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9th Annual Meeting of the TSE EURL
Torino, Italy
17th-18th October 2022



Characterization of emerging CWD strains in Europe

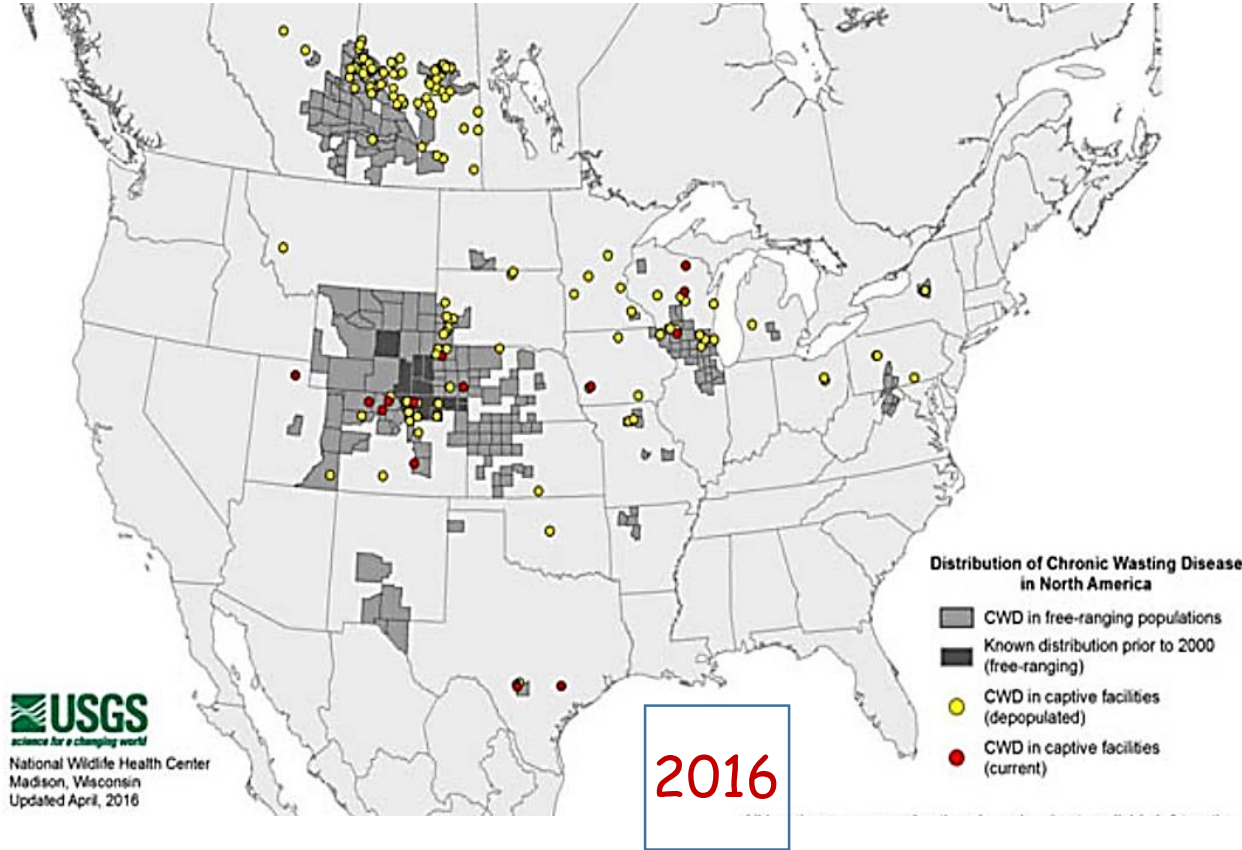
Romolo Nonno, Laura Pirisinu, Michele Di Bari, Claudia D'Agostino
Istituto Superiore di Sanità, Rome

Sylvie Benestad, Norwegian Veterinary Institute, Oslo

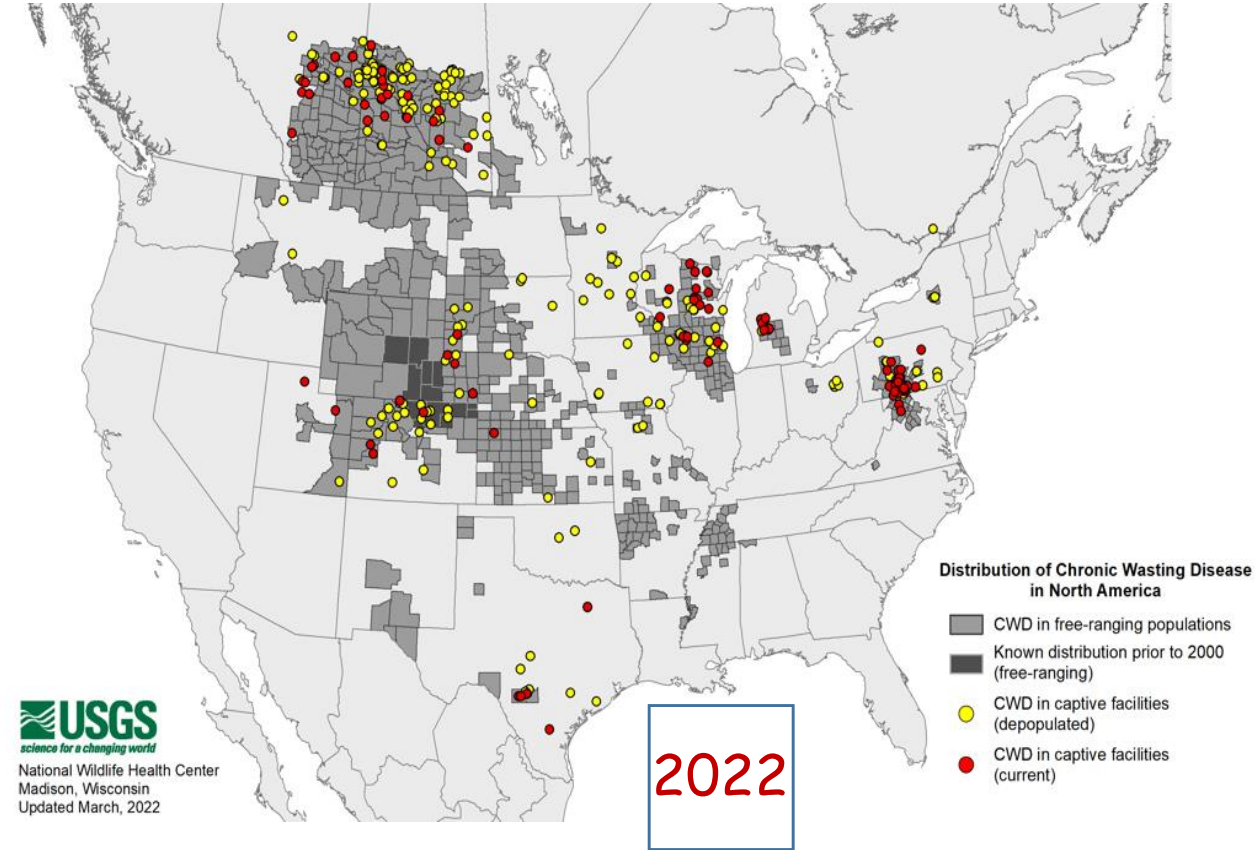
- WB patterns of PK resistant core of PrP^{Sc} (PrP^{res})
(proxy for the conformation of PrP^{Sc})

- Biological strain typing (bioassay in bank voles)

.....epidemiology, PrP genetic and pathology in the original host need to be taken into consideration



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



USGS
science for a changing world
 National Wildlife Health Center
 Madison, Wisconsin
 Updated March, 2022

CWD prevalence increases in affected areas

CWD - host species in North America



Rocky Mountain elk/Wapiti
(*Cervus elaphus nelson/canadensis*)



White-tailed deer (*Odocoileus virginianus*)



Mule deer
(*Odocoileus hemionus*)



Moose
(*Alces alces*)

- ❖ Pathological and PrP^{Sc} features shared among species
- ❖ Wide tissue distribution of prions and lymphoid tissues involvement

Bioassay of North American CWD isolates in bank voles (Bv109I)

OPEN ACCESS Freely available online

PLOS PATHOGENS

Chronic Wasting Disease in Bank Voles: Characterisation of the Shortest Incubation Time Model for Prion Diseases

Michele Angelo Di Bari^{1*}, Romolo Nonno¹, Joaquín Castilla², Claudia D'Agostino¹, Laura Pirisinu¹, Geraldina Riccardi¹, Michela Conte¹, Juergen Richt^{3a}, Robert Kunkle³, Jan Langeveld⁴, Gabriele Vaccari¹, Umberto Agrimi¹

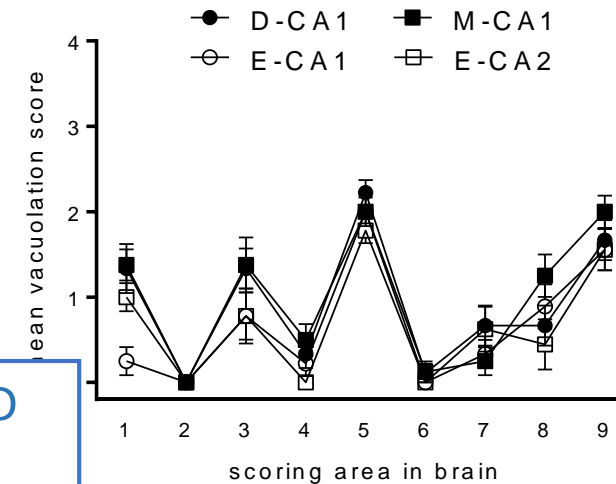
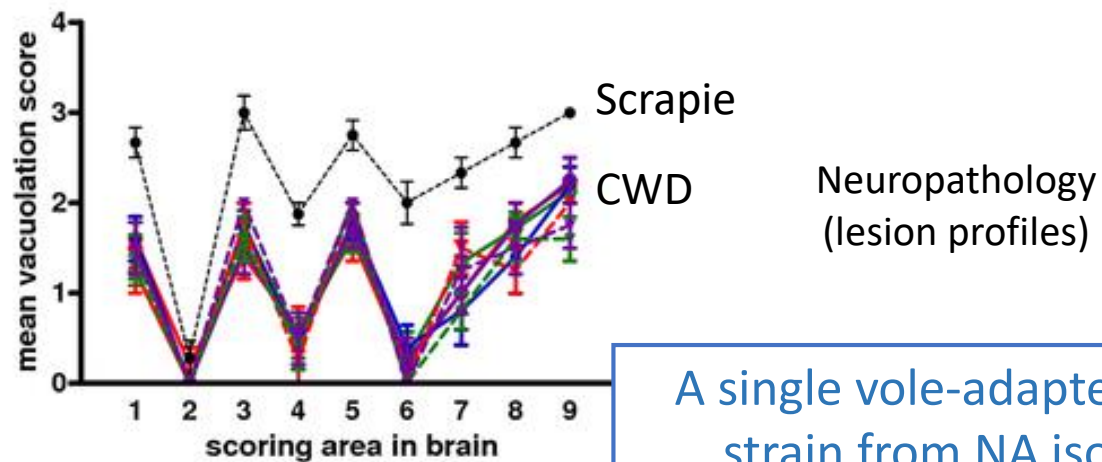
Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America

Romolo Nonno^{a,1}, Michele A. Di Bari^a, Laura Pirisinu^a, Claudia D'Agostino^a, Ilaria Vanni^a, Barbara Chiappini^a, Stefano Marcon^a, Geraldina Riccardi^a, Linh Tran^b, Turid Vikøren^b, Jørn Våge^b, Knut Madslie^b, Gordon Mitchell^c, Glenn C. Telling^d, Sylvie L. Benestad^{b,2}, and Umberto Agrimi^{a,2}

USA (elk, mule deer, WT deer)

Canada (elk, WT deer, moose)

Survival time
Primary transmission ~200 dpi
Second/third passage ~35 dpi



North American CWD in bank voles (Bv109I)

- Our results imply that Bv109I selectively propagated a strain component shared by all NA sources studied so far
- Taken together, «epidemiology + pathology + bioassay» suggest that these NA cases are epidemiologically related by the involvement of a prevalent CWD strain

1° CWD case in EU



Reindeer, 3-4 years
March 2016



Moose, 13 years, pregnant,
abnormal behaviour
May 2016



Moose, 14 years, pregnant,
found dead (trauma)
May 2016

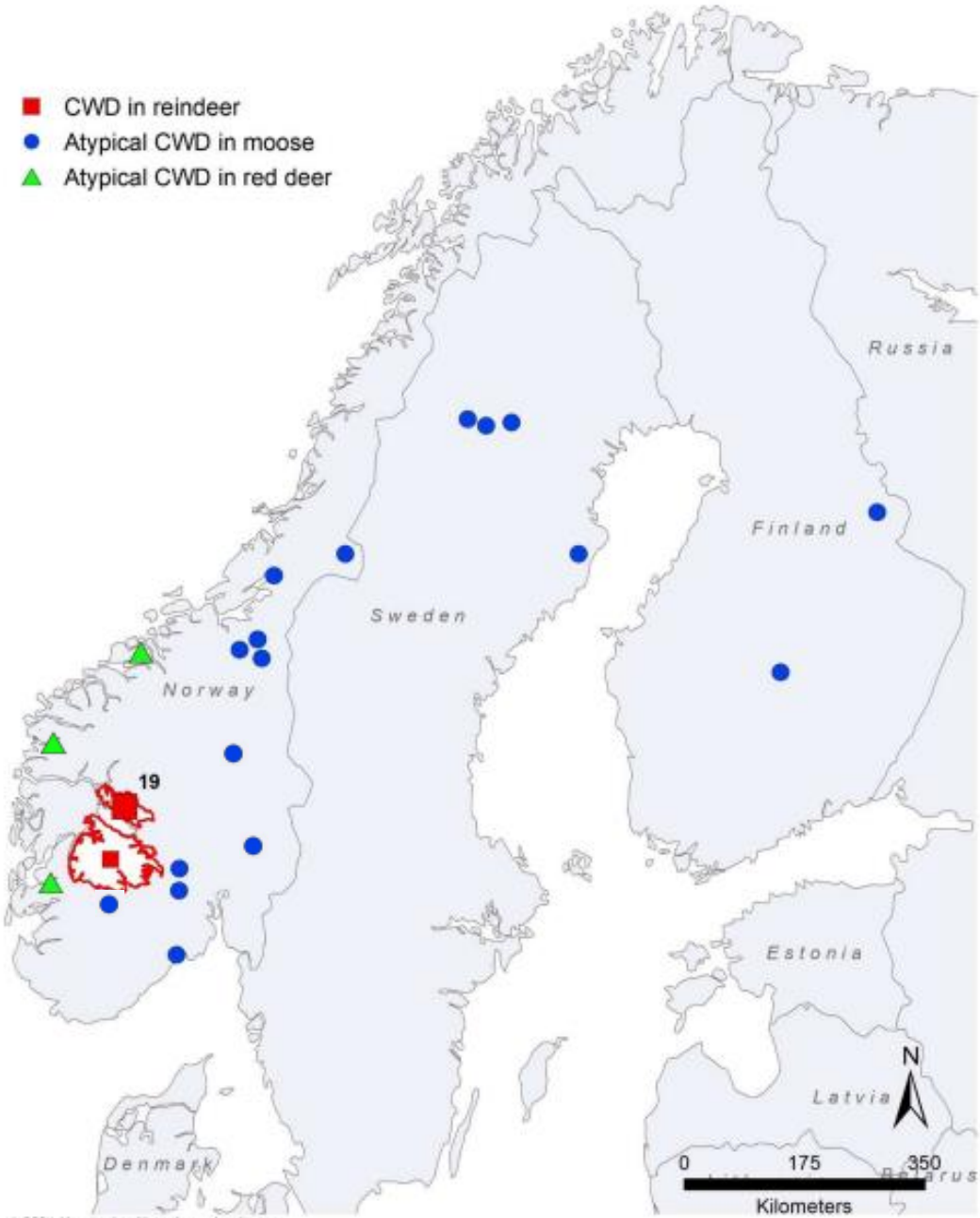


Red deer, 16 years, female,
hunted
October 2017









Cases of CWD detected in Europe

n = 41



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-  =  moose n=17
-  =  reindeer n=21
-  =  red d n=3

	2016 Norway	2018 Finland	2019 Sweden
moose	11	2	4
reindeer	21		
red d	3		
Number cervids tested since 2016	>155.000 (2016-2022)	3.300 (2018-2021)	6.200 (2018-2021)

Norway



	tested	positive	%
Reindeer (wild)	20530	21	0,102289
Nordfjella	2814	19	0,675195
Hardangervidda	6853	2	0,029184
Reindeer (semi-domesticated)	54259		
Red deer (wild)	28366	3	0,010576
Red deer (captive)	3135		
Moose	35247	11	0,031208
Roe deer	11364		
Fallow deer	244		
Total	155875	35	

Daily updated figures available: <http://apps.vetinst.no/skrantesykestatistikk/NO/#omrade>



Depopulation of Nordfjella reindeer (Sept 2017 – May 2018)

2471 reindeer tested

Both Brain and Ln analysed

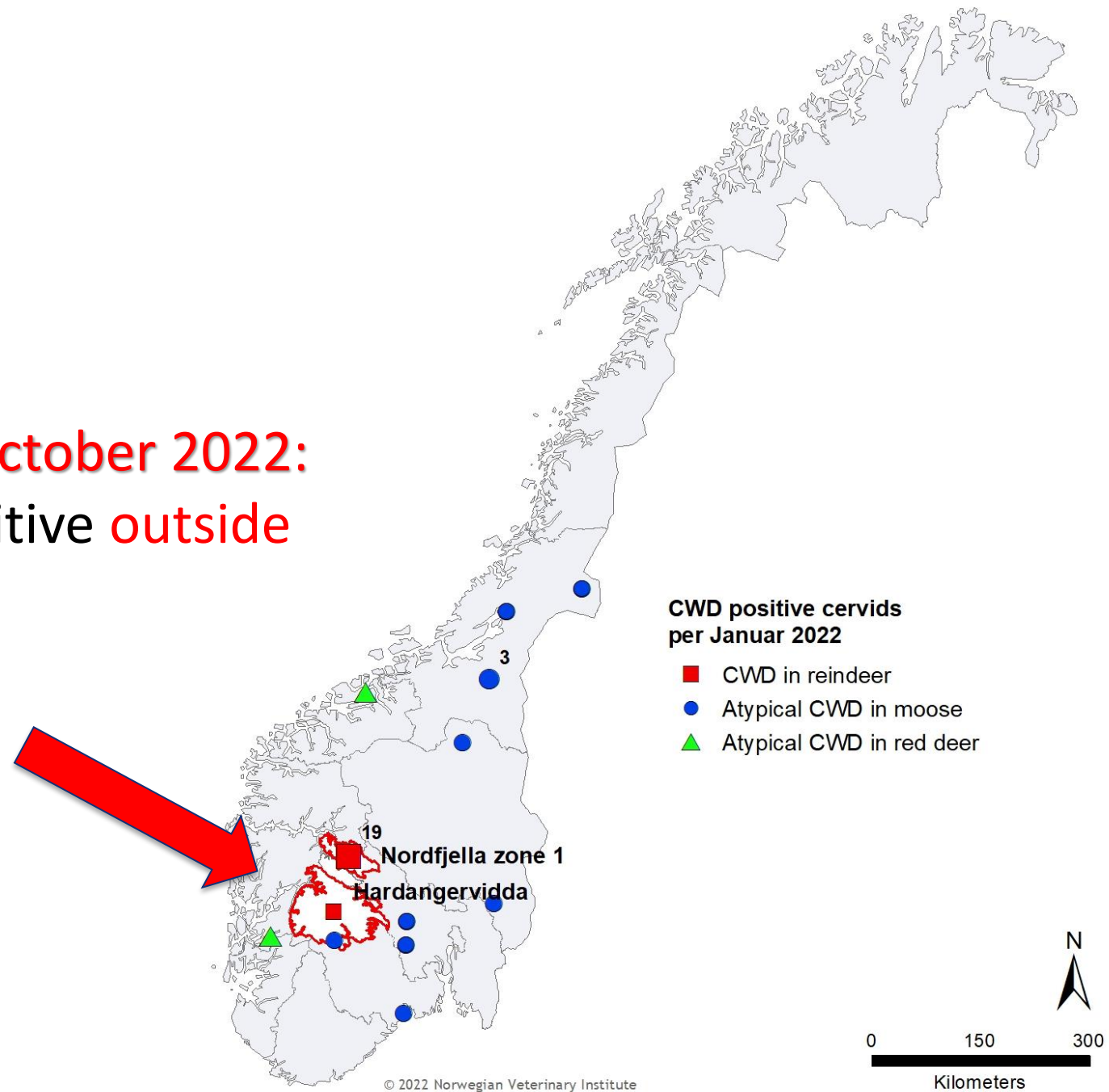
19 positive (9 positives in Ln only)

0,76 % frequency

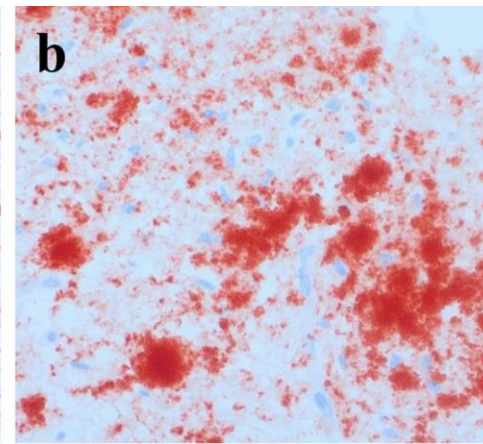
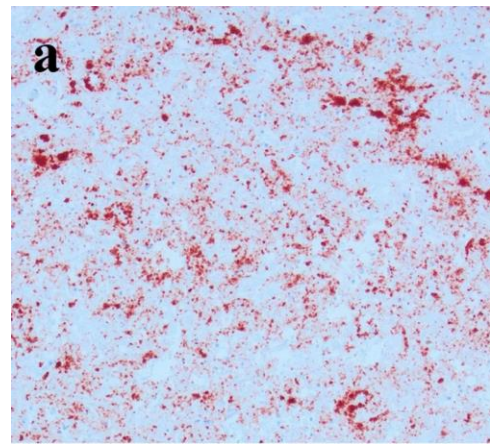
True prevalence estimated as 1.8% in adult males and 0.6% in adult females

**Sex-biased infection pattern:
infection 2.7 times more likely in adult males**

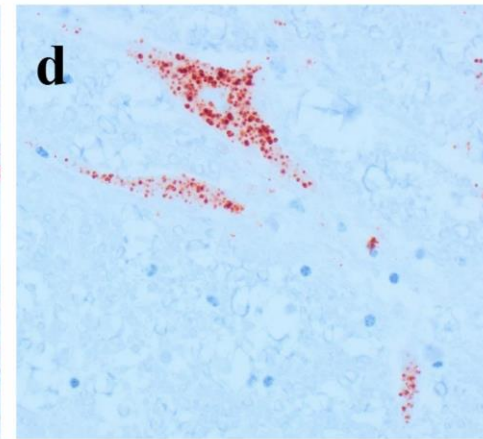
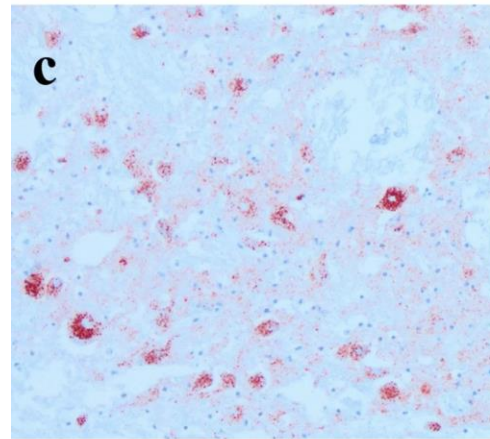
September 2020 and October 2022:
Two reindeer CWD positive **outside**
the depopulated area



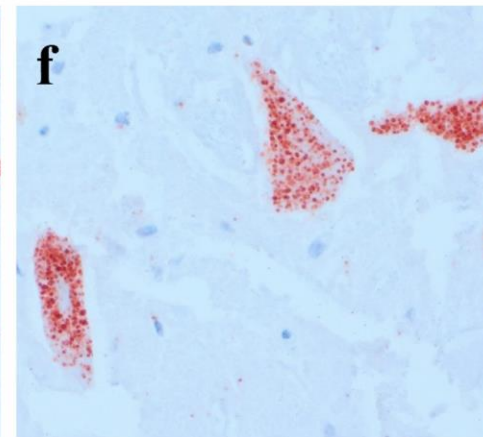
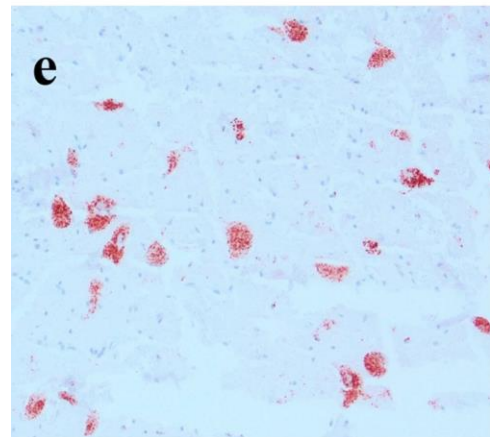
Immunohistochemical labelling of PrP^{Sc} in the brain of reindeer and moose diagnosed with CWD.



a, b Reindeer, medulla oblongata, strong, extracellular thin and coarse granular, coalescing and plaque-like PrP^{Sc} deposition (SAF84 mAb).

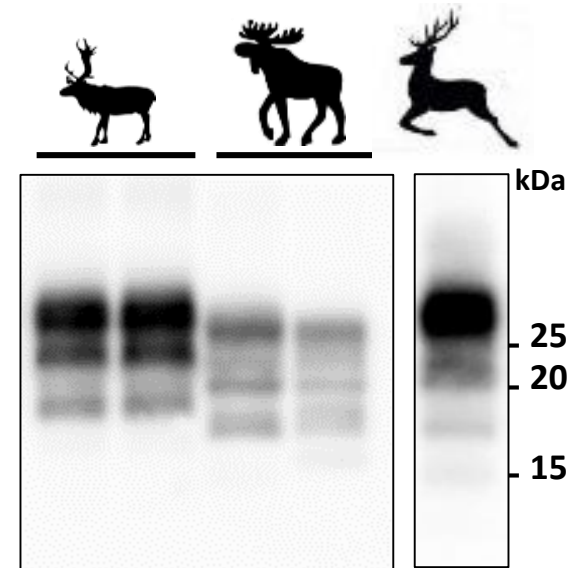
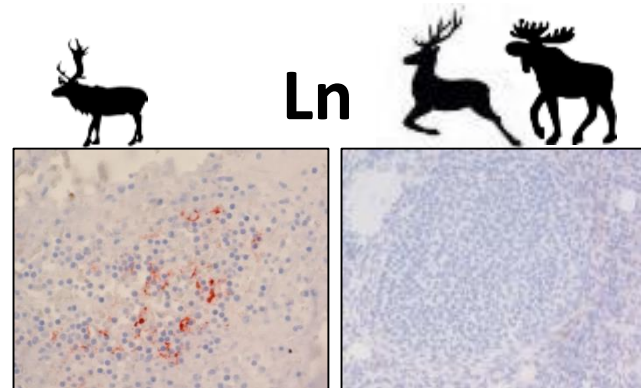
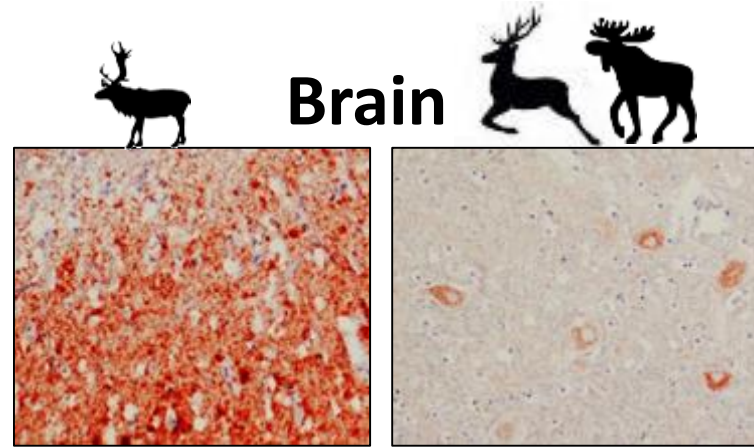


c, d Atypical CWD in a Norwegian moose, medulla oblongata, predominance of intraneuronal PrP^{Sc} deposition (L42 mAb).

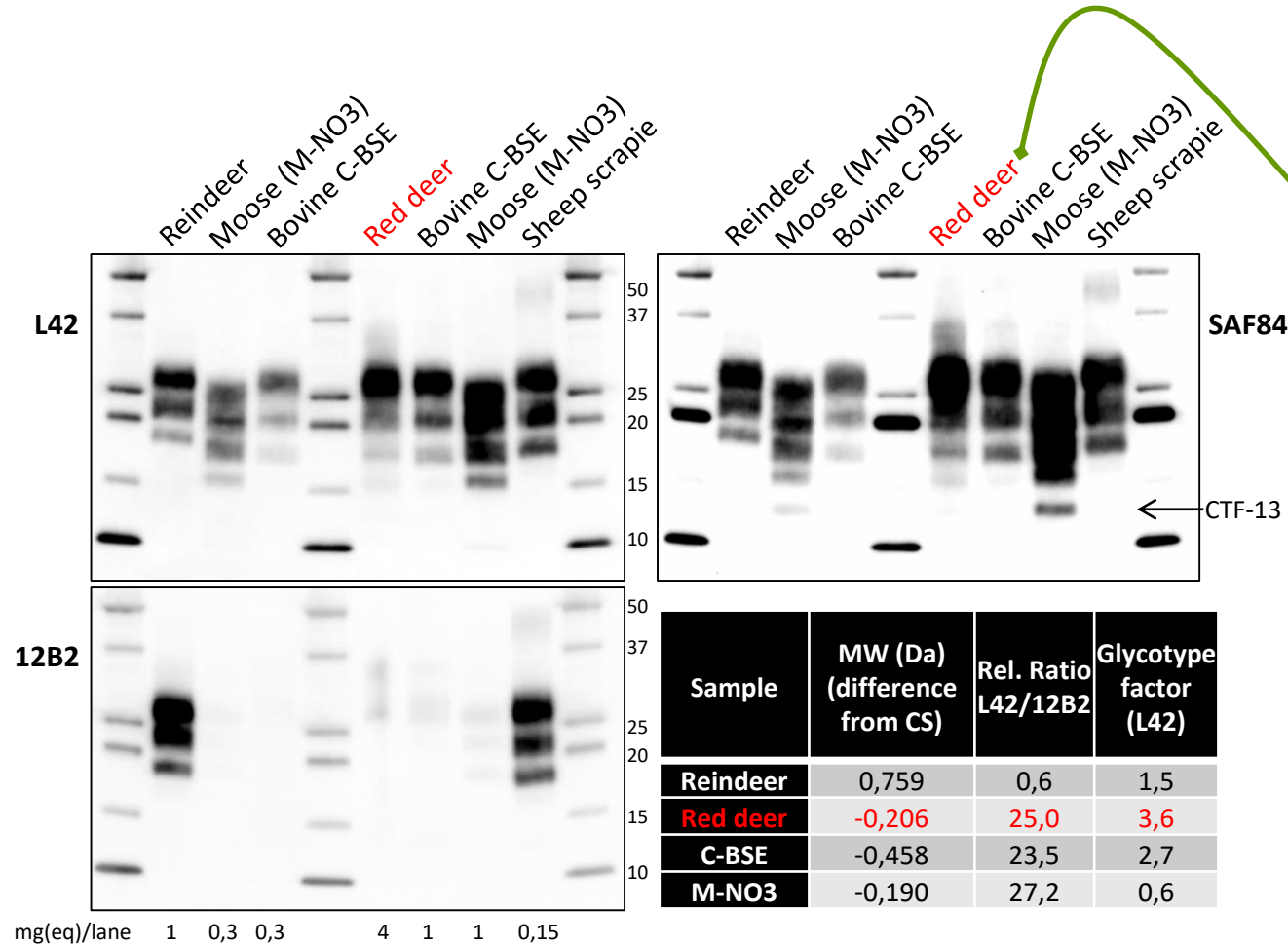


e, f Atypical CWD in a Swedish moose, thalamus, intraneuronal PrP^{Sc} deposition (SAF84 mAb)

Atypical CWD features in moose and red deer in EU



PrP^{Sc} and biological properties in Red deer



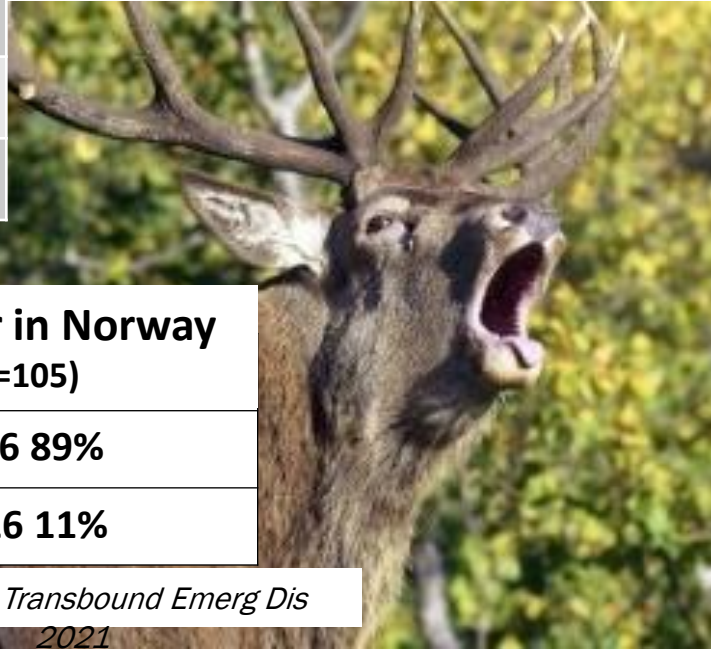
- ✓ Discriminatory IHC (S Benestad, NVI; J Spiropoulos, APHA)
- ✓ PMCA (B Maddison and K Gough, University of Nottingham; J Castilla, CIC BioGUNE; O Andreoletti, INRA)
- ✓ Bioassay (O Andreoletti, INRA; JM Torres, INIA; G Telling, CSU; R Nonno, ISS)
 - ✓ Tg-bov
 - ✓ Tg-ov
 - ✓ Tg-cervid
 - ✓ Bank voles

PMCA and bioassay results show that the CWD strain in this red deer has not the biological properties of C-BSE

CWD in Red deer

Norway, 3 cases

Internal ID	Age	Sex	Genotype
RD-NO1	16	F	EE226
RD-NO2	NA	F	EE226
RD-NO3	NA	F	EE226



**Red deer in Norway
(n=105)**

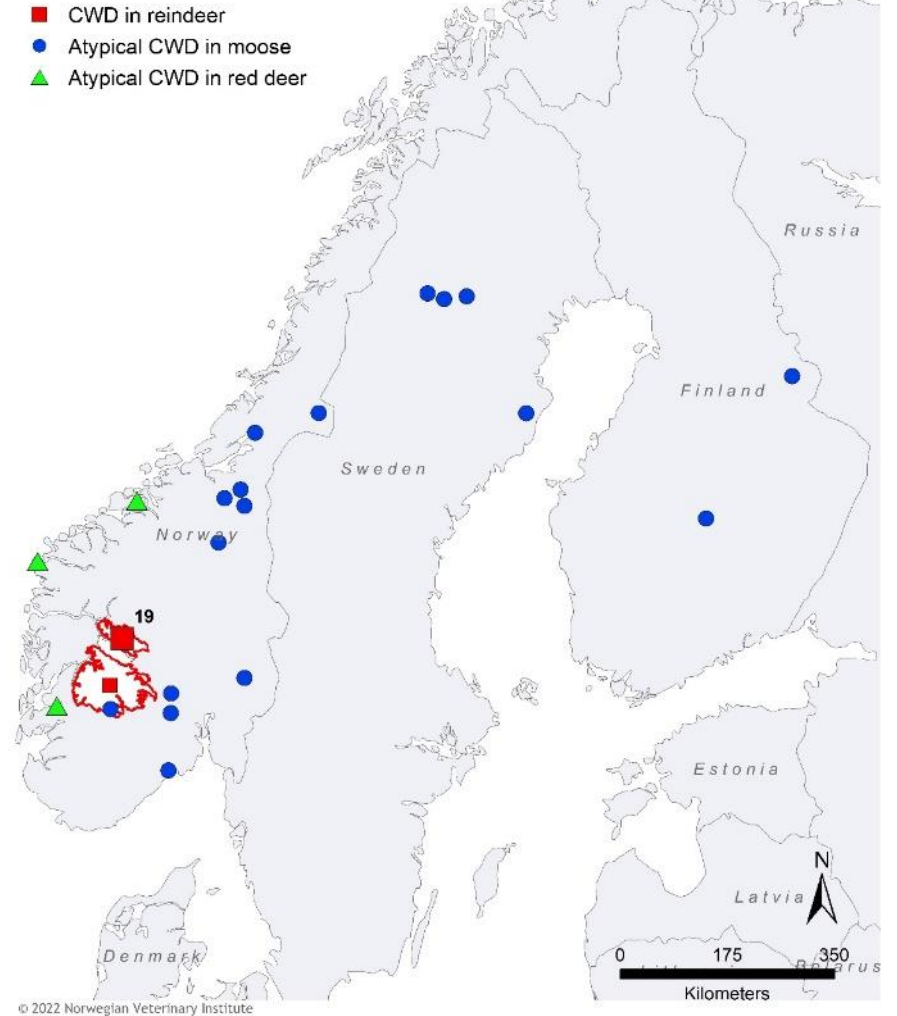
E226 89%

Q226 11%

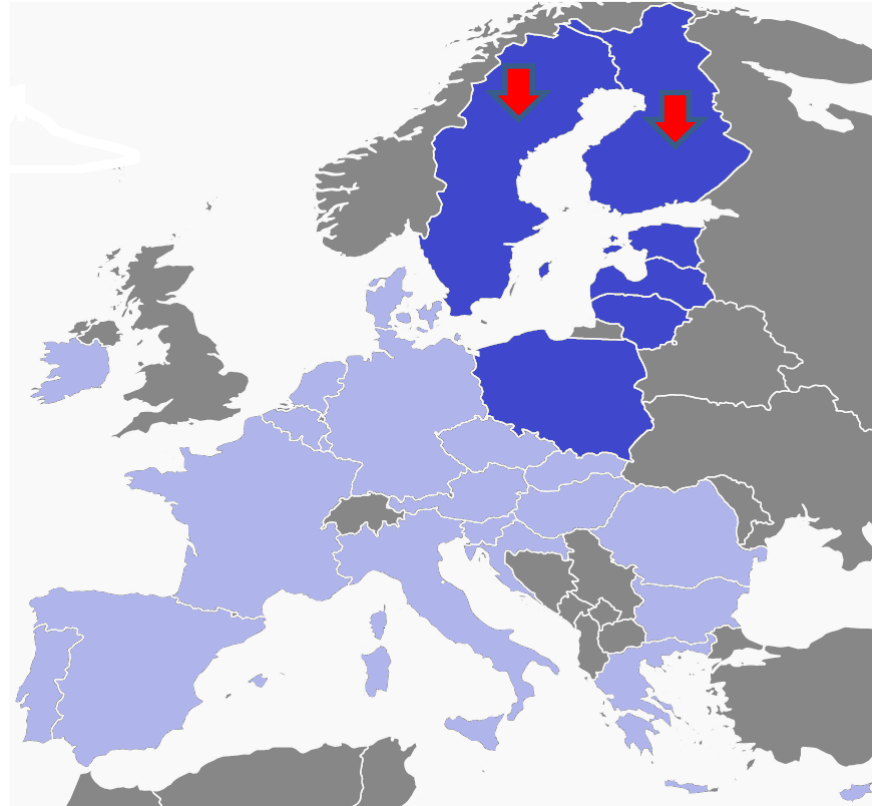
*Güere et al, Transbound Emerg Dis
2021*

All negative for PrP^{Sc} in lymph nodes!

Map: Attila Tarpai NVI

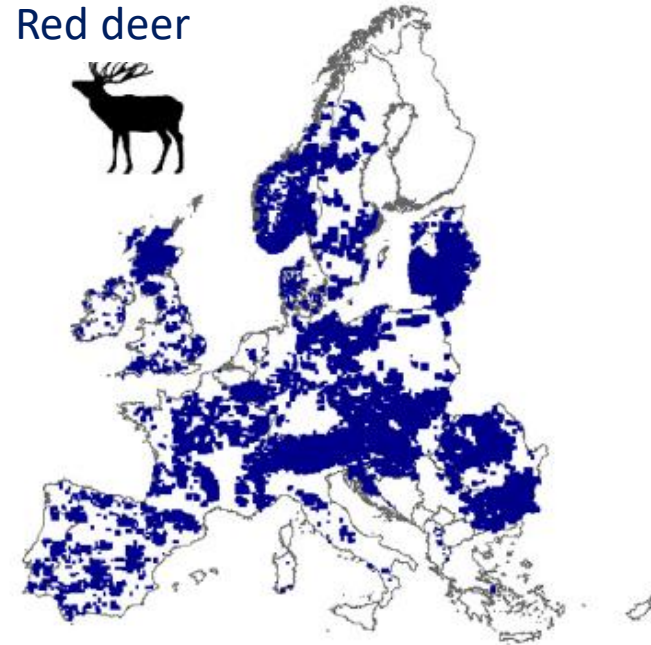


3-year monitoring for CWD only in countries with reindeer and moose, showed CWD also present in **FI, SE**



J.D.C. Linnell, et al. Biological Conservation 244 (2020) 108500

Red deer



What about countries with red deer?

CWD in Moose

Finland
2 cases

Internal ID	Age	Sex	Genotype
M-FI1	15	F	Not available
M-FI2	18	F	Not available

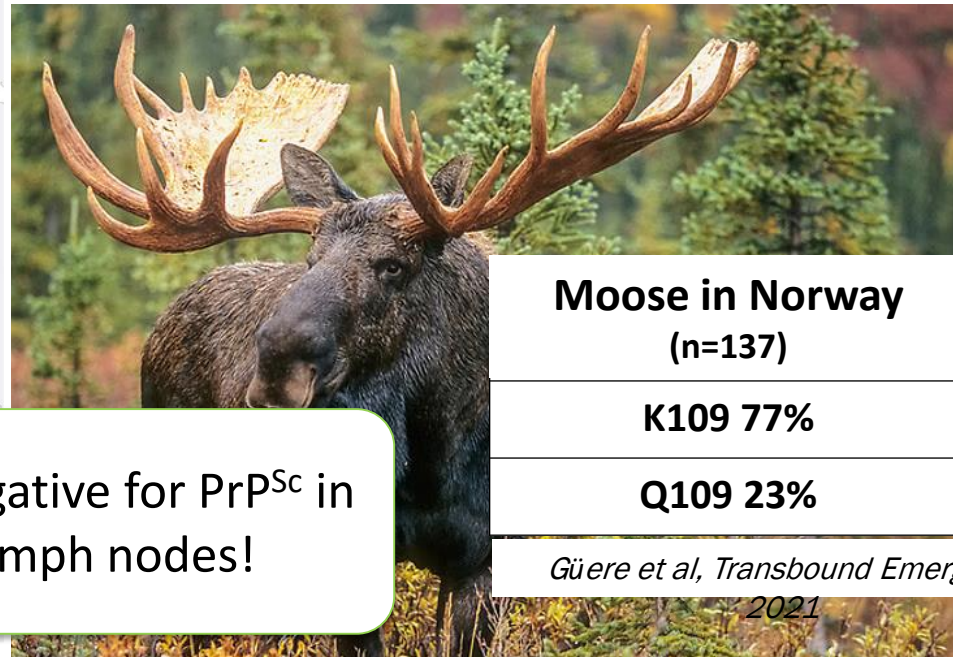
Sweden
4 cases

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	Not available

Norway
11 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

Map: Attila Tarpai NVI



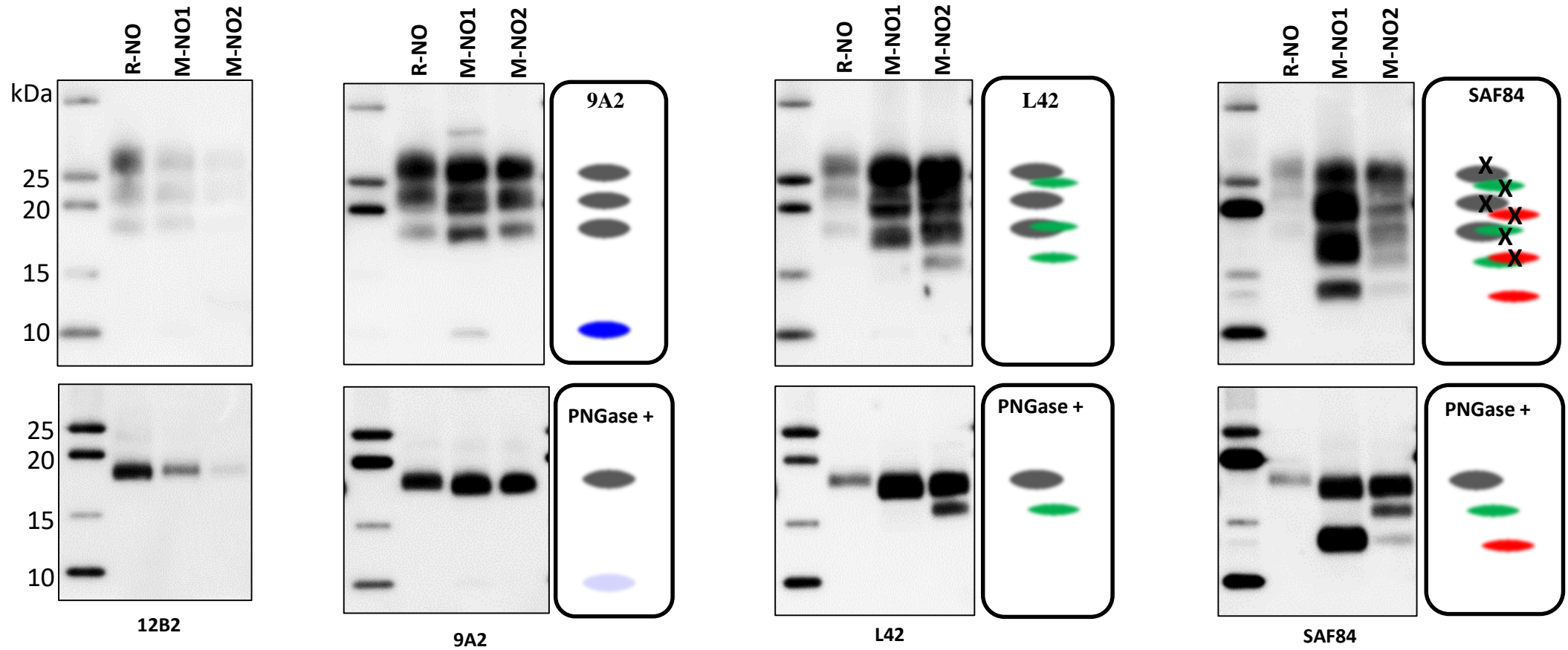
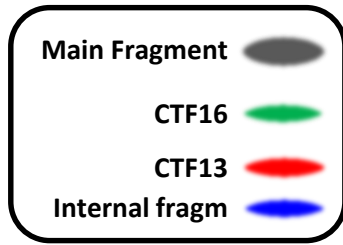
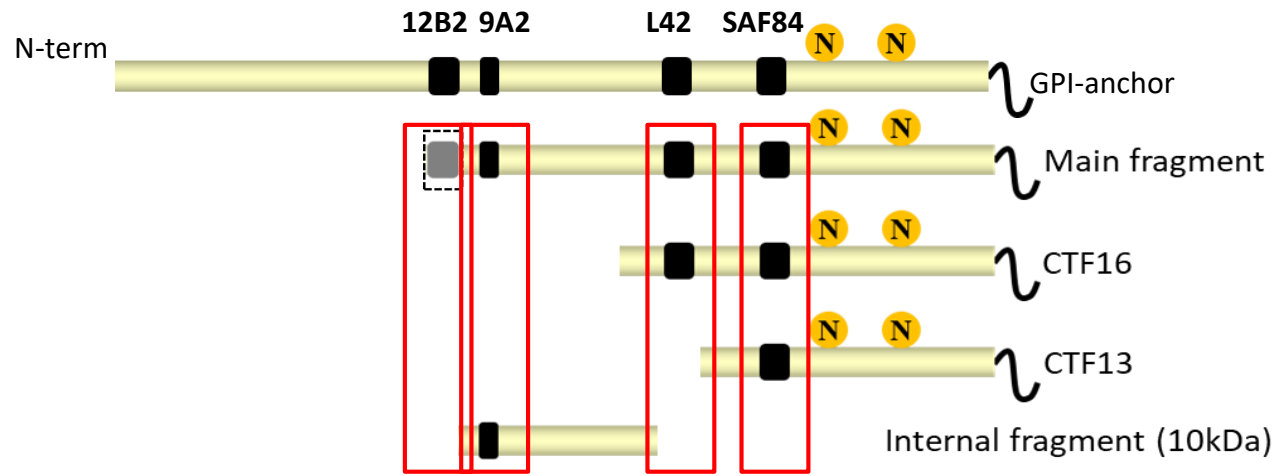
All negative for PrP^{Sc} in lymph nodes!

Moose in Norway
(n=137)

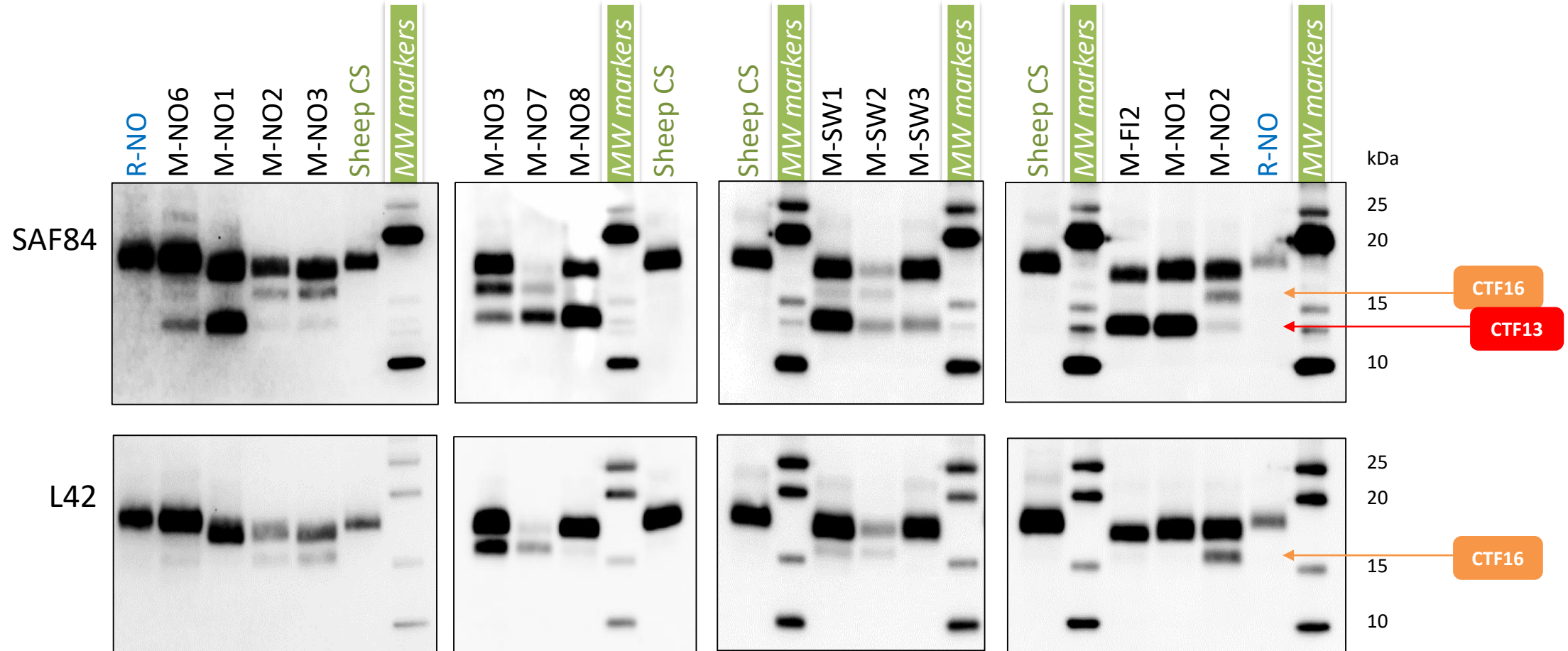
K109 77%

Q109 23%

Güere et al, *Transbound Emerg Dis* 2021



PrP^{res} types in moose



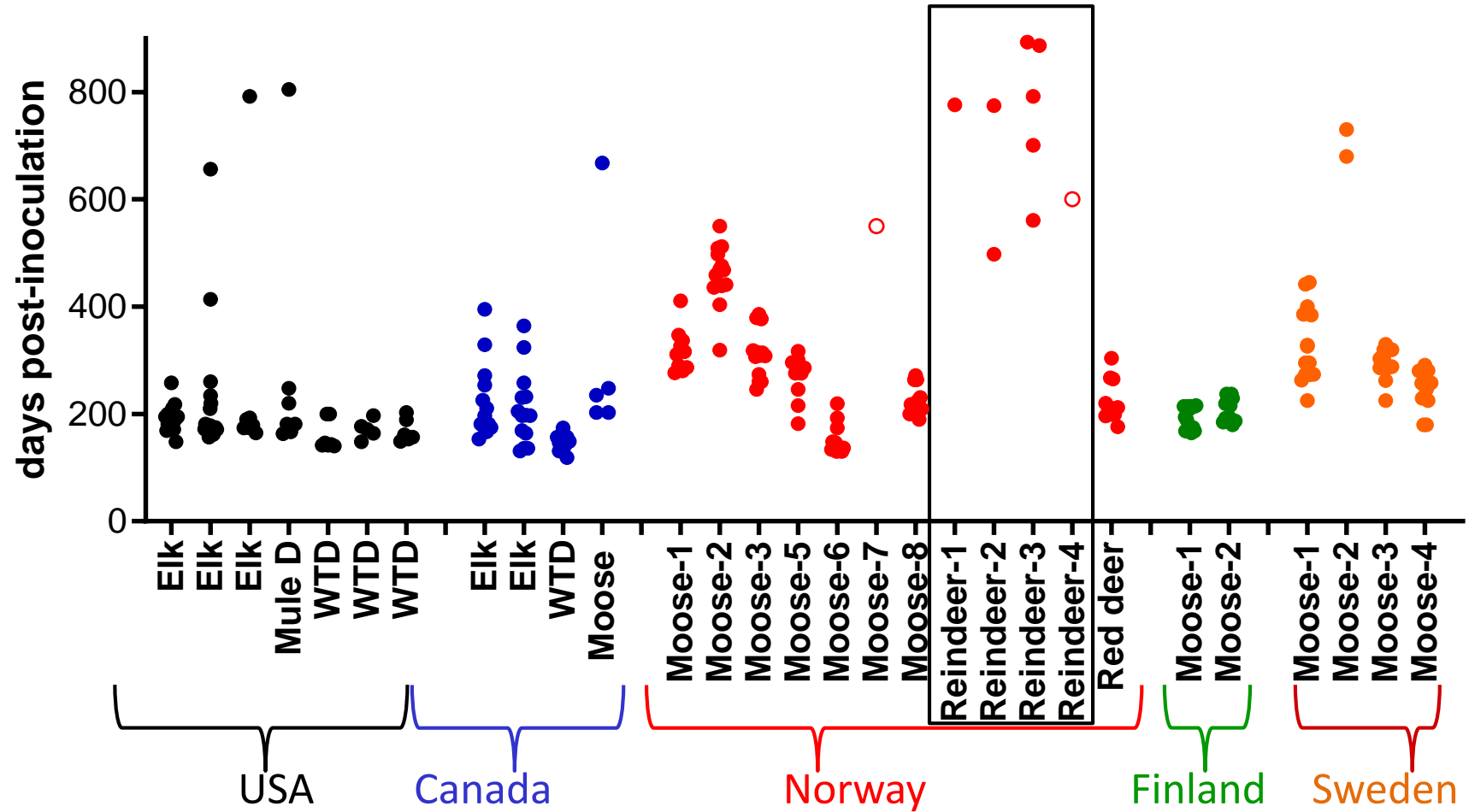
Huge variability!

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	✓		+/-

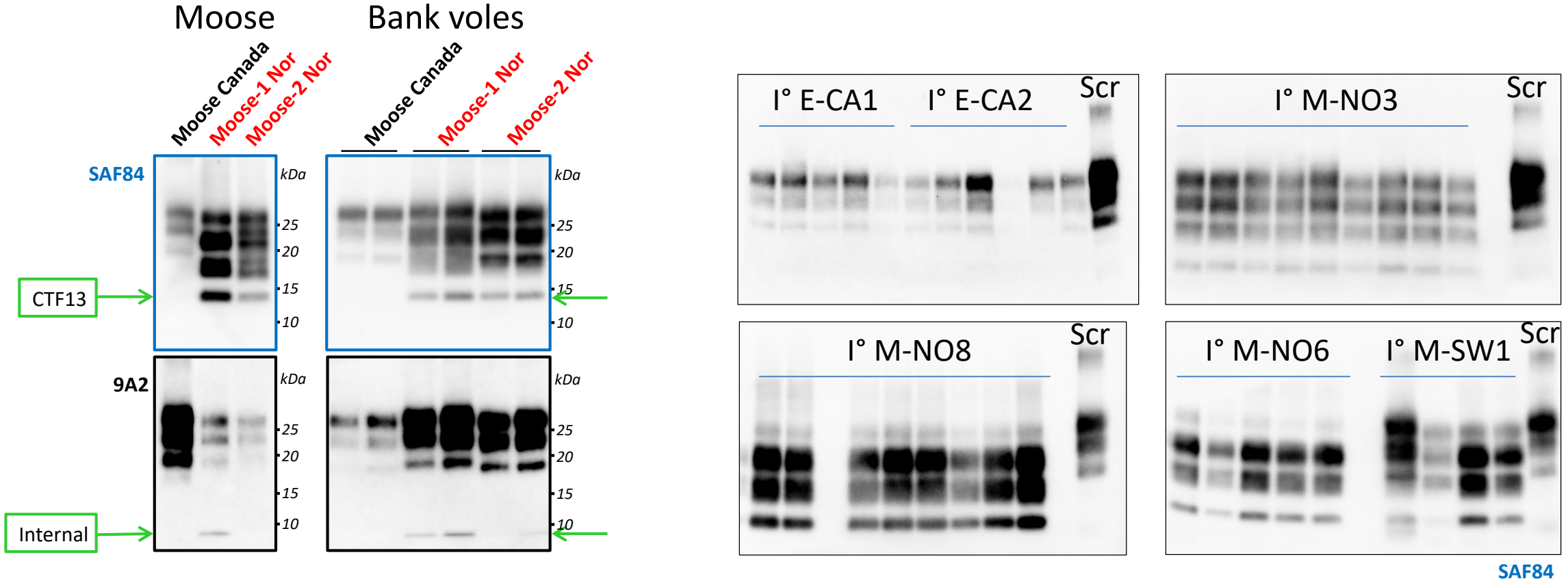
CTF13 seems a conformational signature of CWD in moose



Primary transmission in voles (Bv109I)

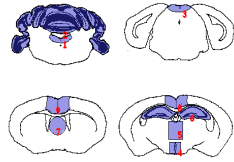


PrP^{res} types in voles: I° transmission

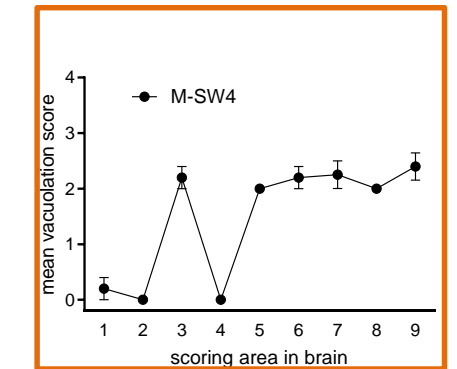
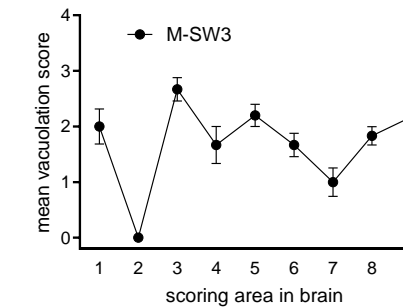
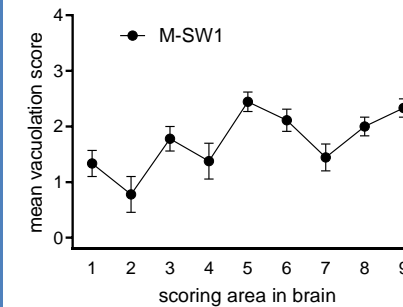
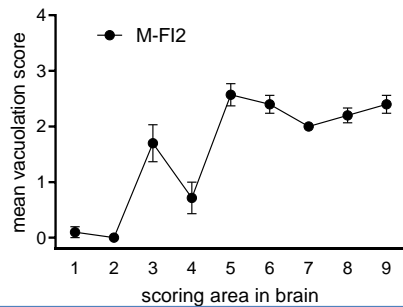
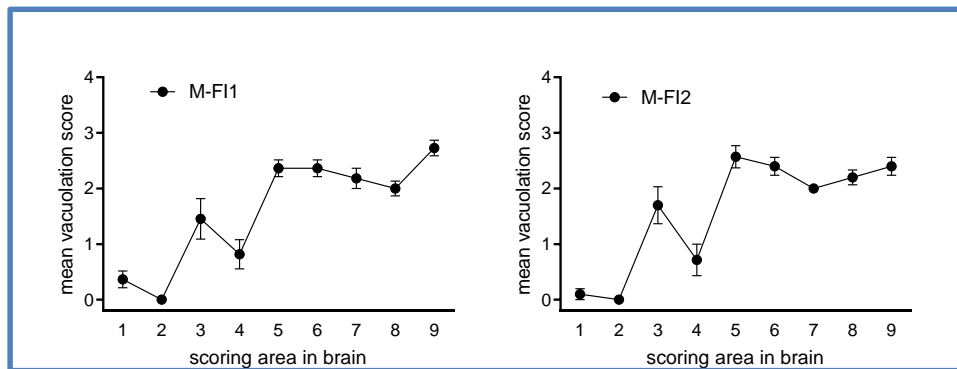
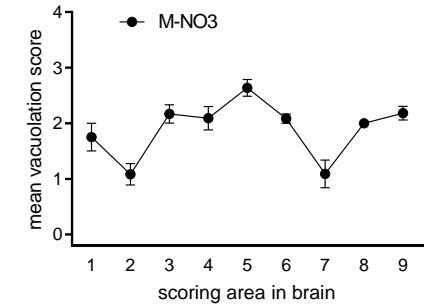
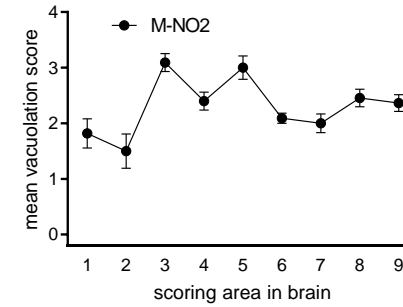
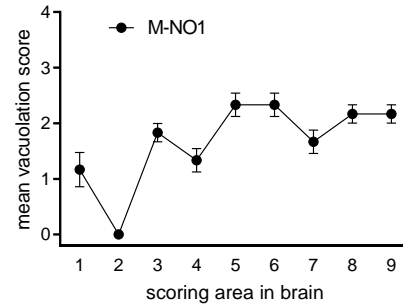
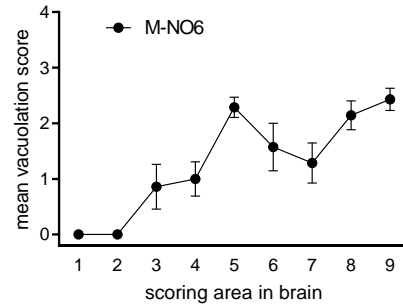
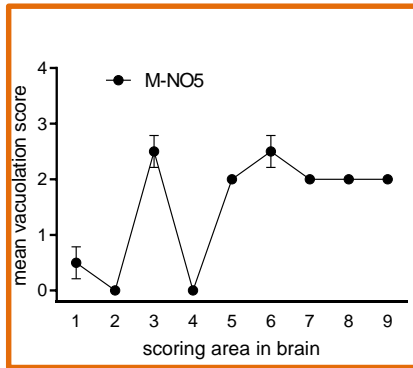
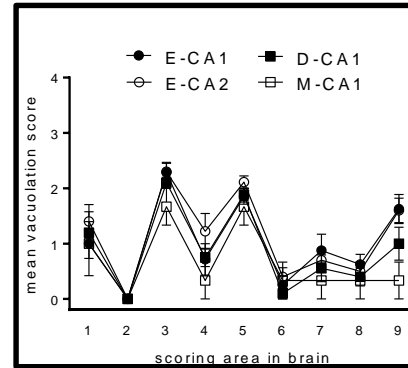


- Overall preservation of PrP^{res} types
- Variation among Bv inoculated with different moose isolates
- Internal variability in some groups

Neuropathology (lesion profiles) in voles: I° transmissions



- 1-medulla
- 2-cerebellum
- 3-superior colliculum
- 4-hypothalamus
- 5-thalamus
- 6-hippocampus
- 7-septum
- 8--cortex (post)
- 9- cortex (ant)



Survival time of vole-adapted CWD

Inocula			Transmission in Bv109I	
Origin	Species	ID	II° passage	III° passage
			Survi. time	Survi. time
North Am. (n=11)			34-52	32-37
Norway	Reindeer	R-NO1	113±4	105±9
Norway	Reindeer	R-NO3	118±11	
Norway	Red Deer	RD-NO1	90±4	88±7
Norway	Moose	M-NO1	78±4	76±3
Norway	Moose	M-NO2	211±18	175±36
Norway	Moose	M-NO3	225±17	263±10
Norway	Moose	M-NO5	149±29	
Norway	Moose	M-NO6	88±6	
Finland	Moose	M-FI1	157±19	125±24
Finland	Moose	M-FI2	162±33	
Sweden	Moose	M-SW1	110±8	
Sweden	Moose	M-SW2	127±5	
Sweden	Moose	M-SW3	~120*	
Sweden	Moose	M-SW3	104±7	
Sweden	Moose	M-SW4	101±5	
Sweden	Moose	M-SW4	179±22	

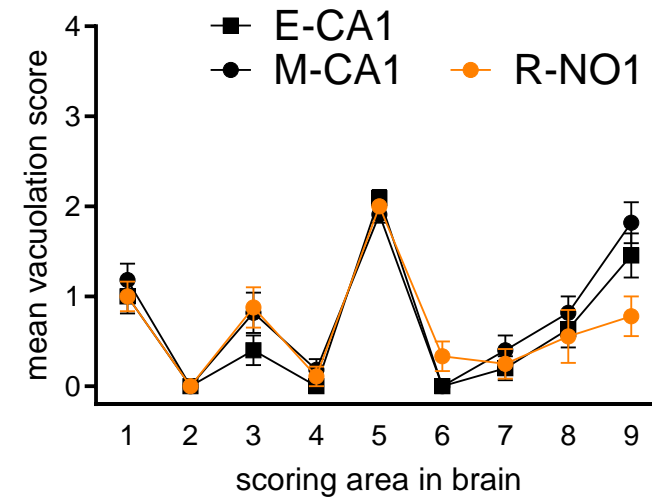
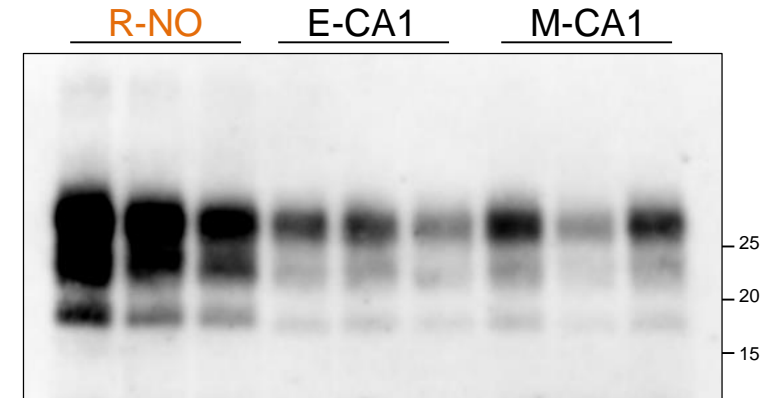
➤ Lack of 35 dpi signature of NA-CWD

➤ Biological variability in moose, suggests strain variation

* Ongoing

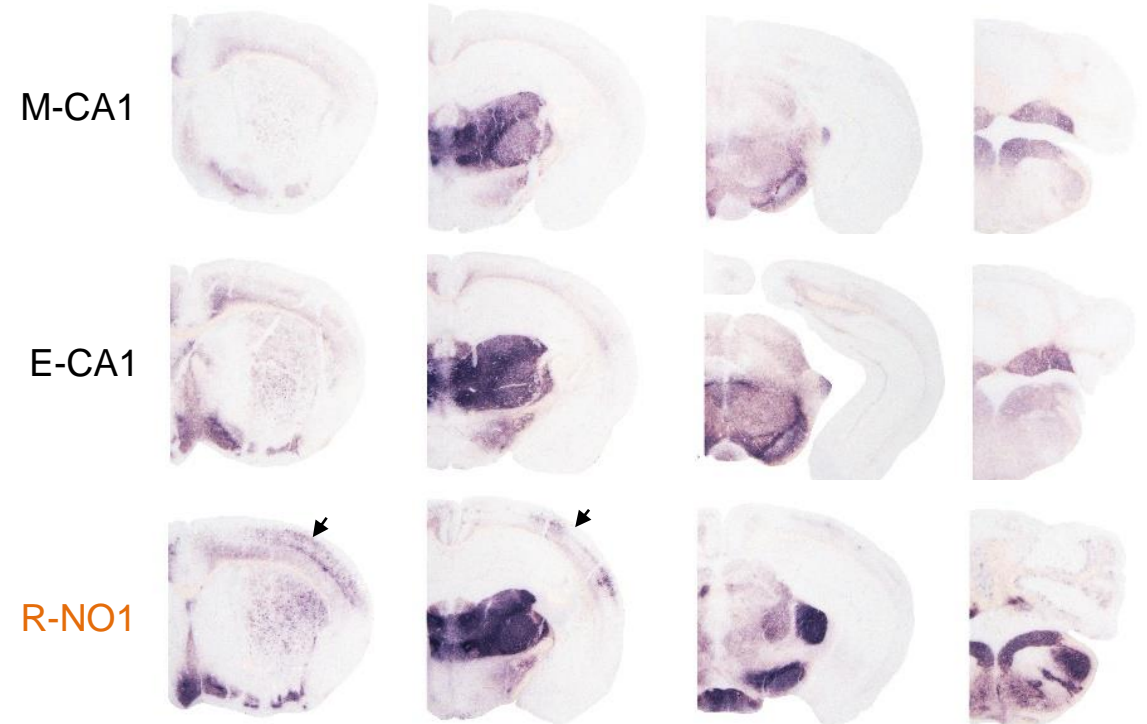
Reindeer CWD vs NA CWD

Inocula			Transmission in Bv109I	
Origin	Species	ID	II° passage Survi. time	III° passage Survi. time
North Am.			34-52	32-37
Norway	Reindeer	R-NO1	113±4	105±9
Norway	Reindeer	R-NO3	118±11	



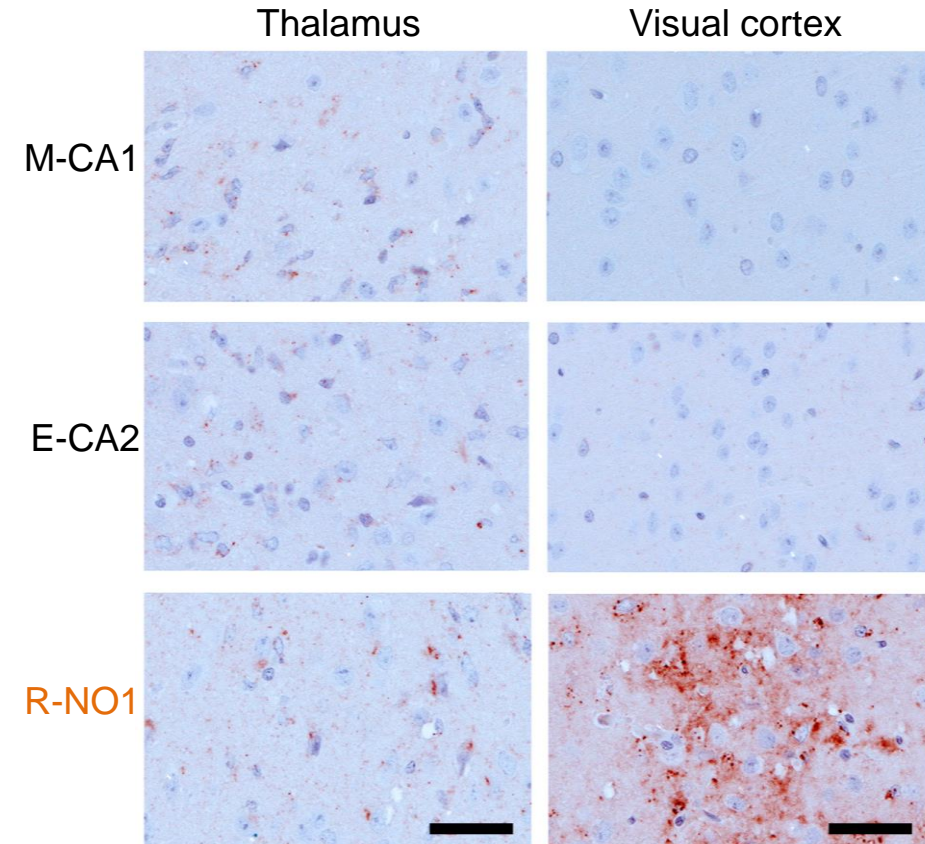
Reindeer CWD vs NA CWD

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Reindeer CWD vs NA CWD

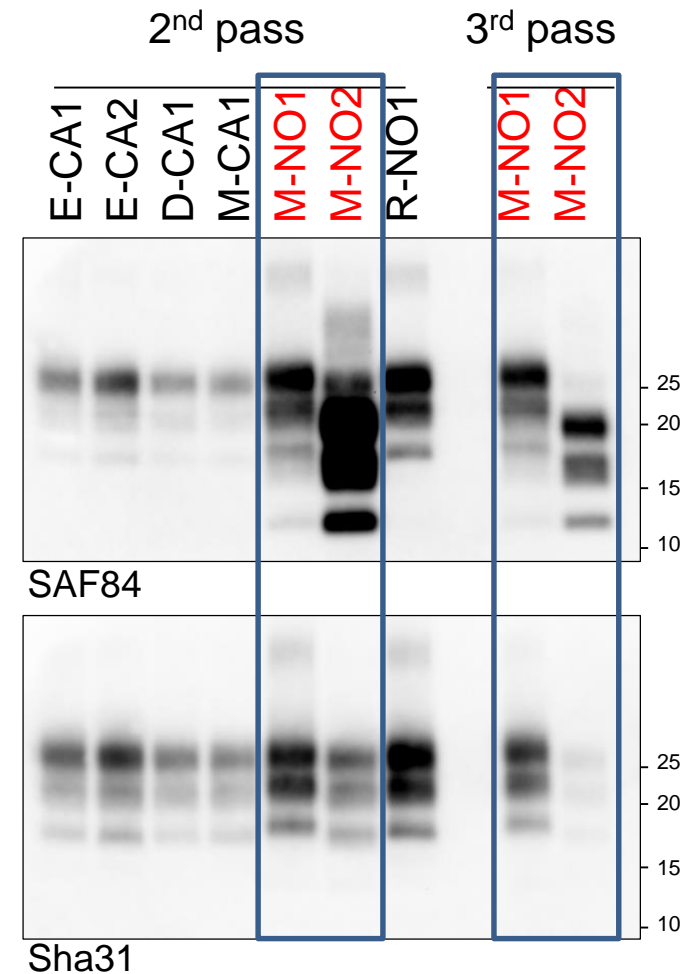
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A new strain of contagious CWD in norwegian reindeer

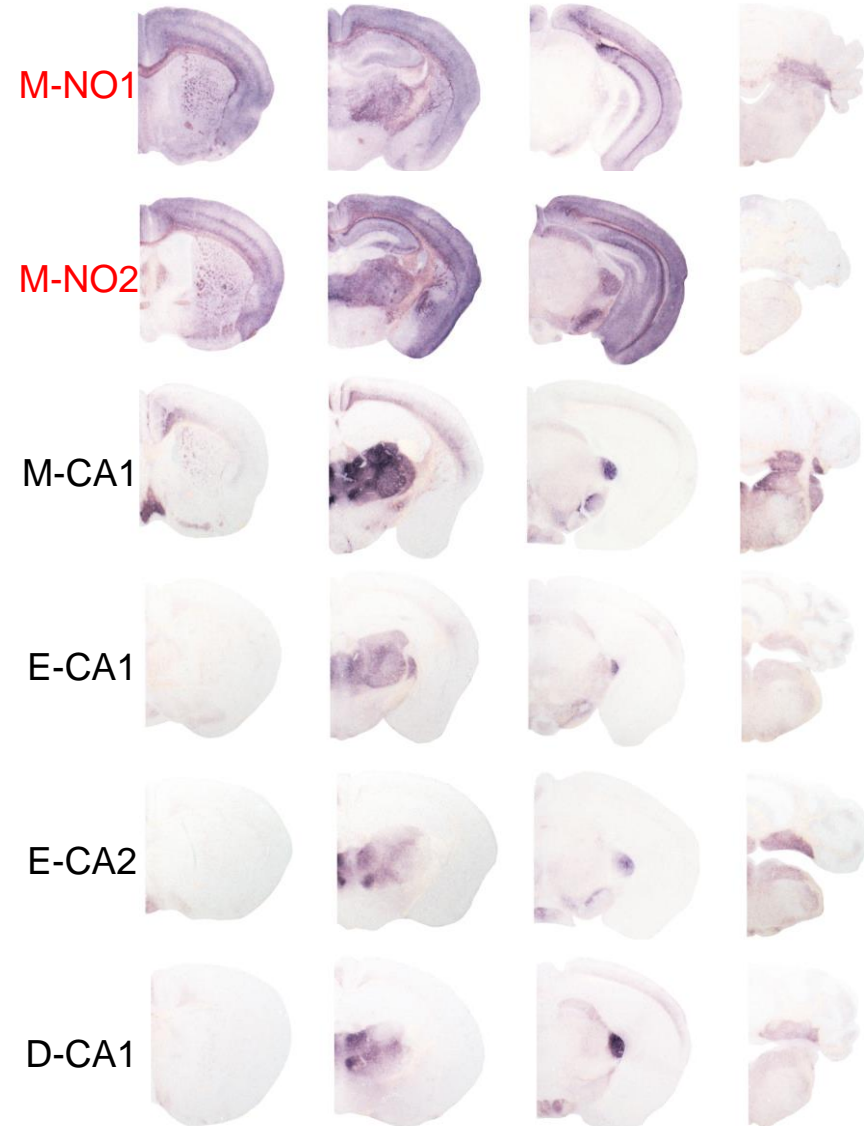
PrP^{Sc} conformations preserved on subpassages in voles

Inocula			Transmission in Bv109I	
Origin	Species	ID	II° passage	III° passage
			Survi. time	Survi. time
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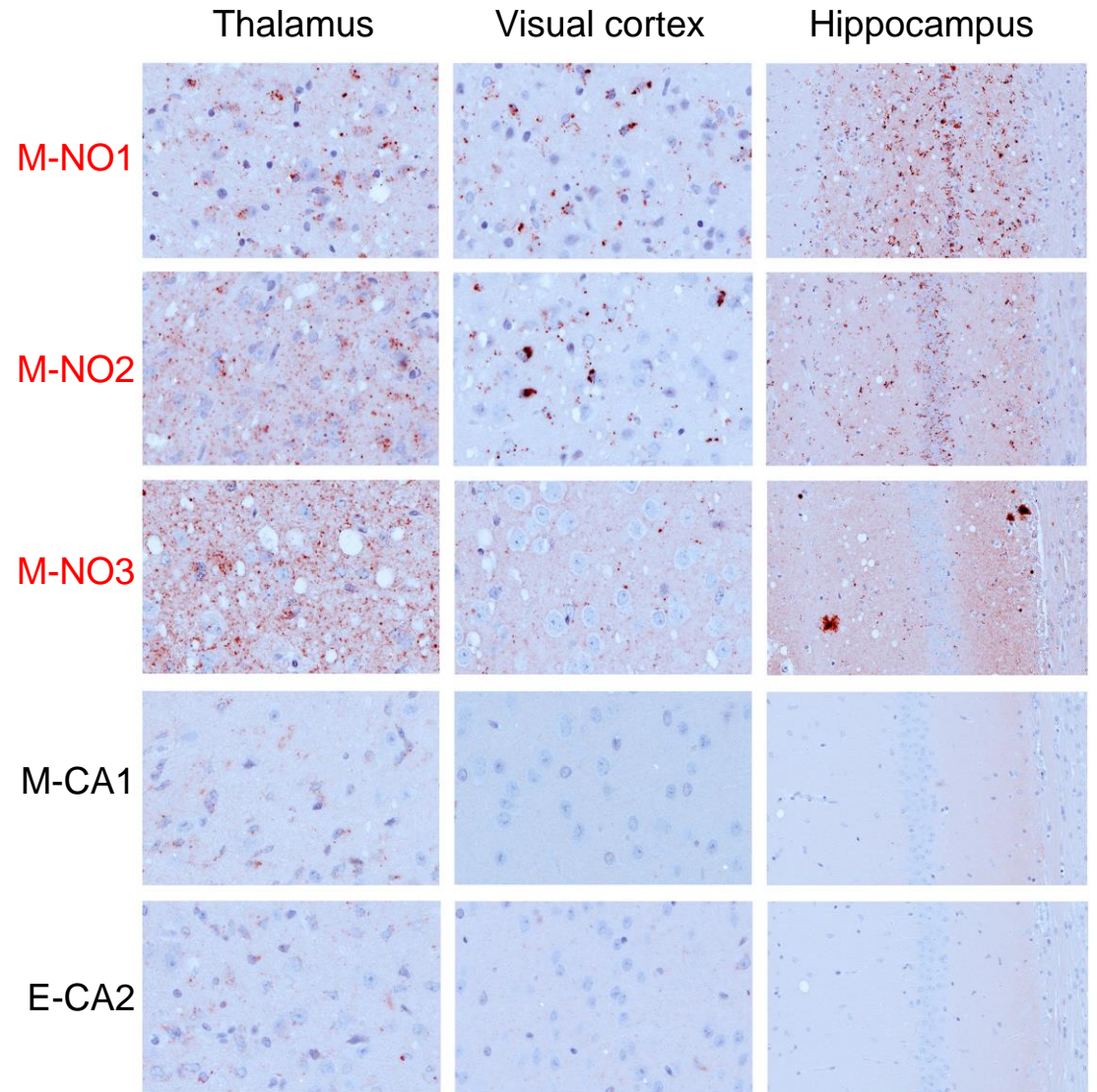
Strain variation in moose CWD

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			Survi. time	Survi. time
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Strain variation in moose CWD

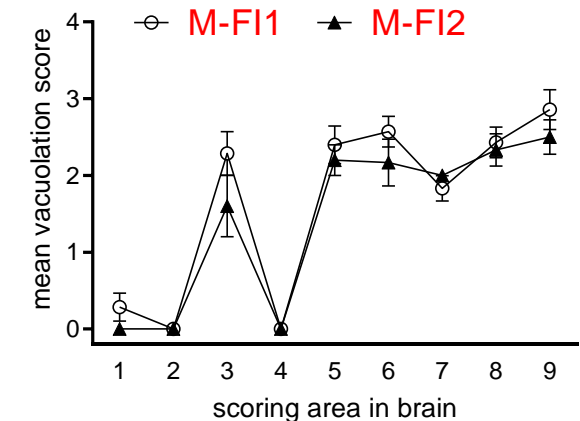
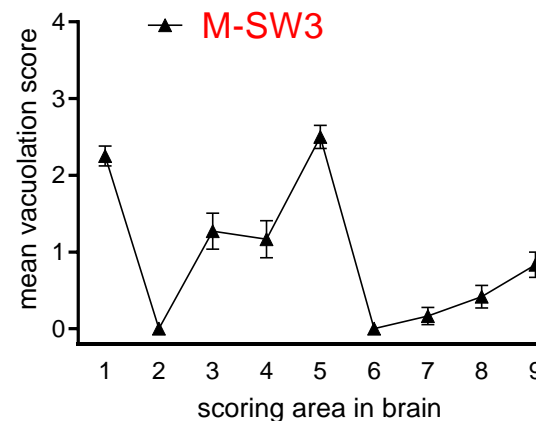
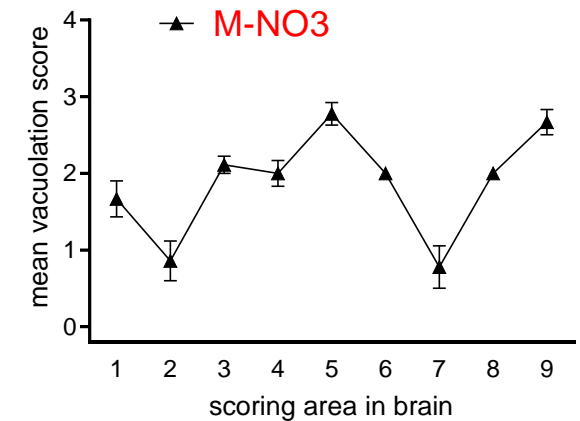
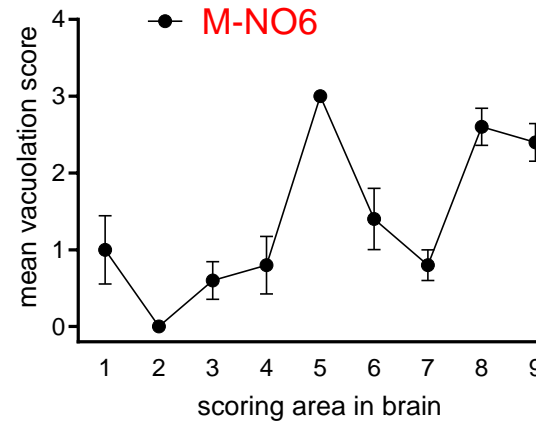
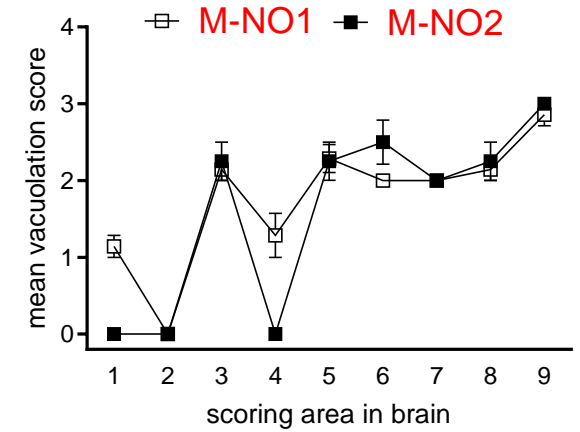
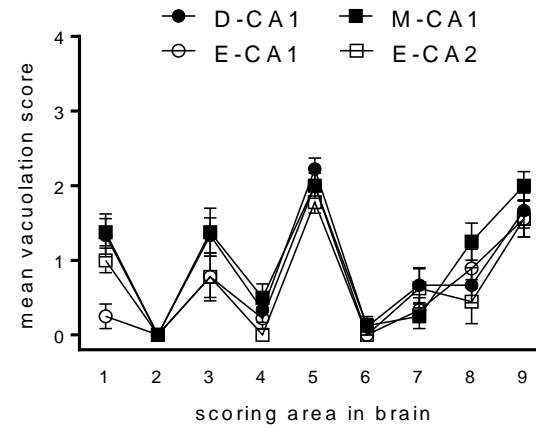
Inocula			Transmission in Bv109I	
Origin	Species	ID	II° passage	III° passage
			Survi. time	Survi. time
North Am.			34-52	32-37
Norway	Moose	M-NO1	78±4	76±3
Norway	Moose	M-NO2	211±18	175±36
Norway	Moose	M-NO3	225±17	263±10
Norway	Moose	M-NO5	149±29	
Norway	Moose	M-NO6	88±6	
Finland	Moose	M-FI1	157±19	125±24
Finland	Moose	M-FI2	162±33	
Sweden	Moose	M-SW1	127±5	
Sweden	Moose	M-SW2	~120	
Sweden	Moose	M-SW3	104±7	
Sweden	Moose	M-SW4	179±22	



Strain variation in moose CWD

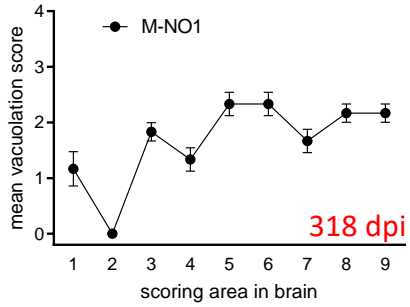
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Multiple CWD strains in moose

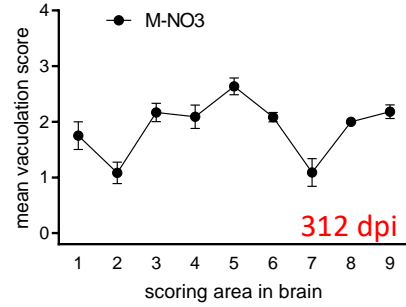


Evolution of red deer and moose strains in voles

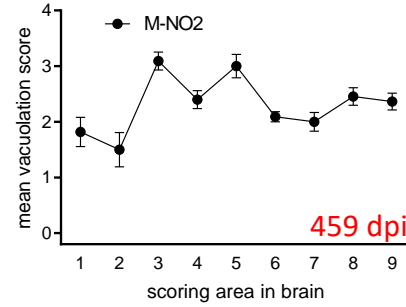
M-NO1



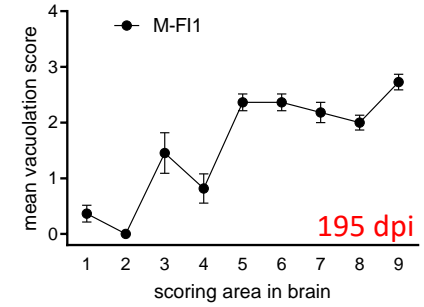
M-NO3



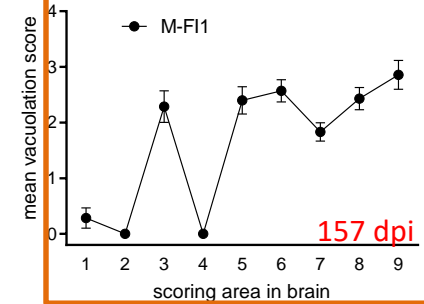
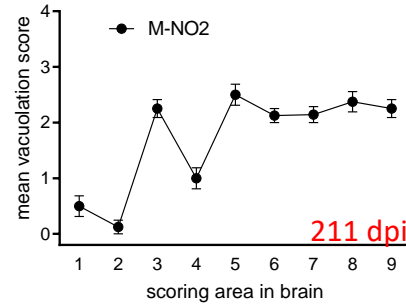
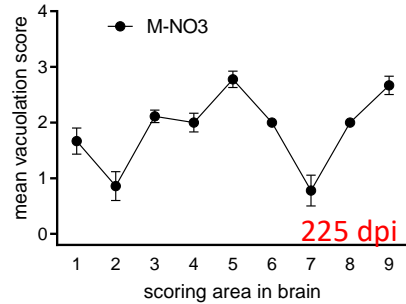
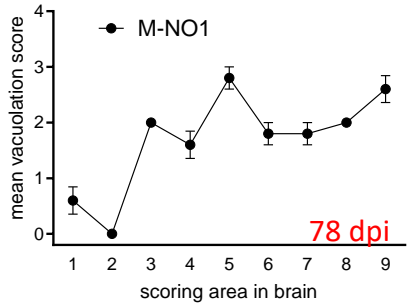
M-NO2



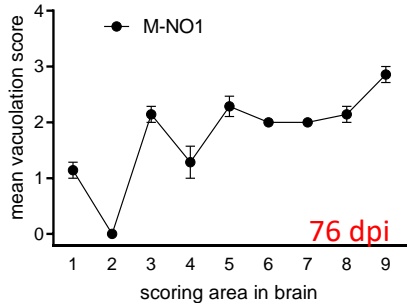
M-FI1



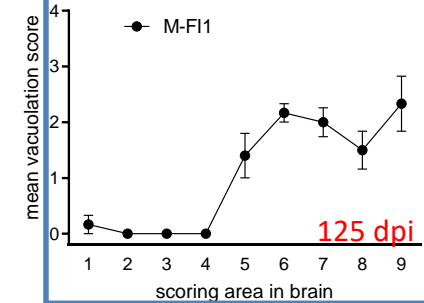
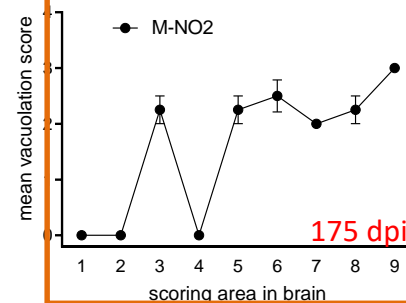
I°



II°



Instability of some vole-adapted strains derived from EU moose and red deer



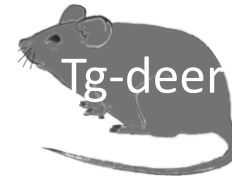
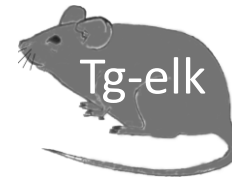
III°

Adaptive selection of a prion strain conformer corresponding to established North American CWD during propagation of novel emergent Norwegian strains in mice expressing elk or deer prion protein

PLoS Pathogens 2021

Jifeng Bian¹, Sehun Kim¹, Sarah J. Kane¹, Jenna Crowell¹, Julianna L. Sun^{1,2}, Jeffrey Christiansen¹, Eri Saijo¹, Julie A. Moreno¹, James DiLisio¹, Emily Burnett¹, Sandra Pritzkow³, Damian Gorski³, Claudio Soto³, Terry J. Kreger⁴, Aru Balachandran⁵, Gordon Mitchell⁵, Michael W. Miller⁶, Romolo Nonno⁷, Turid Vikøren⁸, Jørn Våge⁸, Knut Madslie⁸, Linh Tran⁸, Tram Thu Vuong⁸, Sylvie L. Benestad⁸, Glenn C. Telling^{1,2*}

deer, reindeer and moose express PrP^C with Q226
elk express PrP^C with E226



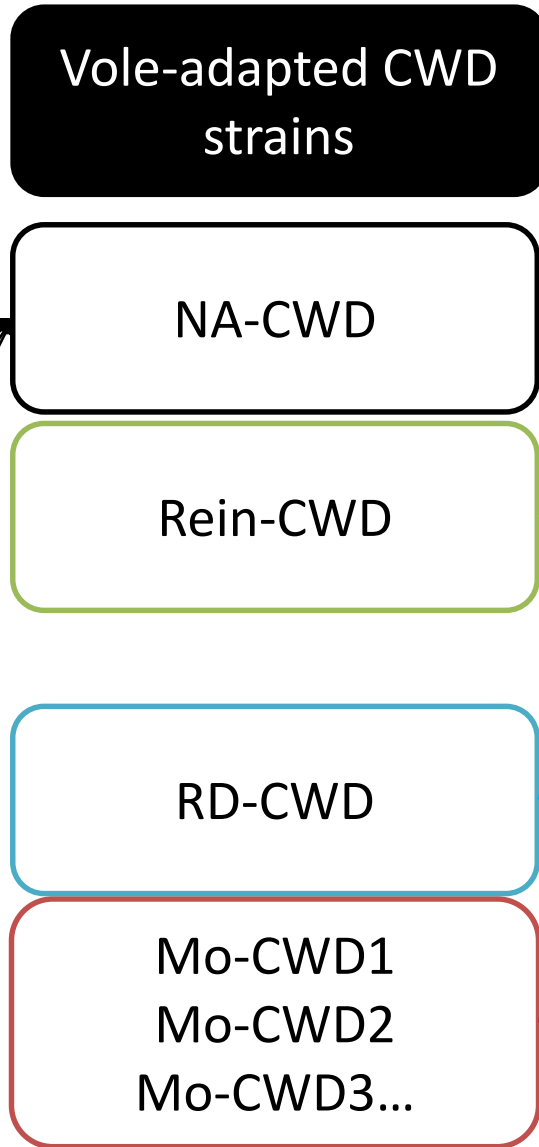
- Transmission (iterative passages)
- PMCA
- Cervid cell assay (RK13 cells)

- NO moose, NO reindeer and NA cervids are infected with distinct strains (M-NO1 ≠ M-NO2)
- NO moose propagates non-lymphotropic CWD strains, while NO reindeer shares lymphotropic properties with NA CWD
- NO moose CWD strain M-NO2, acquired characteristics of the contagious NA CWD strain during its adaptive propagation in Tg mice

Comparison of CWD strains in NA and EU



Moose
Mule deer
WT deer
Elk/Wapiti



Reindeer
Red deer
Moose

Transmissible Spongiform Encephalopathies or Prion diseases

	Humans	Animals
<i>Idiopathic/spontaneous? (sporadic)</i>	85% Sporadic Creutzfeldt–Jakob disease Variably protease sensitive prionopathy	Atypical scrapie (Nor98) Atypical BSE (H-BSE and L-BSE)
<i>Genetic (PrP mutations)</i>	10-15% gCJD Fatal Familial Insomnia Gerstmann-Sträussler-Scheinker disease	<div style="background-color: #800000; color: white; padding: 5px; text-align: center; font-weight: bold;">CWD in moose and red deer?</div>
<i>Acquired</i>	<5% Kuru (foodborne/cannibalism, from sCJD) iCJD (iatrogenic, from sCJD) vCJD (zoonotic, from C-BSE)	Classical BSE (C-BSE)
<i>Acquired and contagious</i>		Classical scrapie CWD (NA and Reindeer/Norway) Camel prion disease (CPD)

- CWD strains in Europe are different from NA-CWD
- Distinct CWD strains in different species in Europe
- CWD in reindeer is similar to NA-CWD: risk of spread
- CWD strains in moose and red deer are different from reindeer CWD and reflect their atypical features: new idiopathic/sporadic forms of CWD?

Implications for

- the origin of CWD in Europe
- the epidemiology and surveillance of CWD in Europe
- the risk for humans

Aknowledgments

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