURA WB FINDINGS

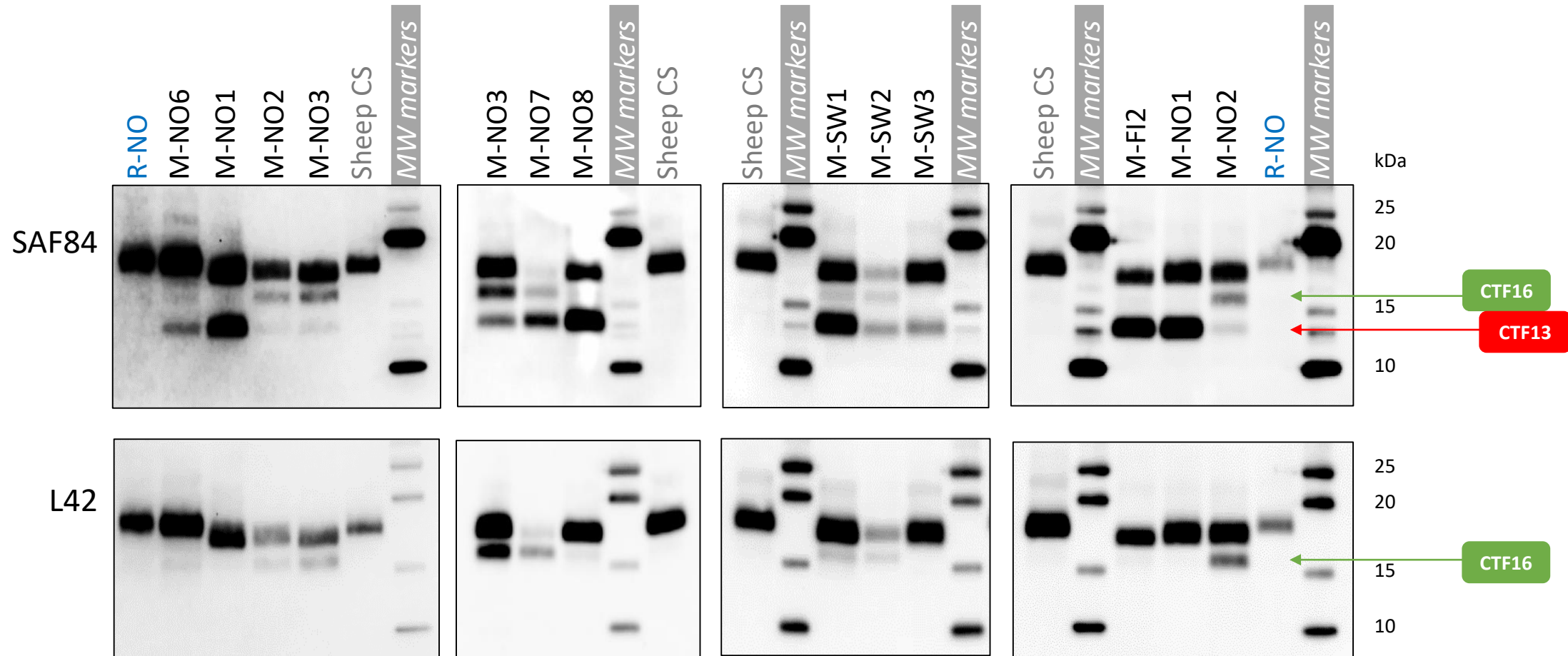
A novel type of CWD in Norwegian moose



- Norwegian moose more N-terminally truncated than reindeer and CWD-NA
- The «deviant» phenotype in Norwegian moose is not an host effect
- Slight WB differences between the 2 Norwegian moose

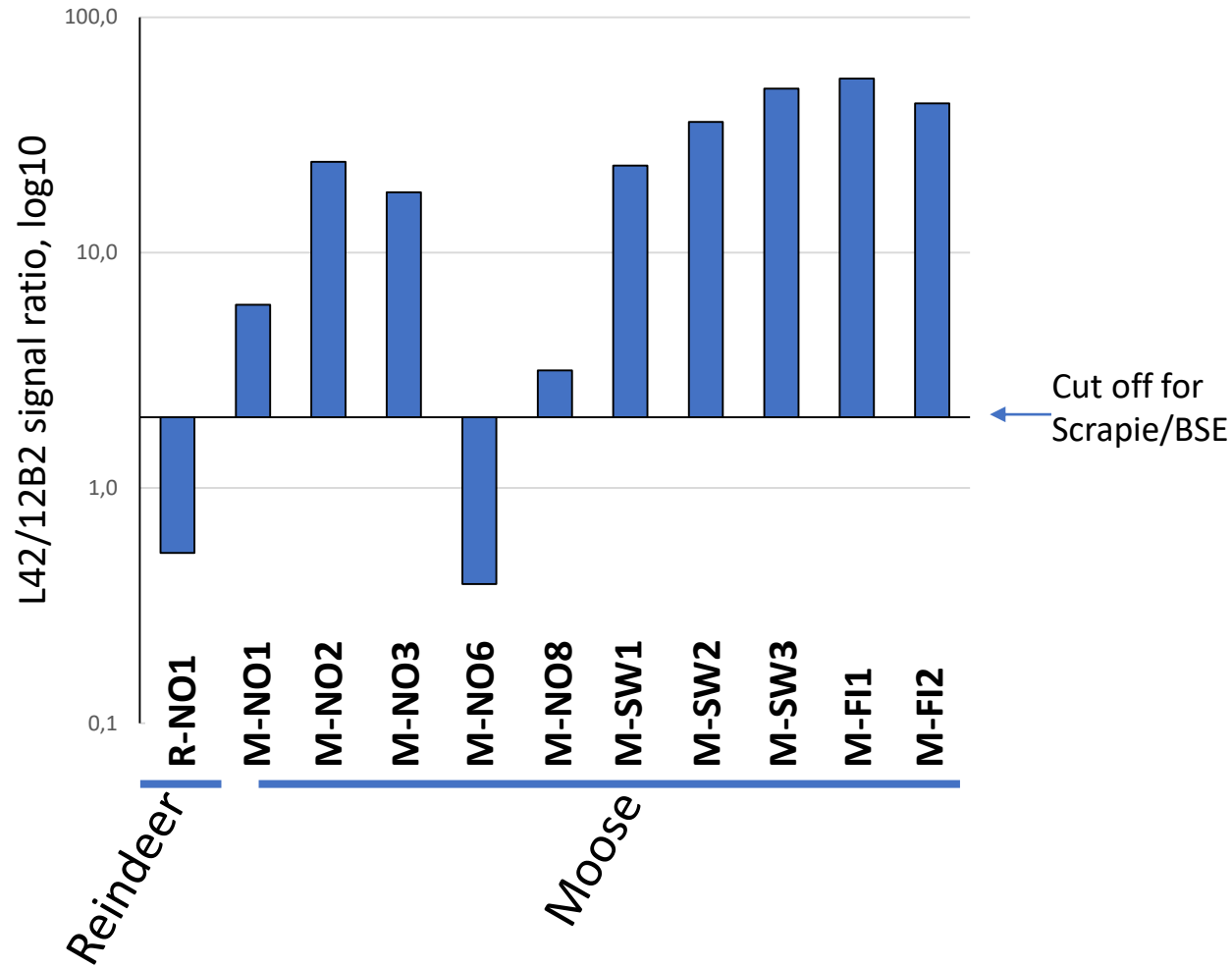


PrP^{res} types in moose



Huge variability!

N-terminal trimming of PrP^{res}



Variability in cleavage site around the 12B2 epitope in moose

- 12B2 preserved in M-NO6 = reindeer!
- 12B2 partially preserved in M-NO8 and M-NO1
- 12B2 mainly lost in all other moose

Origin	Internal ID	Age	Sex	Genotype	Main PrP ^{res} fragment		Minor PrP ^{res} fragments		
					MW	L42/12B2	CTF13	CTF16	Internal
Nor	M-NO1	13	F	KK109-MM209	~ 17 kDa	2 ÷ 10	✓		✓
Nor	M-NO2	14	F	KK109-MM209	~ 17 kDa	>10	✓	✓	
Nor	M-NO3	13	F	KK109-MM209	~ 17 kDa	>10	✓	✓	✓
Nor	M-NO4	15	F	QQ109-MM209					
Nor	M-NO5	20	F	KK109-MM209					
Nor	M-NO6	12	F	QQ109-MM209	~ 19 kDa	<1	✓		✓
Nor	M-NO7	17	F	KK109-MM209	~ 17 kDa	>10	✓	✓	
Nor	M-NO8	13	M	NA	~ 17 kDa	2 ÷ 10	✓		✓
Swe	M-SW1	16	F	KK109-MM209	~ 17 kDa	>10	✓	✓	✓
Swe	M-SW2	16	F	KK109-MM209	~ 17 kDa	>10	✓	✓	✓
Swe	M-SW3	10	F	KK109-MM209	~ 17 kDa	>10	✓		+/-
Swe	M-SW4	14	F	NA	?		✓		
Fi	M-FI1	15	F	NA	~ 17 kDa	>10	✓		
Fi	M-FI2	18	F	NA	~ 17 kDa	>10	✓		+/-

CTF13 seems a conformational signature of CWD in moose

Moose characteristics using IHC

Pirisinu et al. Em. Inf. Dis 2018

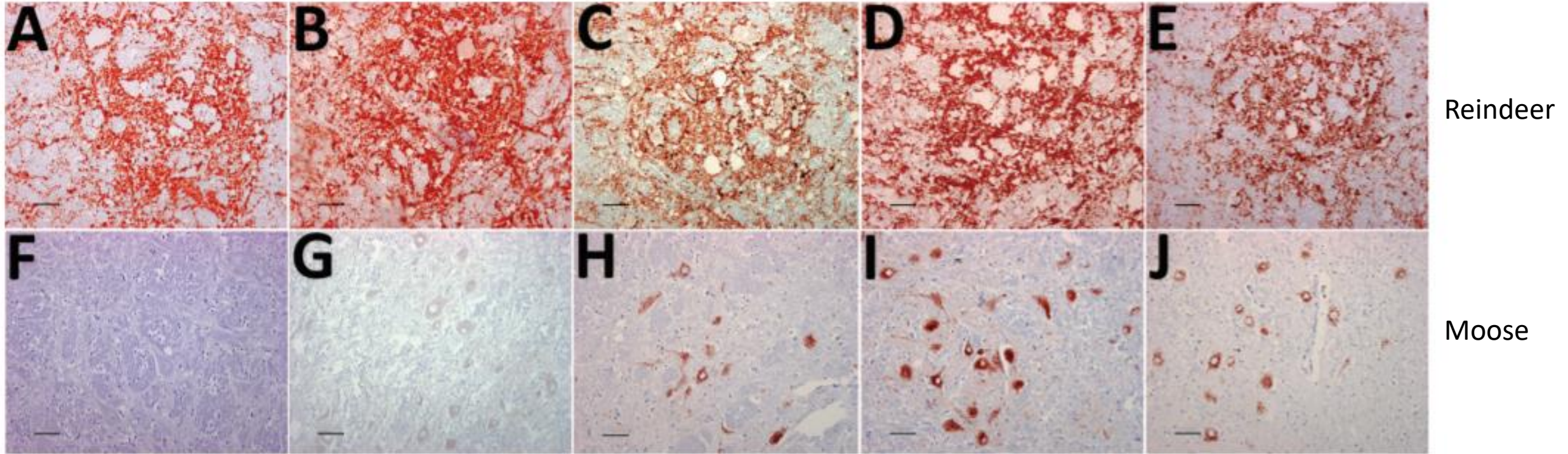
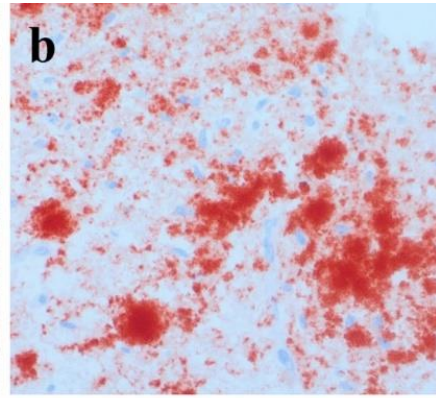
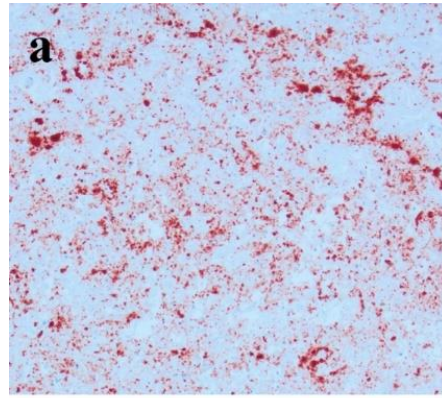
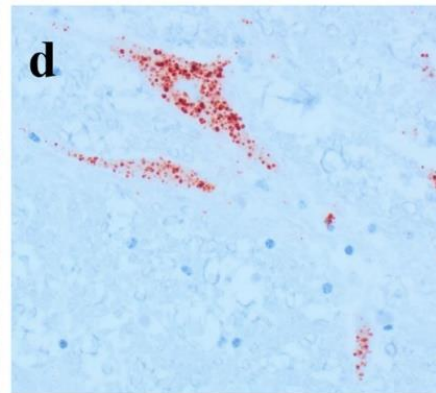
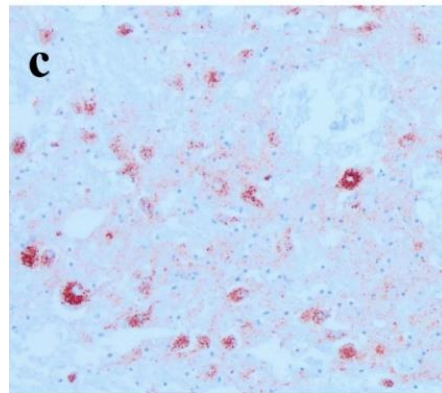


Figure 1. Immunohistochemical detection of disease-associated prion protein in brain sections at the level of the obex in cervids with chronic wasting disease, Norway. A–E) Reindeer; F–J) moose. mAbs used were 12B2 (A, F), 9A2 (B, G), L42 (C, H), SAF 84 (D, I), and F99/97.6 (E, J). Staining obtained in the reindeer tissues is similar regardless of mAbs used (A–E). Conversely, for moose tissues, the staining was primarily observed intraneuronally with L42, SAF84, and F99/97.6 (H–J) but was not observed using the more N-terminal mAbs 12B2 and 9A2 (F, G). Scale bars indicate 40 μ m. mAbs, monoclonal antibodies.

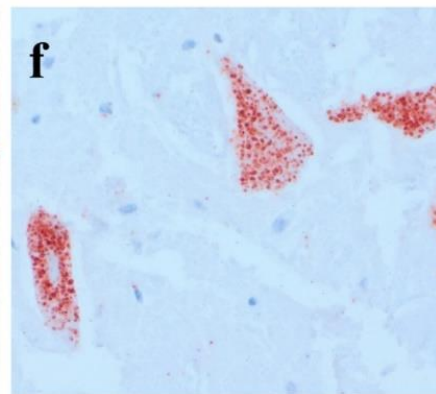
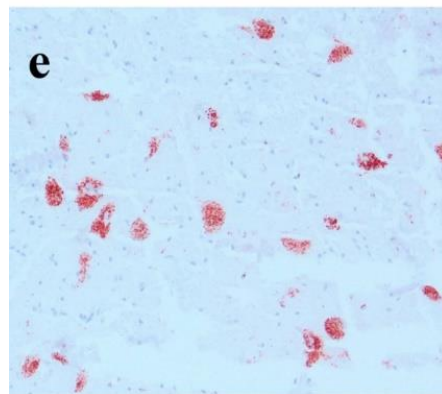
← N-Term C-Term →



Reindeer



Moose Norway




Moose Sweden

RESEARCH ARTICLE

Open Access

Heterogeneity of pathological prion protein accumulation in the brain of moose (*Alces alces*) from Norway, Sweden and Finland with chronic wasting disease



Diego Sola^{1*} , Linh Tran⁴, Jørn Våge⁴, Knut Madslie⁴, Tram T. Vuong⁴, Sirkka Liisa Korpenfelt⁵, Erik O. Ågren², Gustav Averhed², Maria Nöremark², Kaisa Sörén², Mats Isaksson², Cristina Acín¹, Juan José Badiola¹, Dolores Gavier-Widén^{2,3} and Sylvie L. Benestad⁴

❖ 13 Moose :
 7 from Norway (No)
 4 from Sweden (Sw)
 2 from Finland (Fi)

Table 1 Identification of the moose investigated.

ID	Country	Age (years)	Sex	Clinical observations
No1: 16-P138	Norway	13	Female	Abnormal behaviour, killed
No2: 16-P153	Norway	14	Female	Found dead with trauma
No3:17-CD11399	Norway	13	Female	Hunted but with abnormal behaviour (post-mortem detected hip dislocation)
No4: 18-CD24724	Norway	15	Female	Abnormal behaviour, killed (post-mortem detected hip dislocation and ethmoid tumour)
No5: 19-CD14225	Norway	20	Female	Hunted, no clinical signs
No6: 19-CD24854	Norway	12	Female	Found dead
No7: 21-CD41	Norway	13	Male	Killed due to a broken leg
Sw1: 19-VLT000541	Sweden	16	Female	Emaciated, walking in circles, seemed blind
Sw2: 19-VLT000876	Sweden	16	Female	Emaciated, behavioural changes
Sw3: 19-VLT002322	Sweden	> 10	Female	Hunted. Clinically healthy (but altered behaviour)
Sw4: 20-VLT002459	Sweden	14	Female	Lame, indolent
Fi1: 1256/20	Finland	15	Female	Found dead
Fi2: 2054/20	Finland	18	Female	Emaciated, in lying position

❖ All moose were KK109, except 2 Norwegian moose, No4 and No6 which were QQ109.

❖ IHC with 5 monoclonal mAbs:

3 on N-Terminal:

12B2

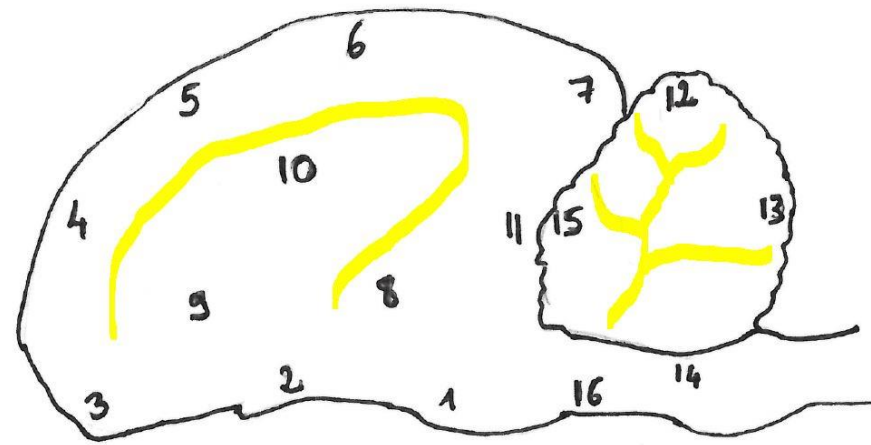
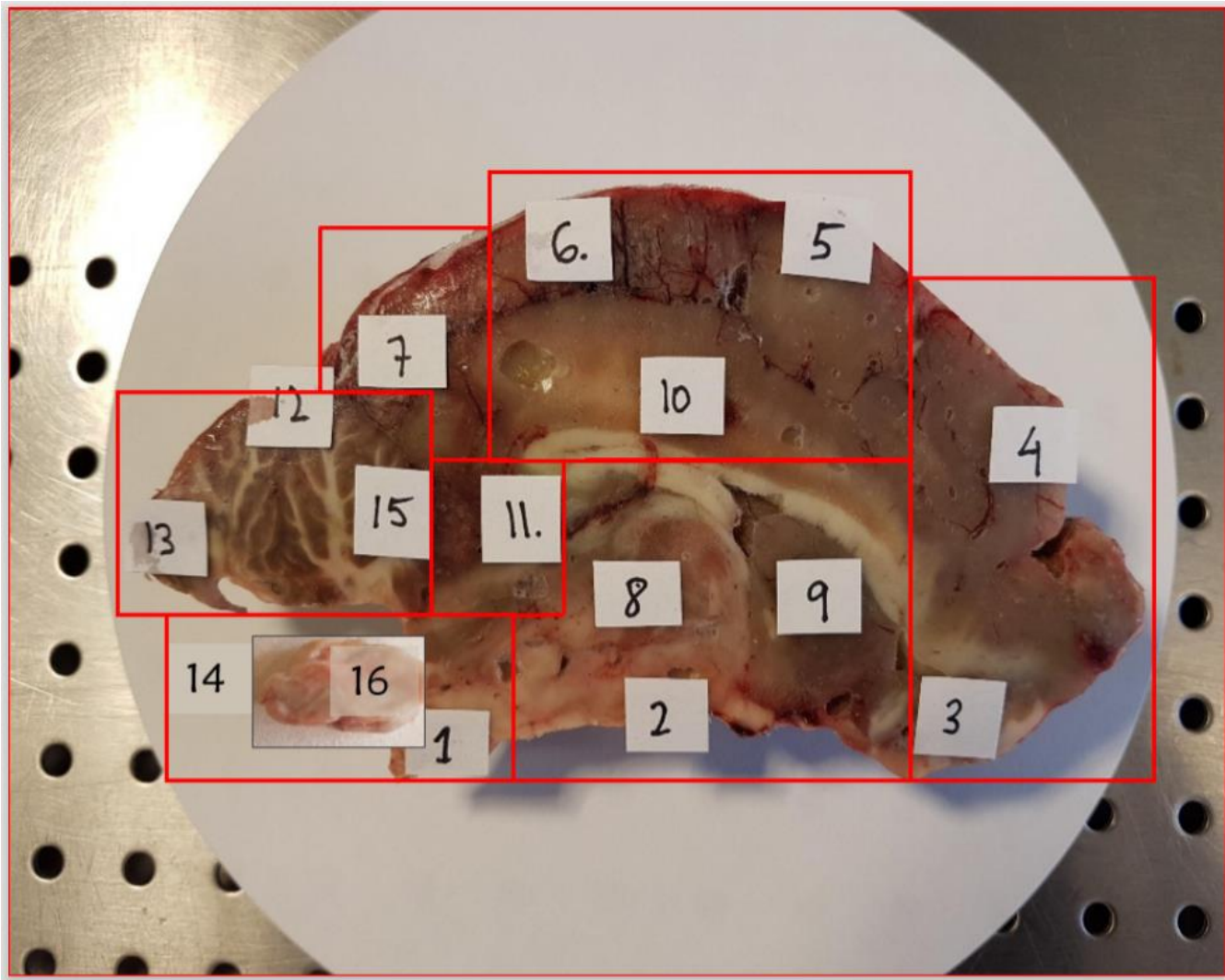
9A2

P4

2 in the core protein:

L42

SAF 84



Mango sampling

16 areas for ELISA investigation (white)

7 major brain areas (for No and Sw): frontal cortex, temporal cortex, occipital cortex, thalamus, mesencephalon, obex and cerebellum for IHC (red rectangles)

No suitable material for histopathology

Scoring system assesses the intensity and extent of distribution of PrP^{Sc} accumulation:

0 no stain;

1 mild

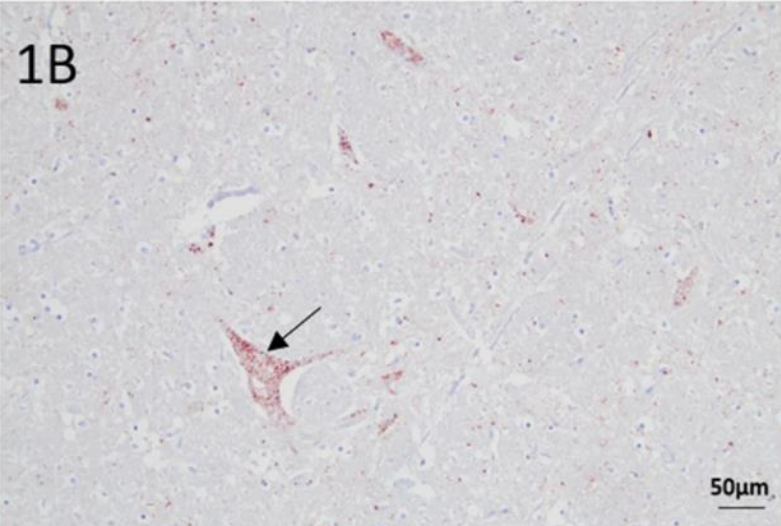
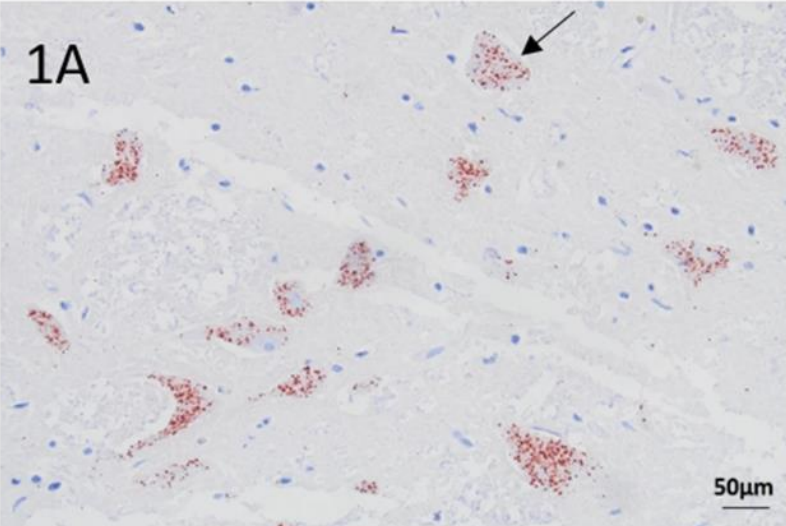
2 moderate

3 striking

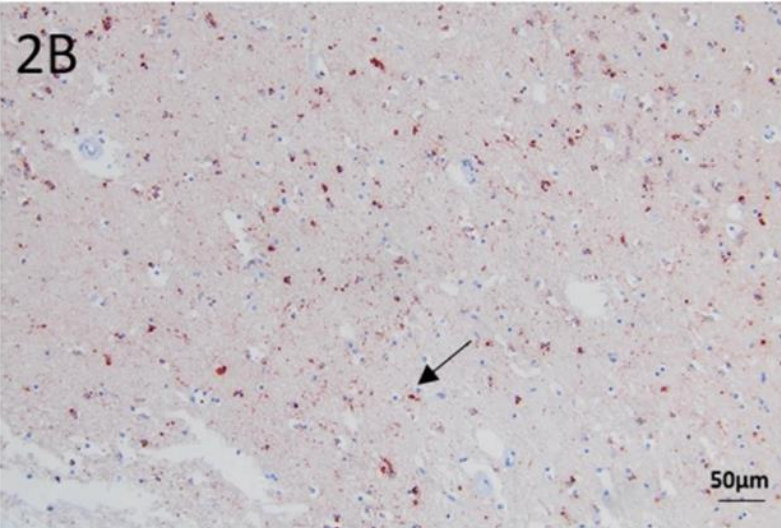
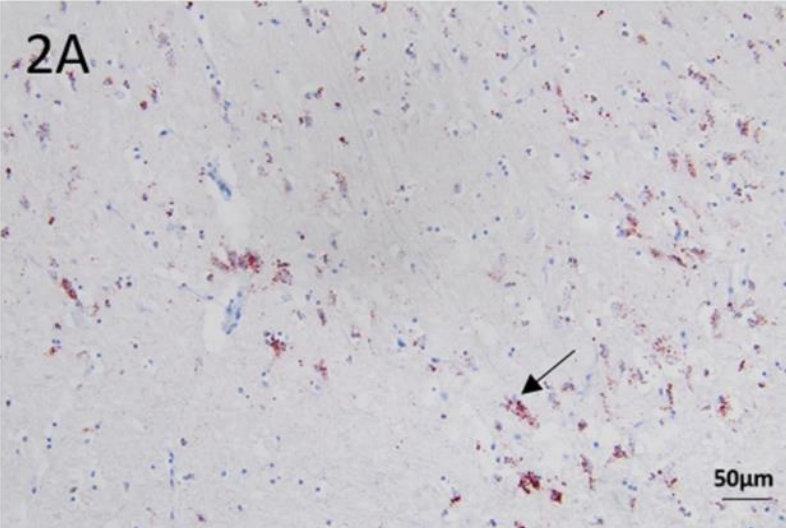
Intracellular staining

L42

SAF84



Intraneuronal

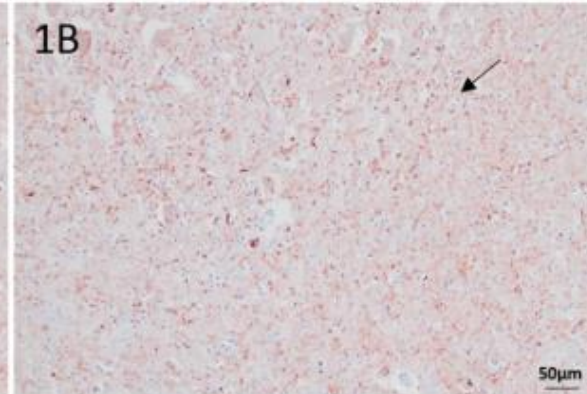
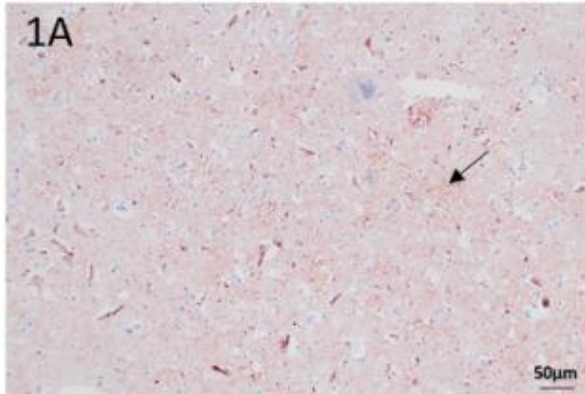


Intraglial

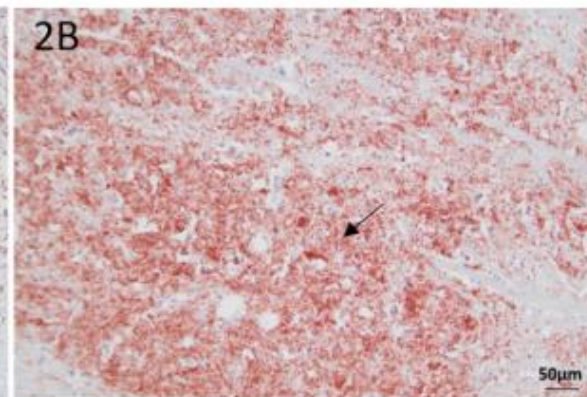
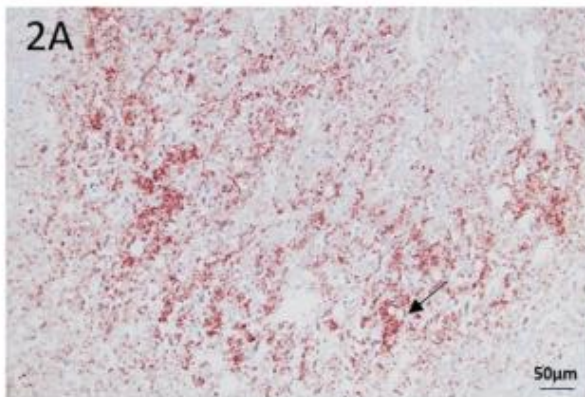
Extracellular staining

L42

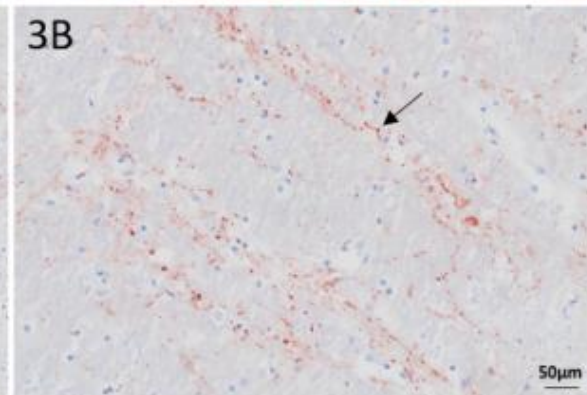
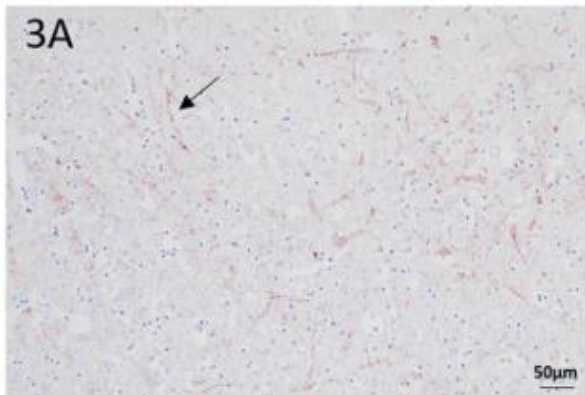
SAF84



Fine punctuate

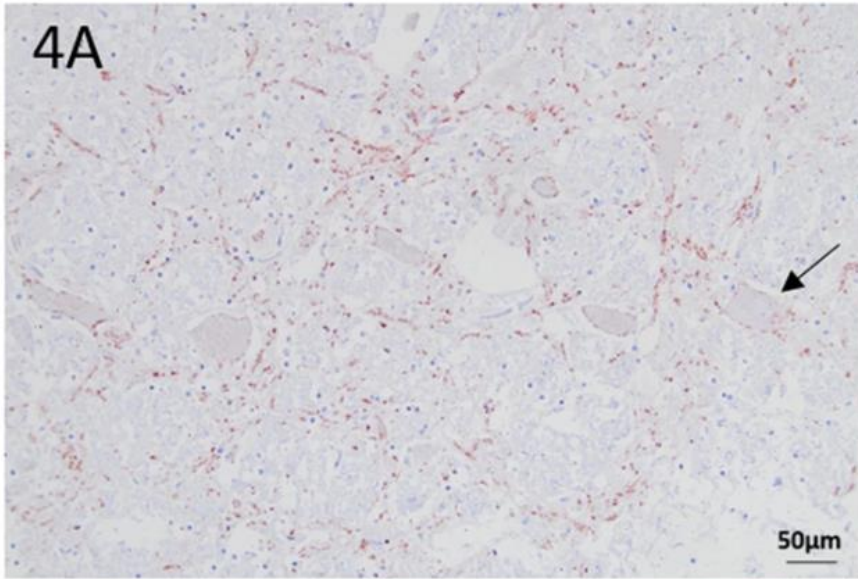


Coarse granular

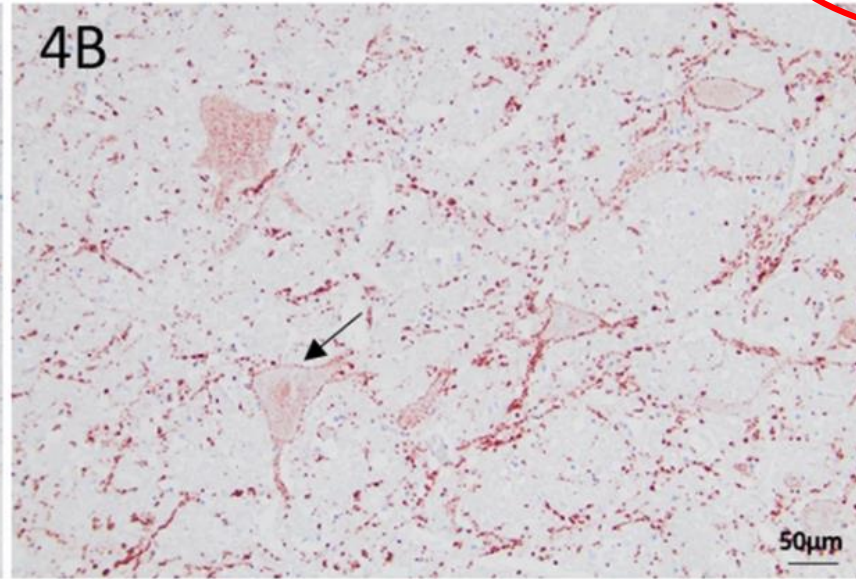


Linear

L42

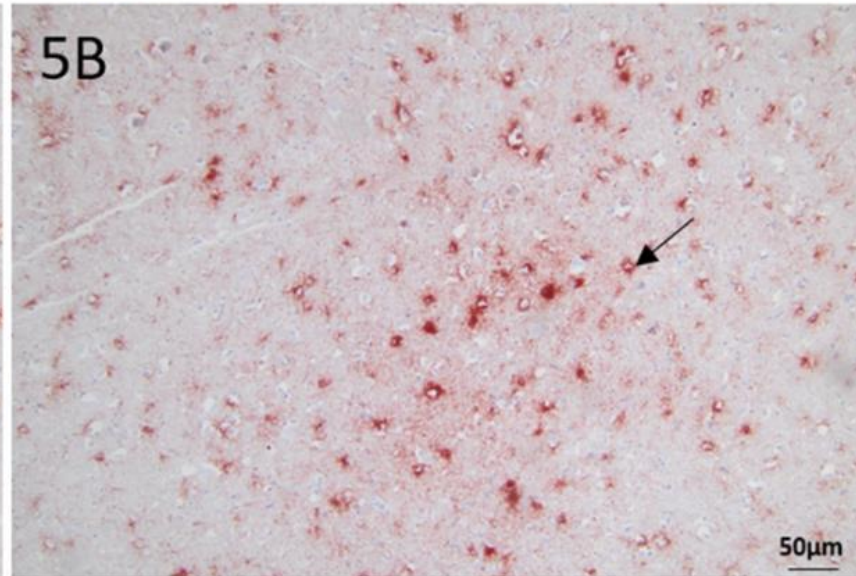
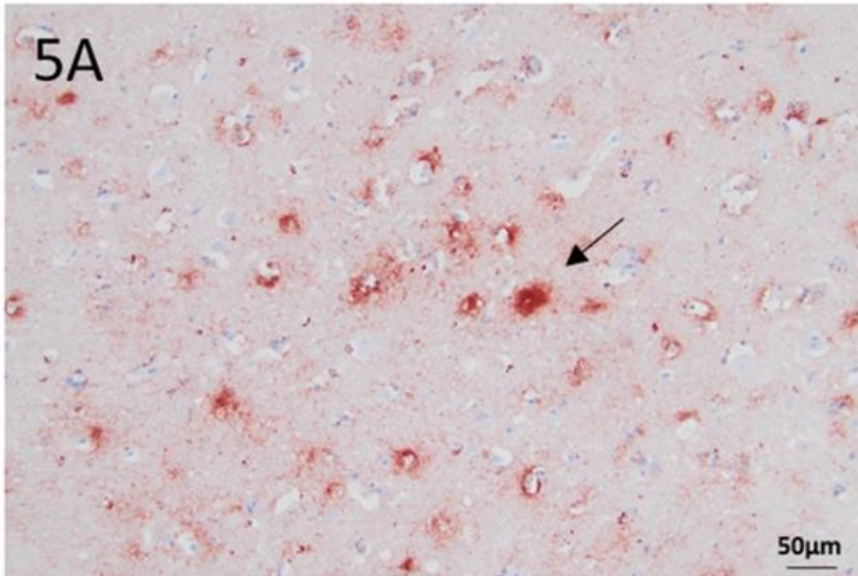


SAF84



Rare staining

Perineuronal
only in 1 moose

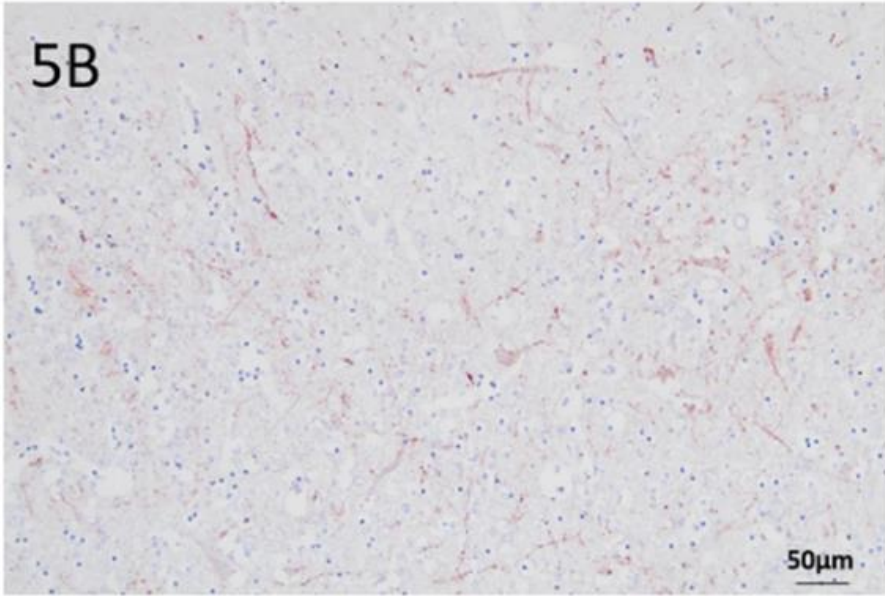
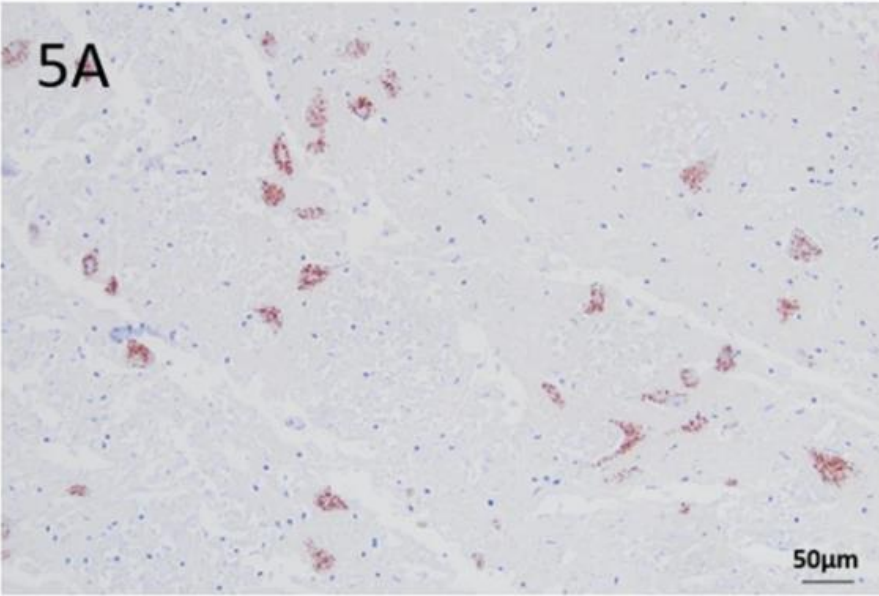


Stellate
only in 1 moose

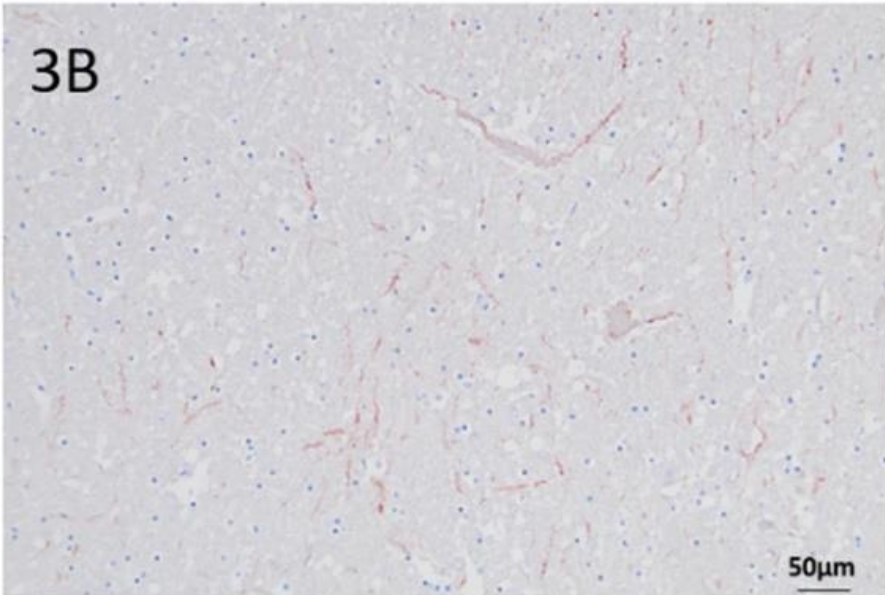
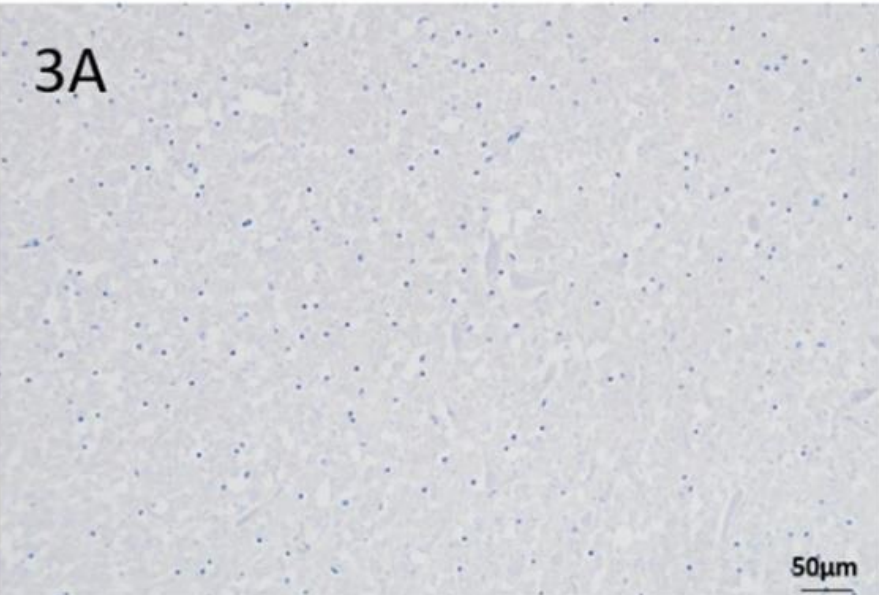
Differences not dependent of the anatomical structure

Thalamus-Sw1

Thalamus-Sw3

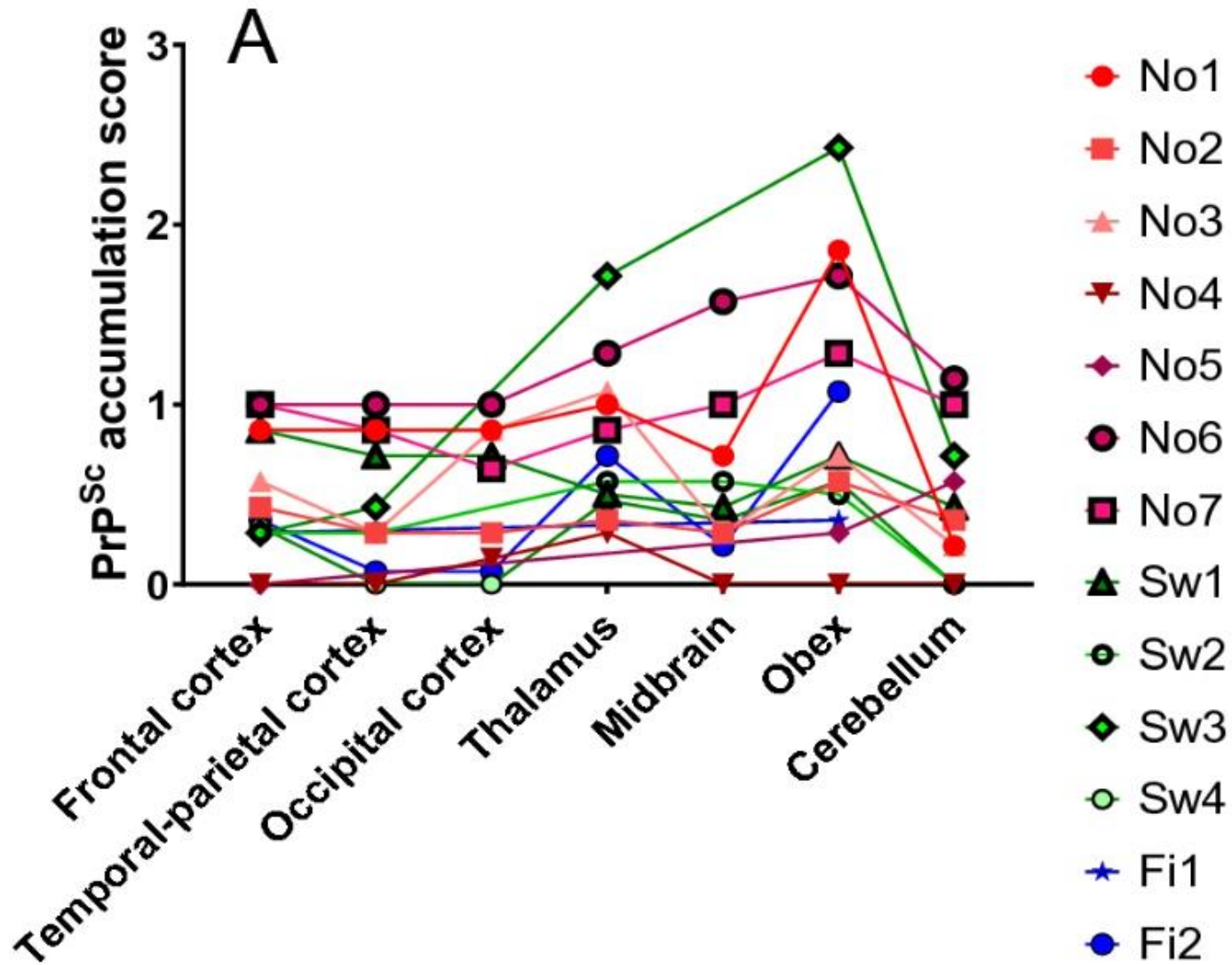


L42

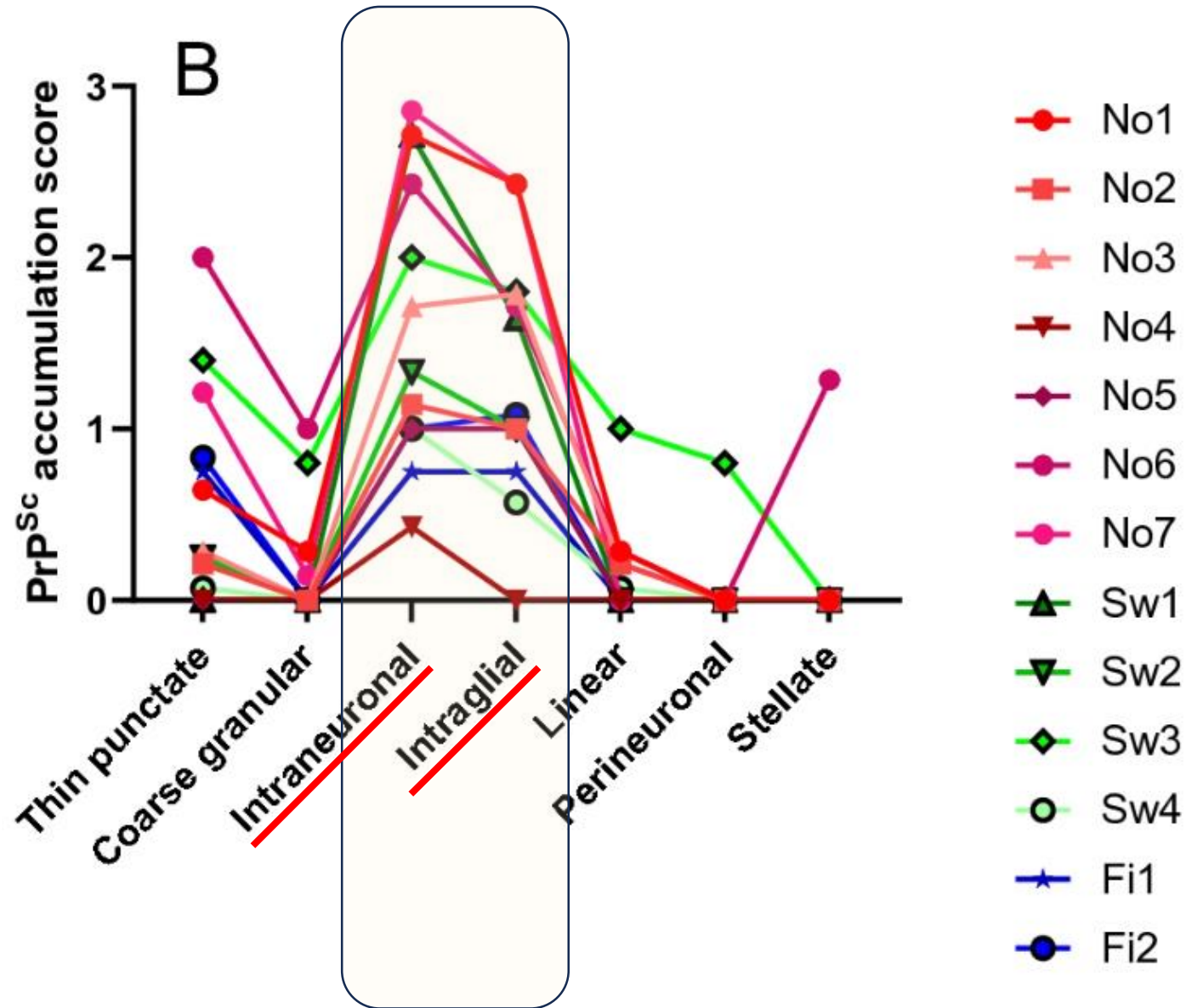


P4

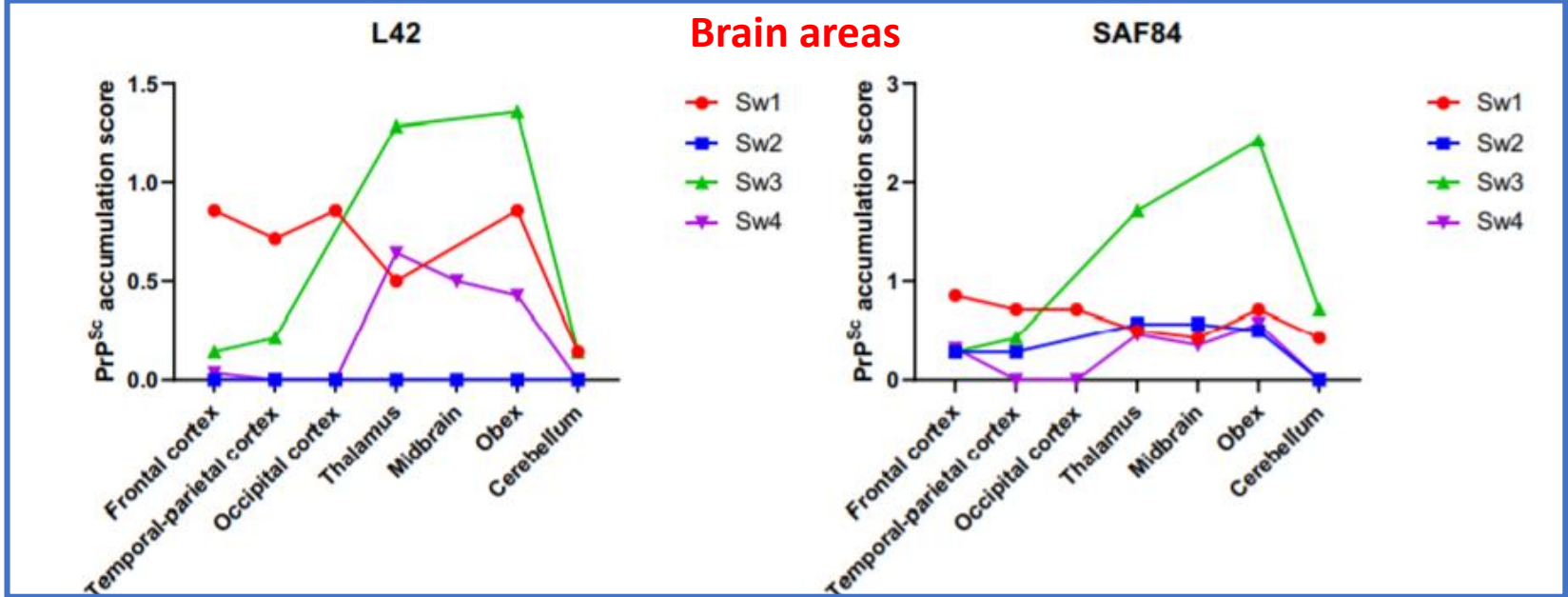
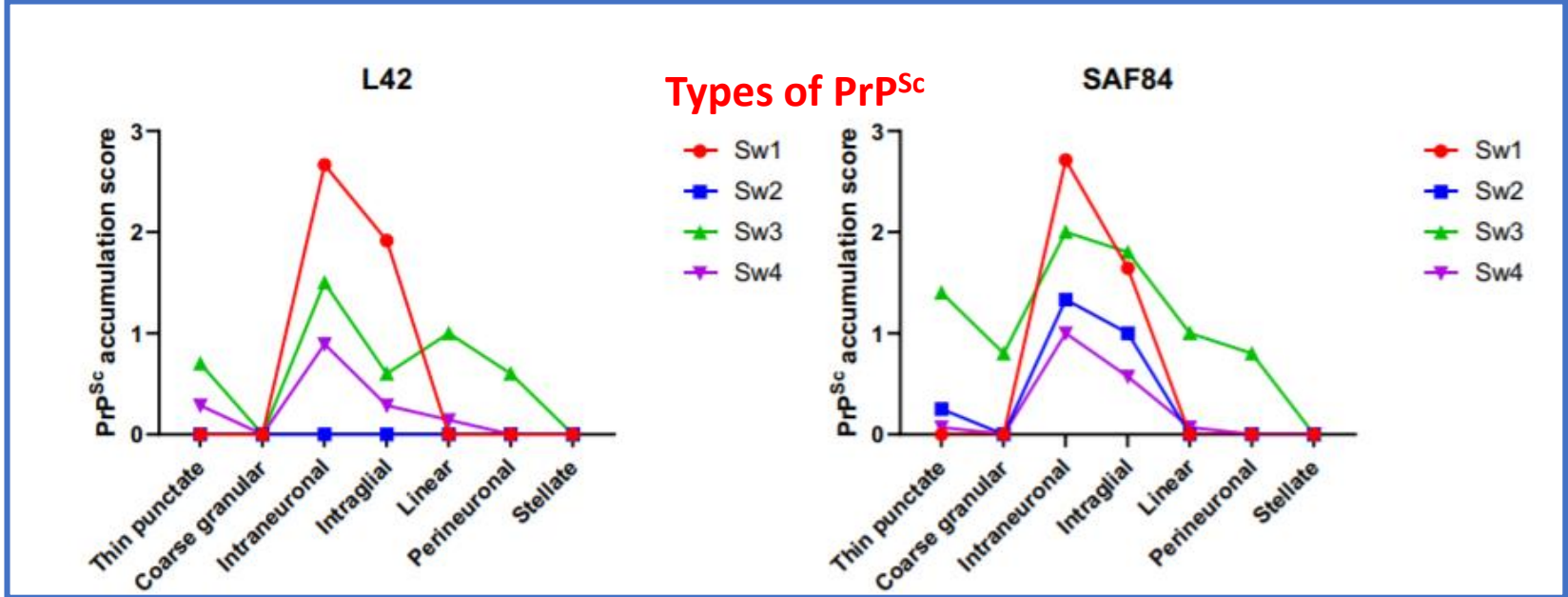
Intensity of staining varies in the same area between moose



Frequency of the PrP^{Sc} types varies between the moose



Differences regardless the country of origin (Sweden here)



The Nordic CWD moose have differences

- PrP^{Sc} glycoprofiles
- PrP^{Sc} distribution within the brain
- PrP^{Sc} IHC patterns in the brain
- differences not related to their geographical origin

But they have common points

- presence of the 13 CT fragment (WB)
- PrP^{Sc} IHC patterns: intraneuronal and intraglial predominant
- negative diagnostic test results in the lymphoid tissues
- older animals

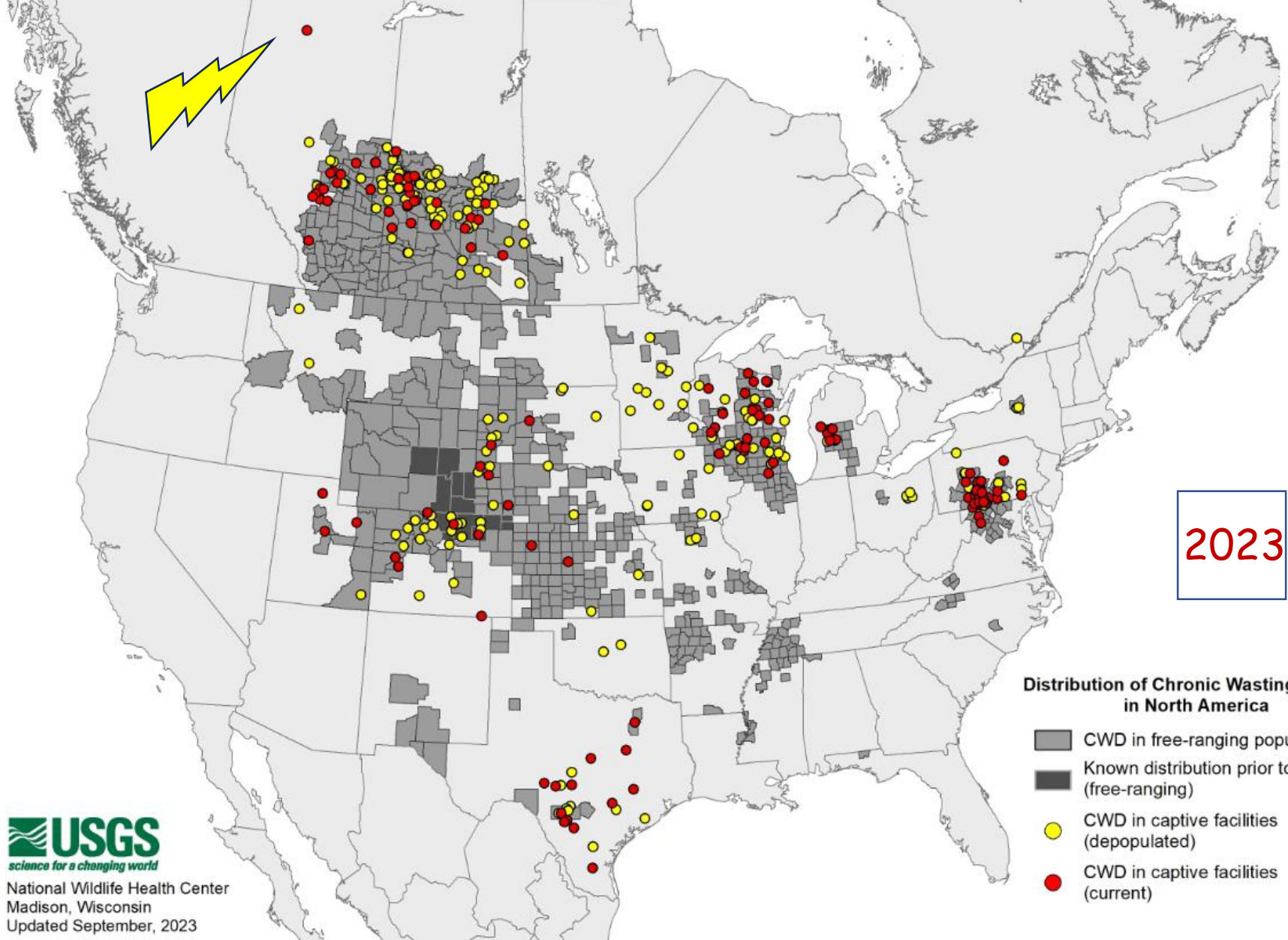


Thank you to the collaborators:

- ISS Roma (Romolo Nonno's group)
- SVA Sweden (Dolores Gavier-Widen, Maria Nöremark)
- Finnish Food Authority (Sirikka Liisa Korpenfelt)
- Diego Sola (Zaragoza University)

Thank you for your attention





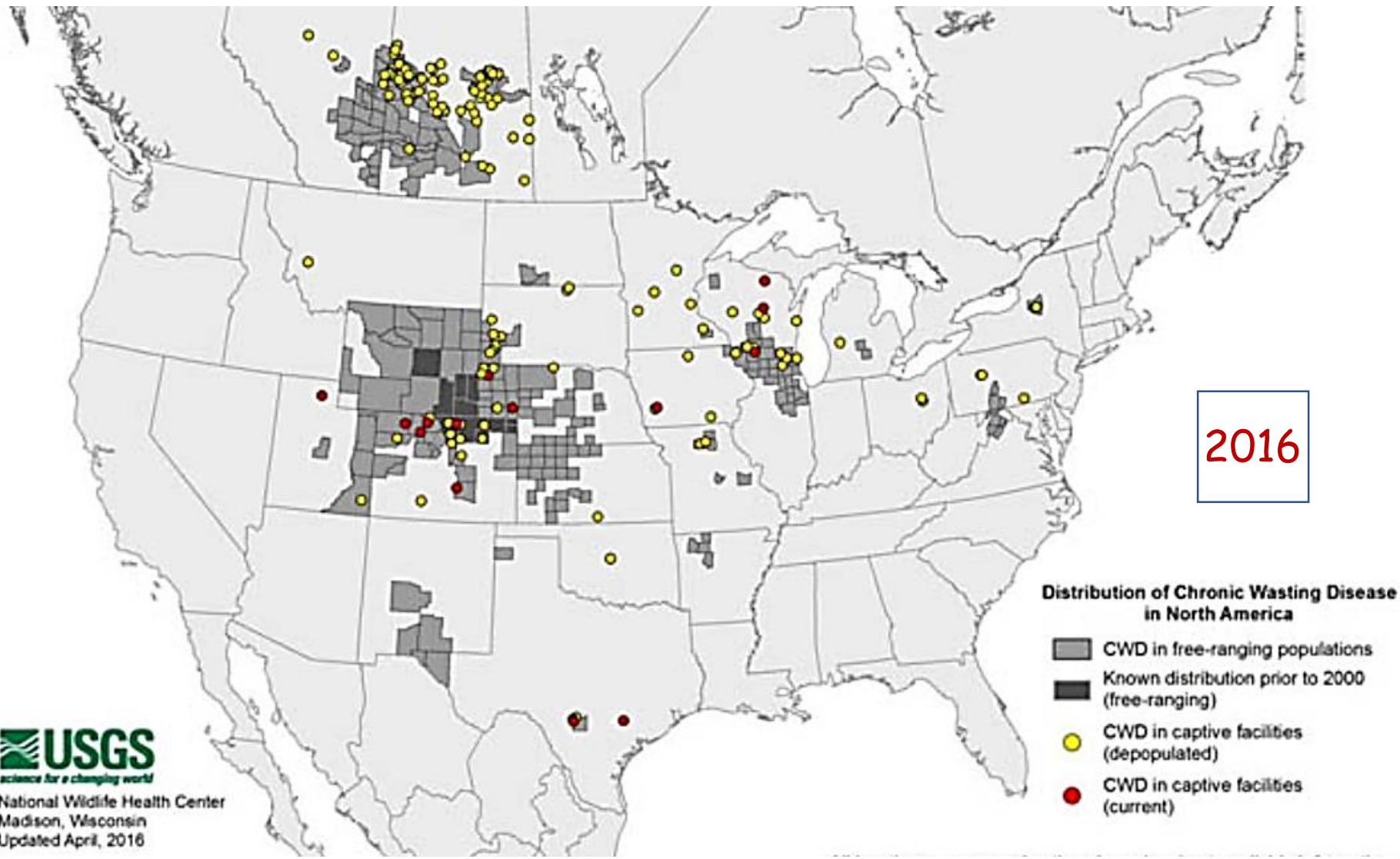
2023

Distribution of Chronic Wasting Disease in North America

- CWD in free-ranging populations
- Known distribution prior to 2000 (free-ranging)
- CWD in captive facilities (depopulated)
- CWD in captive facilities (current)



National Wildlife Health Center
Madison, Wisconsin
Updated September, 2023



USGS
science for a changing world
 National Wildlife Health Center
 Madison, Wisconsin
 Updated April, 2016