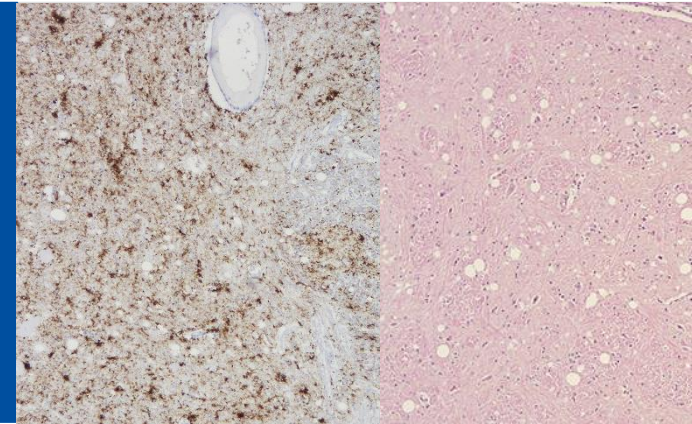


Unravelling Goat Prion Isolates by Studying Ovine Models

Sonja Ernst, Romolo Nonno, Jan Langeveld, Olivier Andreoletti, Cristina Acin, Penelope Papasavva-Stylianou, Theodoros Sklaviadis, Pier Luigi Acutis, Lucien van Keulen, John Spiropoulos, Markus Keller, Martin H. Groschup, Christine Fast



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SCRAPIE IN EUROPEAN SHEEP

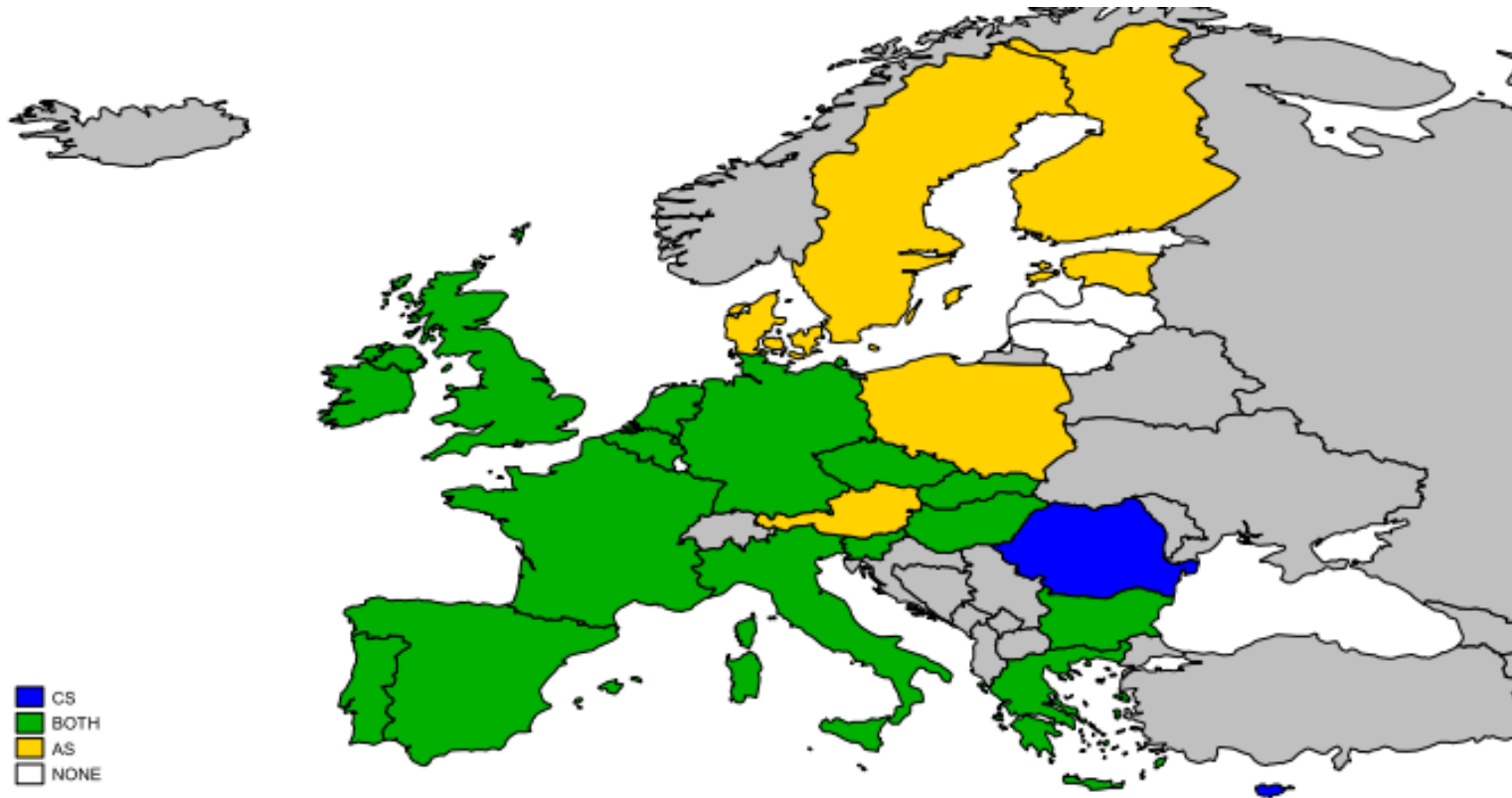


Figure 1: Geographical distribution of ovine CS and AS within EU27. Countries in green reported both CS and AS; countries in blue reported only CS; countries in yellow reported only AS; white is used for countries where scrapie has been never reported

© EFSA J 2014

But how is the picture in European goat populations?



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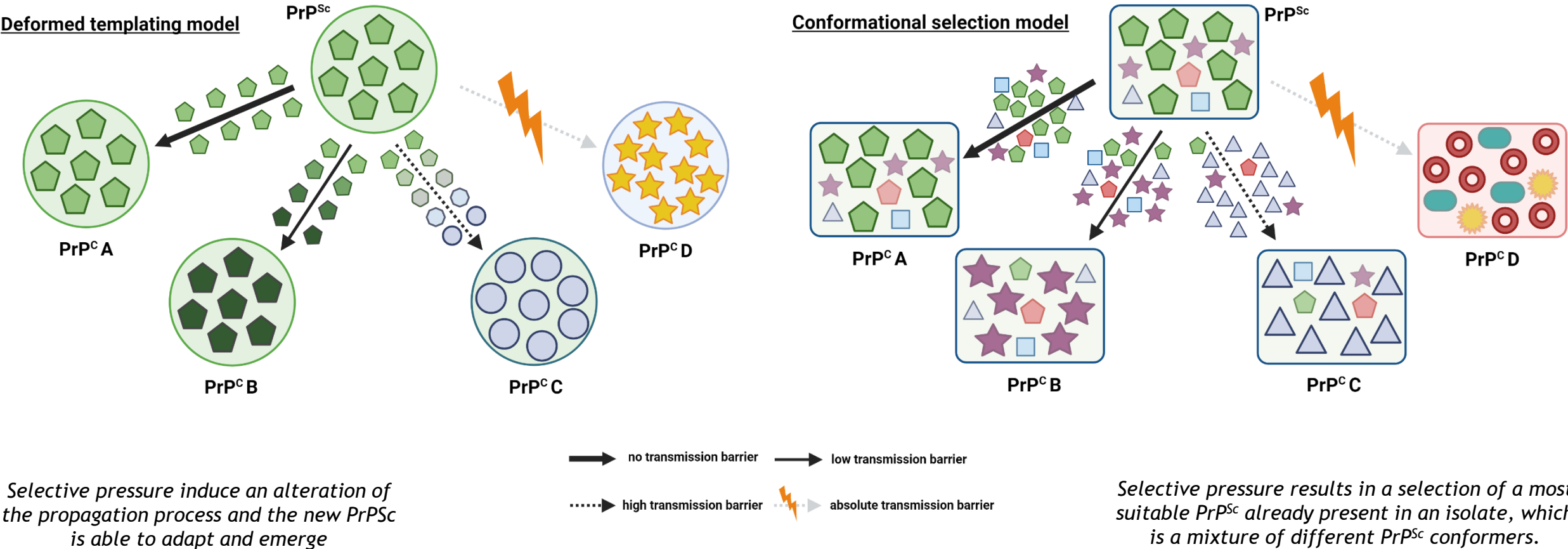
© FLI

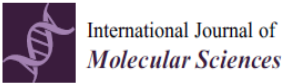
Goat scrapie

- Investigation of the transmission efficiency of European goat scrapie to the ovine Tgshp IX mouse model
- Defining parameters for prion discrimination in the Tgshp IX mouse model using reference scrapie strains
- Prion strain typing of field goat scrapie isolates to get an overview of circulating goat scrapie strains

PRION STRAIN PROPAGATION AND TRANSMISSION BARRIER

Two hypothesis are described to explain, how a prion strain adapt/ evolve and cross the transmission barrier





Article

Strain Typing of Classical Scrapie and Bovine Spongiform Encephalopathy (BSE) by Using Ovine PrP (ARQ/ARQ) Overexpressing Transgenic Mice

Olanrewaju I. Fatola ^{1,2}, Markus Keller ², Anne Balkema-Buschmann ², James Olopade ¹, Martin H. Groschup ² and Christine Fast ^{2,*}



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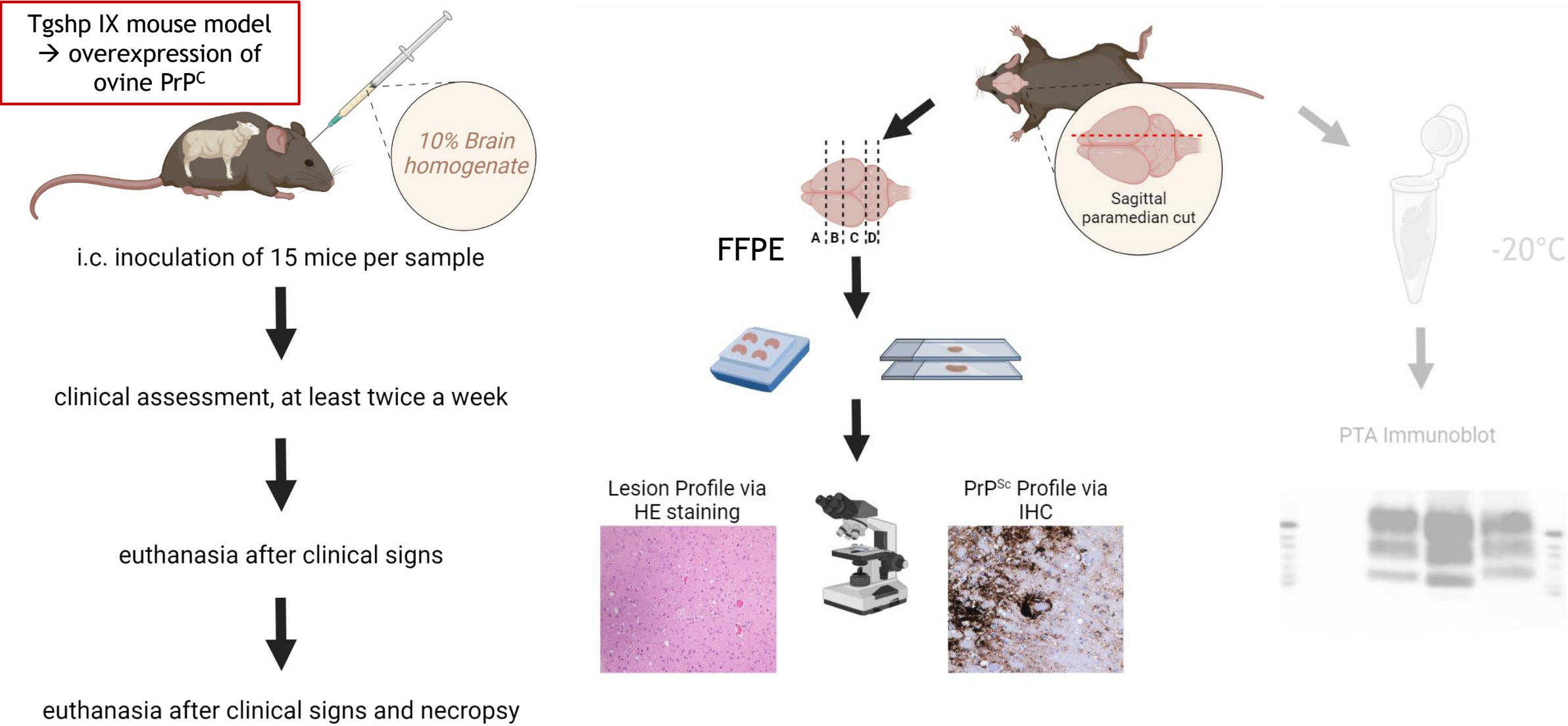
	Tgshp IX	Tgshp XI
Genetic background	B6CBAx1290la	
Transgene	Ovine ARQ (wildtype) PrP	
Expression rate	2-4x overexpression	4-8x overexpression

Inocula	Mean incubation period (dpi)	
	Tgshp IX	Tgshp XI
BSE	351 ±165*	445 ±185
ovBSE	230 ±63	231 ±9
CS LAN	190 ±3	195 ±11
CS S805	160 ±18	158 ±20
CS DAW	371 ±44	342 ±39
AS	317 ±5	272 ±32

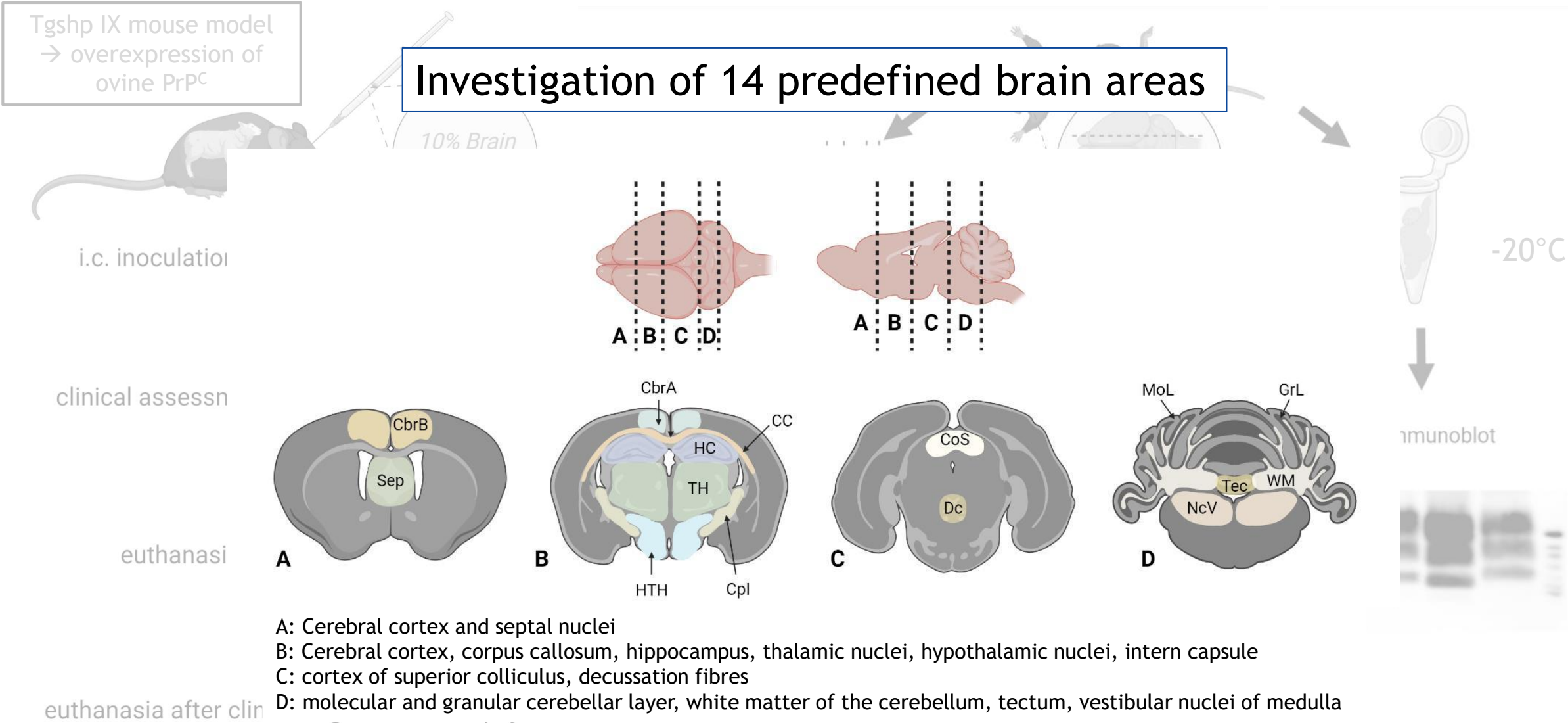
*only one mouse with very high IC, without that animal 286 ±86

Lower expression level of ovine PrP in Tgshp IX = lower transmission barrier for interspecies transmission
→ Tgshp IX is the more suitable model for transmission studies to novel hosts

PRION STRAIN TYPING BY BIOASSAYS



PRION STRAIN TYPING BY BIOASSAYS

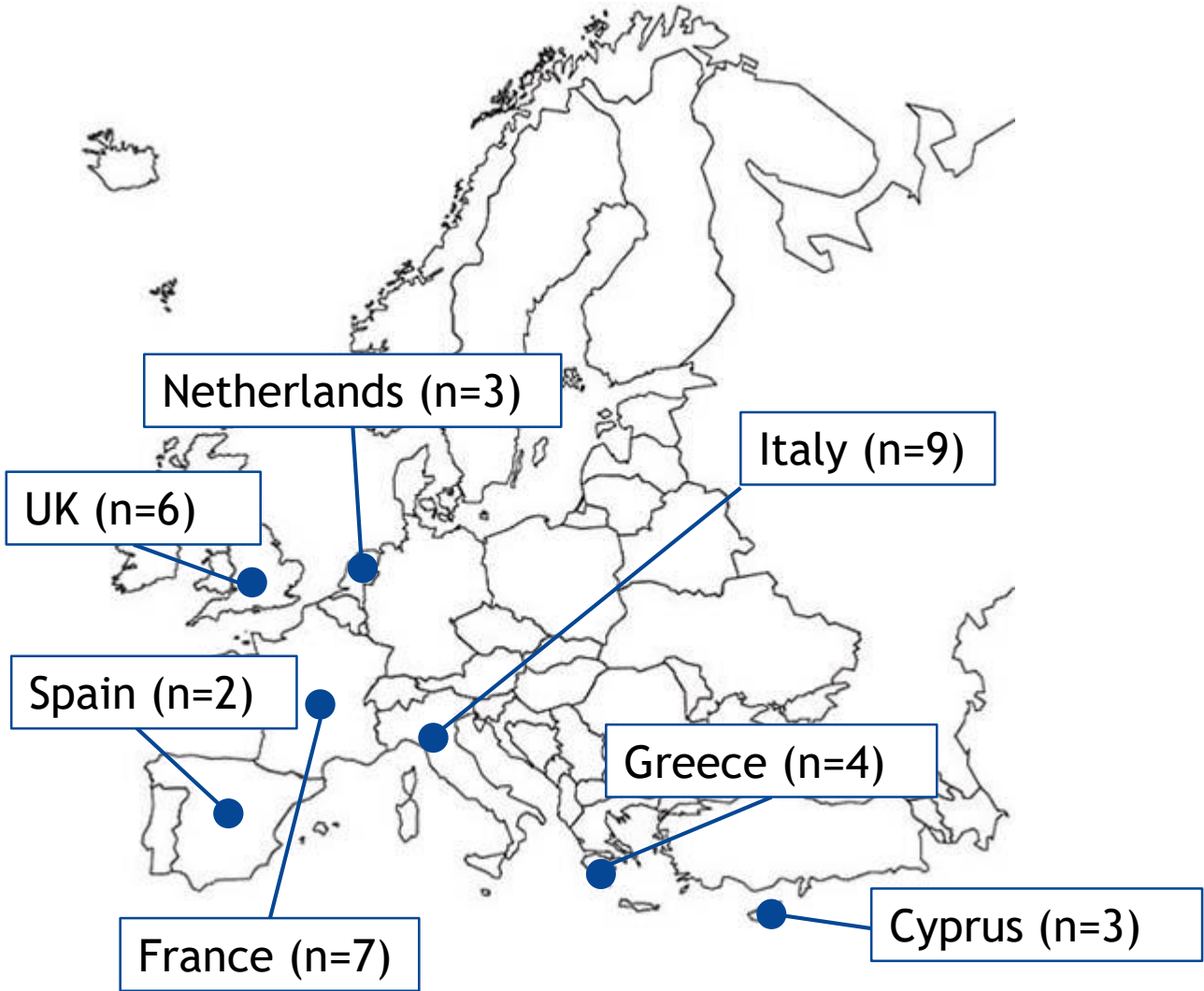


EUROPEAN FIELD GOAT INOCULA - RESULTS



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EUROPEAN FIELD GOAT INOCULA



Reference Strains:

Table 1. Isolate information and transmission features of the TgshpIX (tg-shARQ) mouse model (in parts already published by Nonno et al. 2020 [20]).

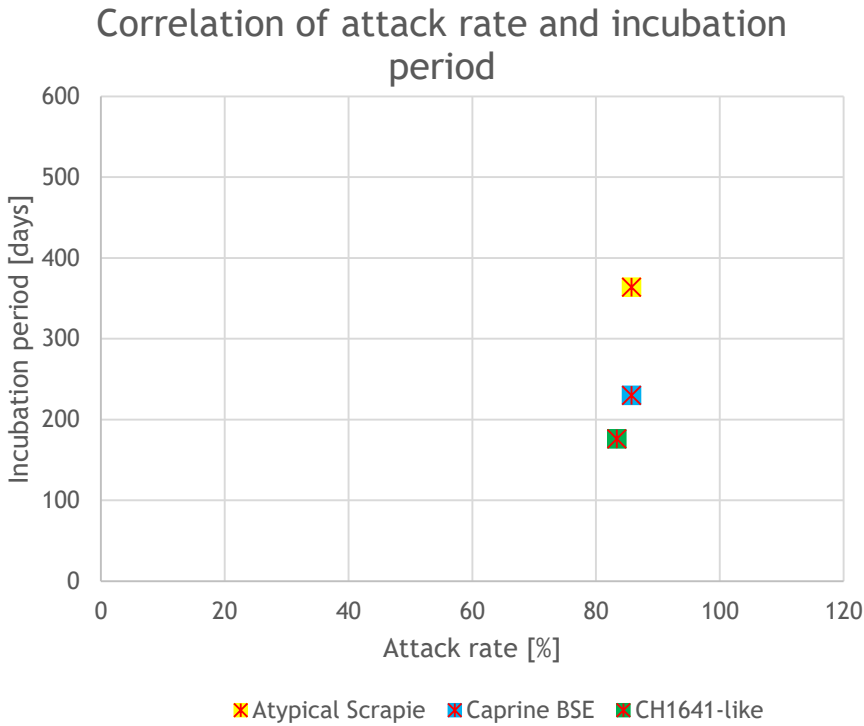
Isolate Code	Country of Origin	PrP Genotype [#]	PrP ^{Sc} Type	Attack Rate	Mean Incubation Period (dpi)
I2	Italy	240PP	CS	4/12	410 ± 209
I3	Italy	240PP	CS	7/7	464 ± 72
I4	Italy	211QR, 240PS	CS	3/5	477 ± 58
I5	Italy	240PP	CS	4/5	493 ± 66
I7	Italy	240PP	CS	12/12	417 ± 70
I9	Italy	143HR, 240PS	CS	4/5	400 ± 67
I11	Italy	240PS	CS	12/14	380 ± 116
I12	Italy	240PS	CS	14/15	408 ± 91
I15	Italy	154RH, 240PS	AS	6/7 [^]	364 ± 32
S2	Spain	240PS	CS	14/14	222 ± 44
S3	Spain	240PP	CS	13/13	210 ± 33
N1	Netherlands	143HR, 240PS	CS	14/15	276 ± 66
N2	Netherlands	143HR, 240PS	CS	8/9	321 ± 76
N3	Netherlands	240PP	CS	11/14	270 ± 14
F2 [*]	France	240PS	CS	11/11	177 ± 25
F3	France	240PP	CS	13/15	232 ± 68
F6	France	240PS	CS	13/14	235 ± 38
F10	France	240PS	CS	11/11	251 ± 66
F11 [*]	France	142IM, 240PP	CS	9/10	297 ± 67
F14	France	142IM, 240PS	CS	9/11 [*]	380 ± 66
F16	France	240PS	CS	5/9	238 ± 78
gtBSE [*]	France	211RQ, 240PS	caprine BSE	12/14	230 ± 59
G1	Greece	240PP	CS	2/5 [^]	493 ± 45
G2	Greece	240PP	CS	8/9	295 ± 5
G3	Greece	143HR, 240PP	CS	6/6	292 ± 54
G4	Greece	240PP	CS	5/7	399 ± 33
C1	Cyprus	240PP	CS	13/14 [^]	295 ± 15
C2	Cyprus	240PP	CS	14/14	292 ± 70
C3	Cyprus	240PP	CS	14/15	292 ± 49
UKA1 [§]	UK	240PS	CS	6/9	281 ± 128
UKA2	UK	240PS	CS	14/14	224 ± 53
UKB1 [§]	UK	240PS	CS	7/7	418 ± 68
UKB2	UK	127GS, 240PP	CH1641-like	10/12	176 ± 28
UKC1 [§]	UK	127GS, 240PP	CS	7/9	363 ± 129
UKD2	UK	211RQ, 240PP	CS	3/7 [^]	503 ± 30

Atypical
Scrapie

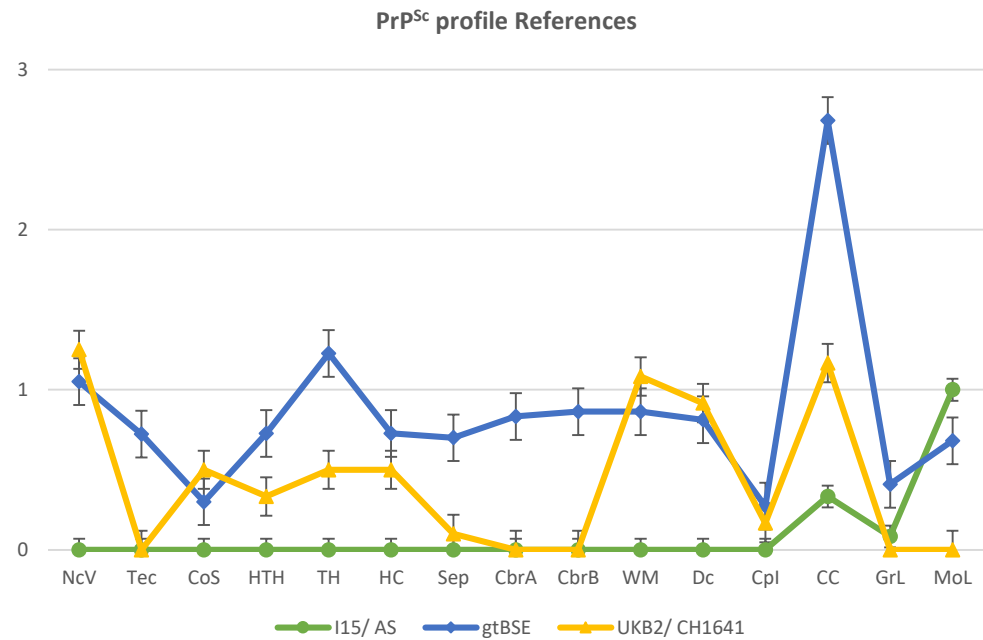
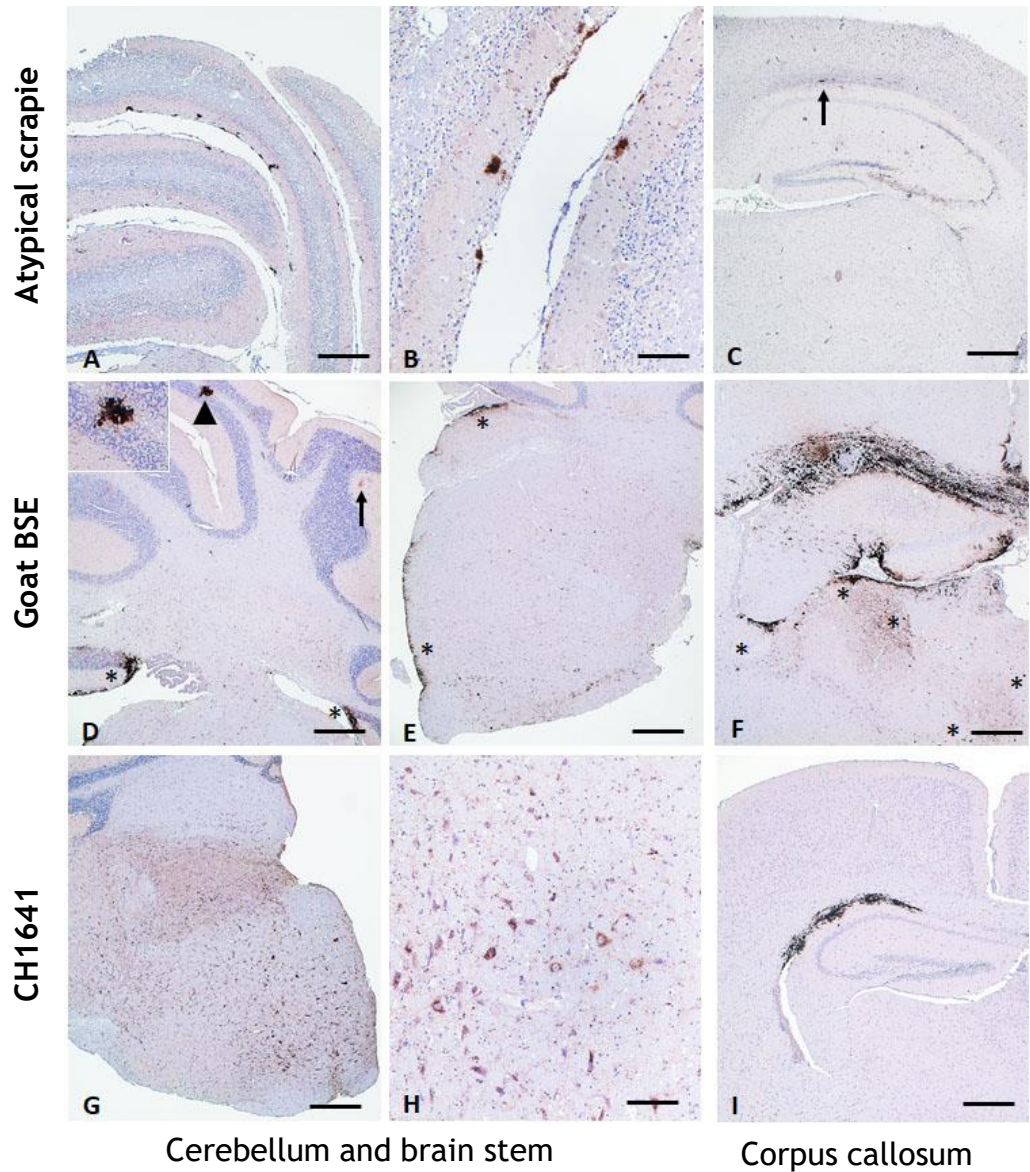
Goat BSE

CH1641

Isolate code reflecting the country of origin was first introduced by Langeveld et al. (2019) [25] and will be consecutively used in the main text; ^{*} isolates obtained after experimental infection; [§] only codons with polymorphisms compared to the homogenous wild-type are given in a one-letter amino



REFERENCE STRAINS

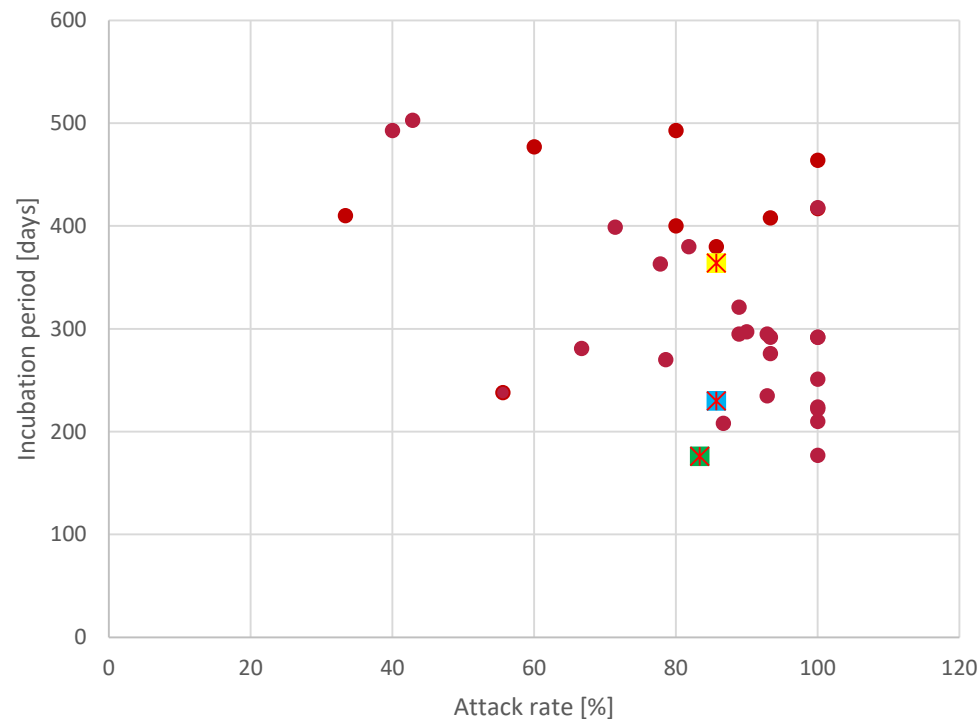


Clear-cut discrimination of all three reference strains in the Tgshp IX mouse model along

- Attack rate
- Incubation period
- PrP^{Sc} profile

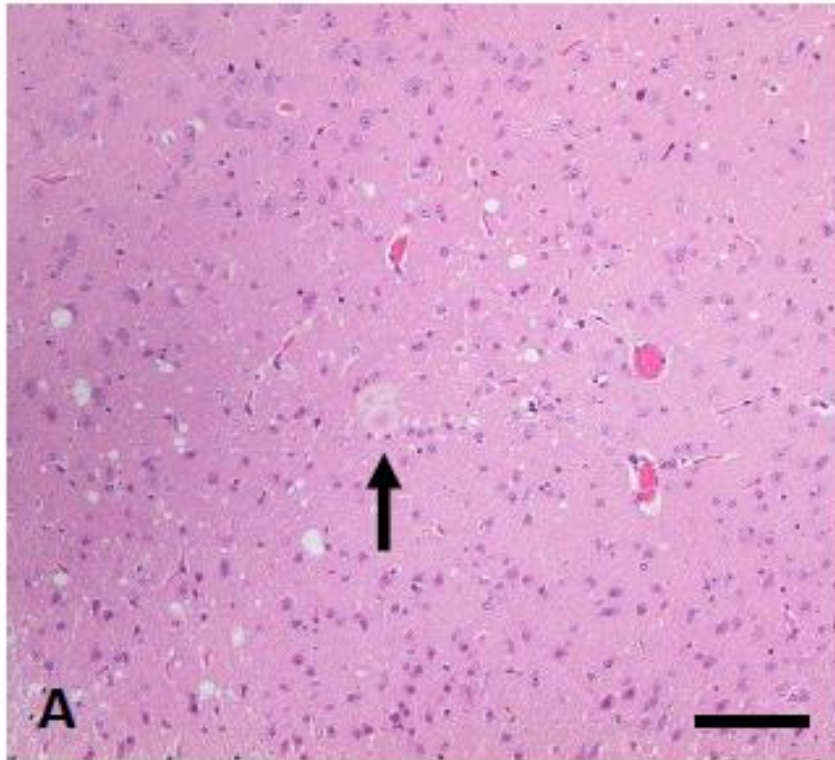
FIELD SCRAPIE CASES - ATTACK RATE AND INCUBATION PERIOD

Correlation of attack rate and incubation period



- broad variability between the 35 field scrapie isolates and reference strains
- impossible to distinguish reference strains from field isolates by attack rate and incubation period only

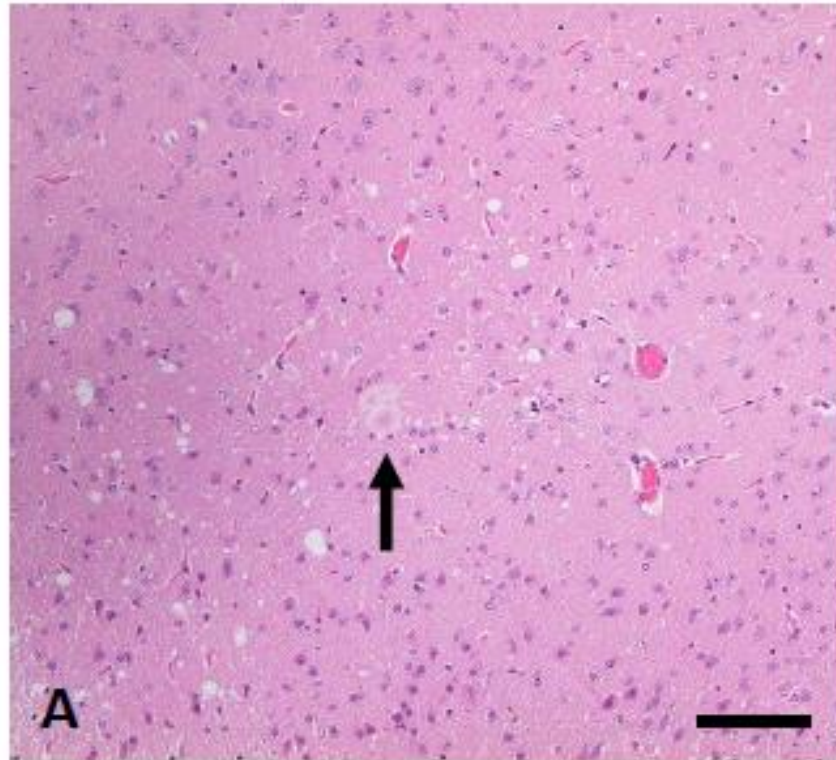
Lesion profile (HE)



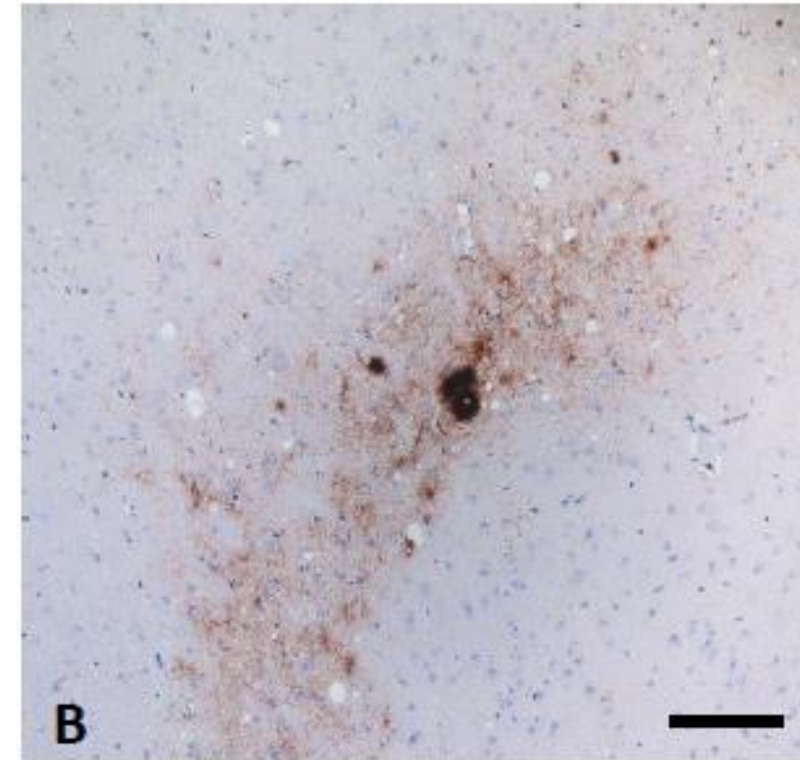
- Up to severe multifocal spongiform encephalopathy but no prion strain specific pattern detectable
- Plaques (arrow) detected in gtBSE and several scrapie isolates

LESION PROFILE VS. PrP^{Sc} PROFILE

Lesion profile (HE)

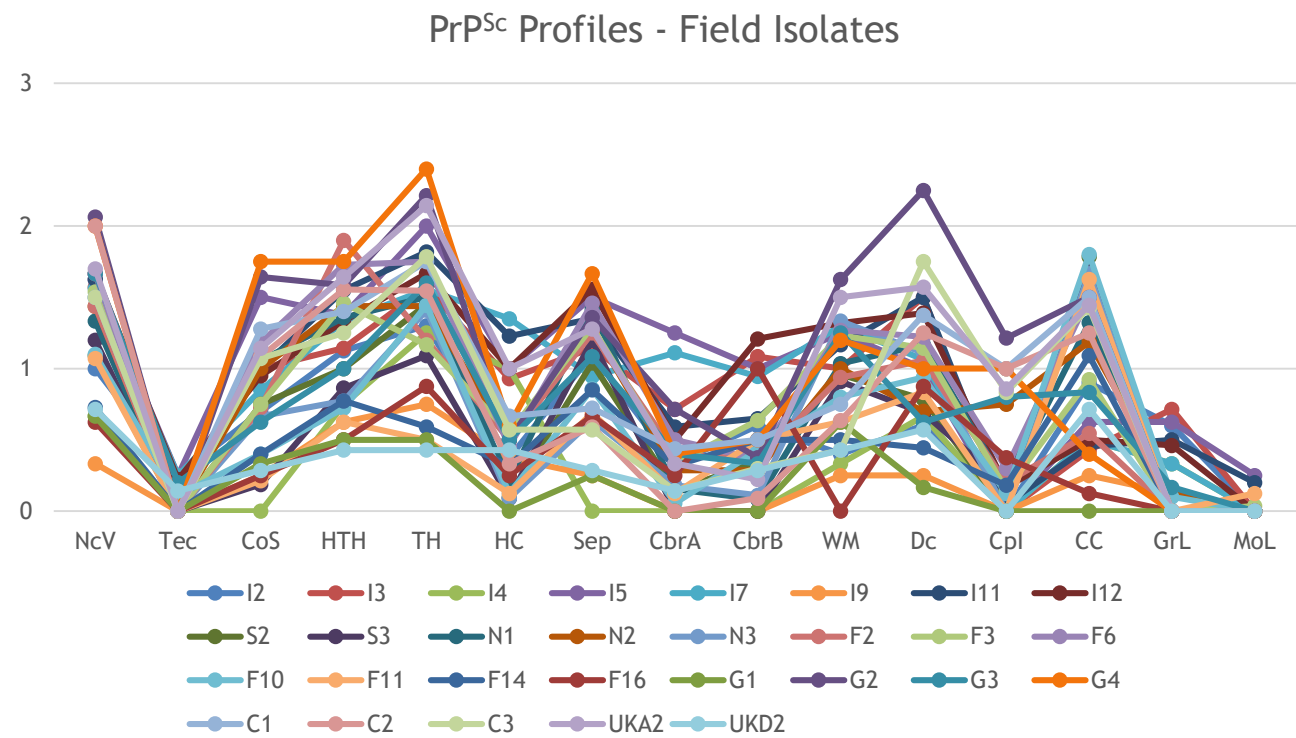


PrP^{Sc} profile (IHC, mab R145)

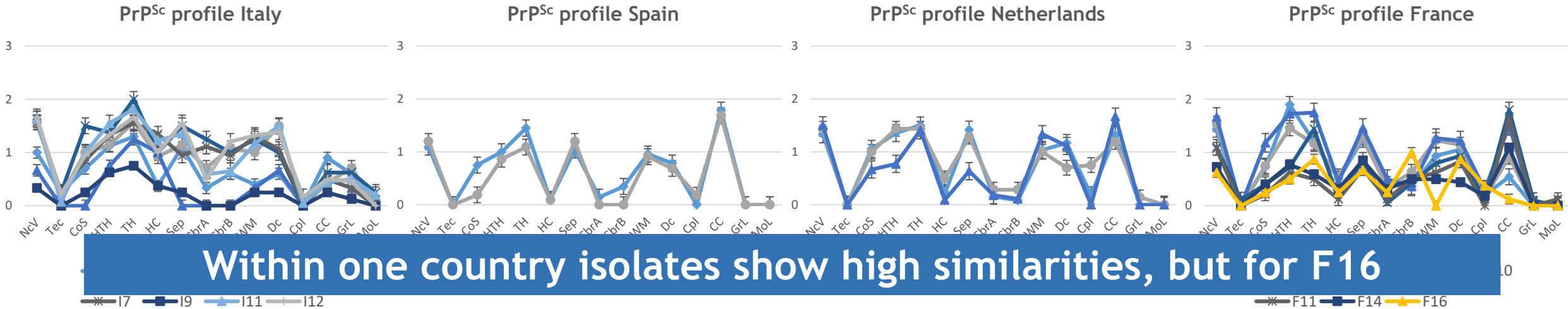


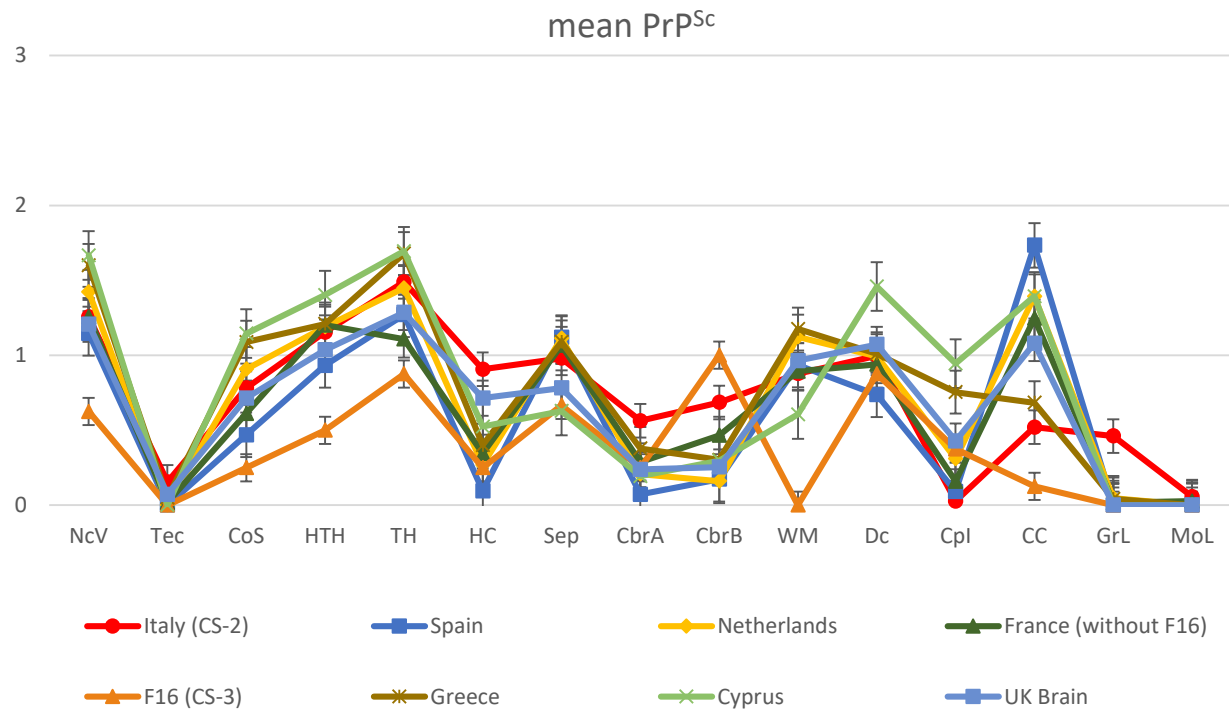
- Up to severe multifocal spongiform encephalopathy but no prion strain specific pattern detectable
- Plaques (arrow) detected in gtBSE and several scrapie isolates
- Severe spongiform lesions often indicated severe PrP^{Sc} accumulation

FIELD SCRAPIE CASES - PrP^{Sc} PROFILE

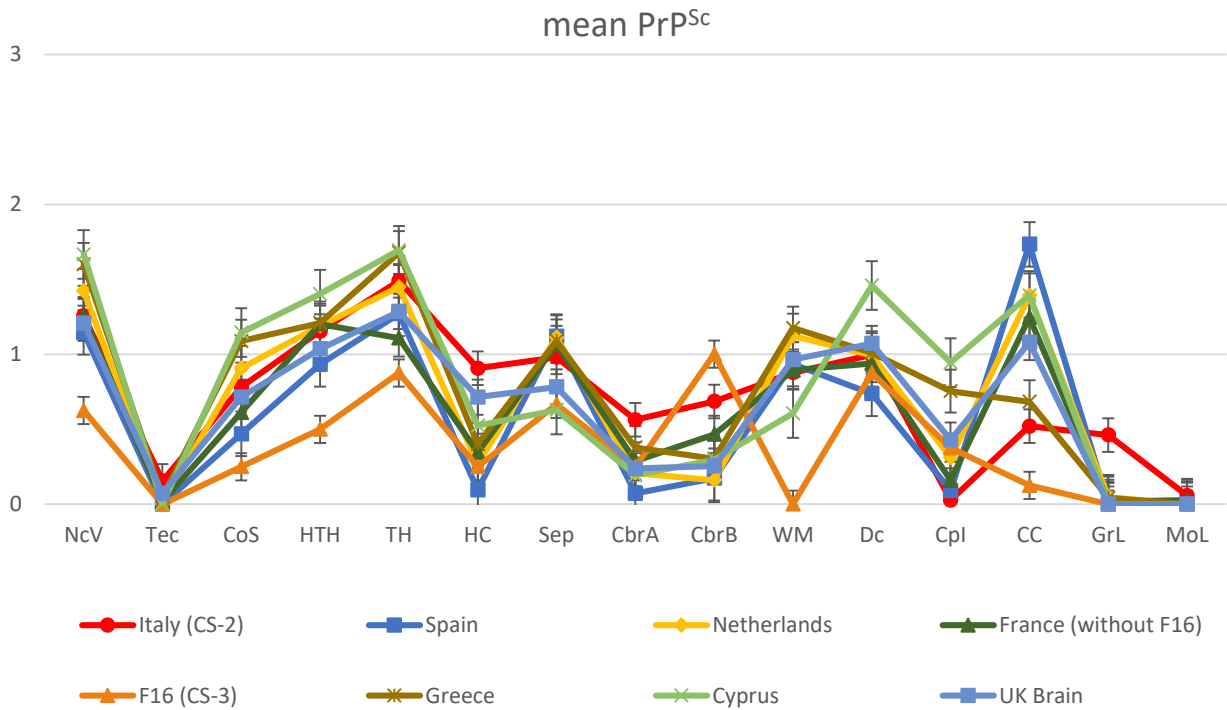


GOAT ISOLATES - GEOGRAPHICAL ANALYSIS

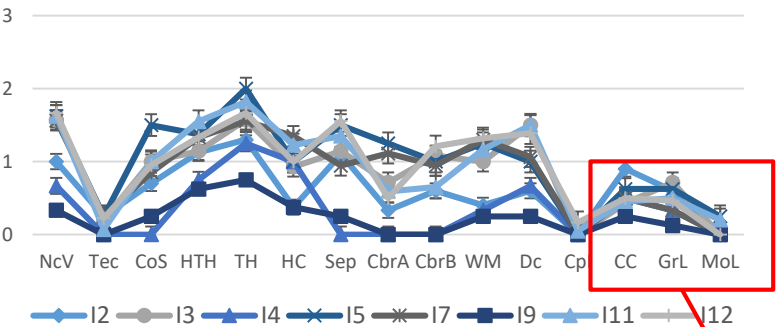




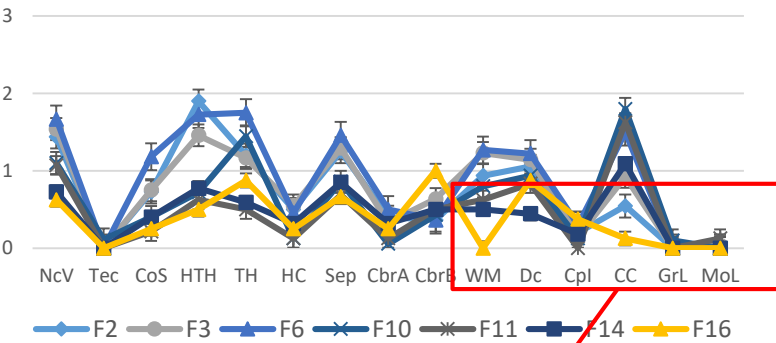
Comparison of mean PrP^{Sc} profiles of all countries



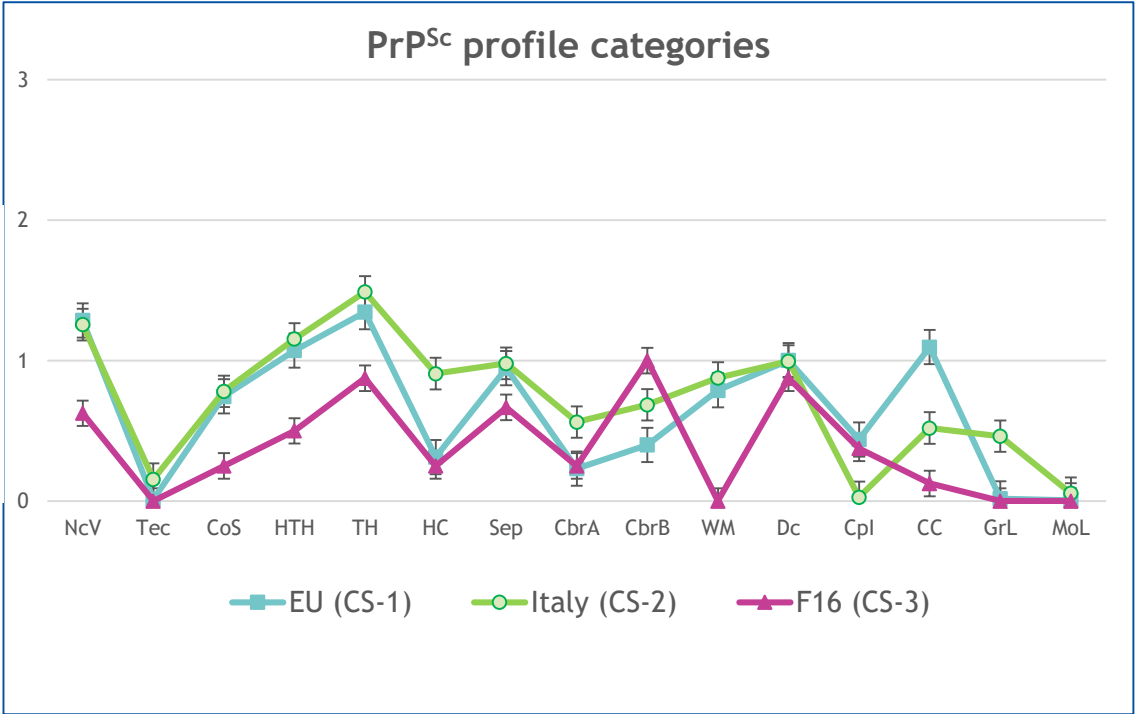
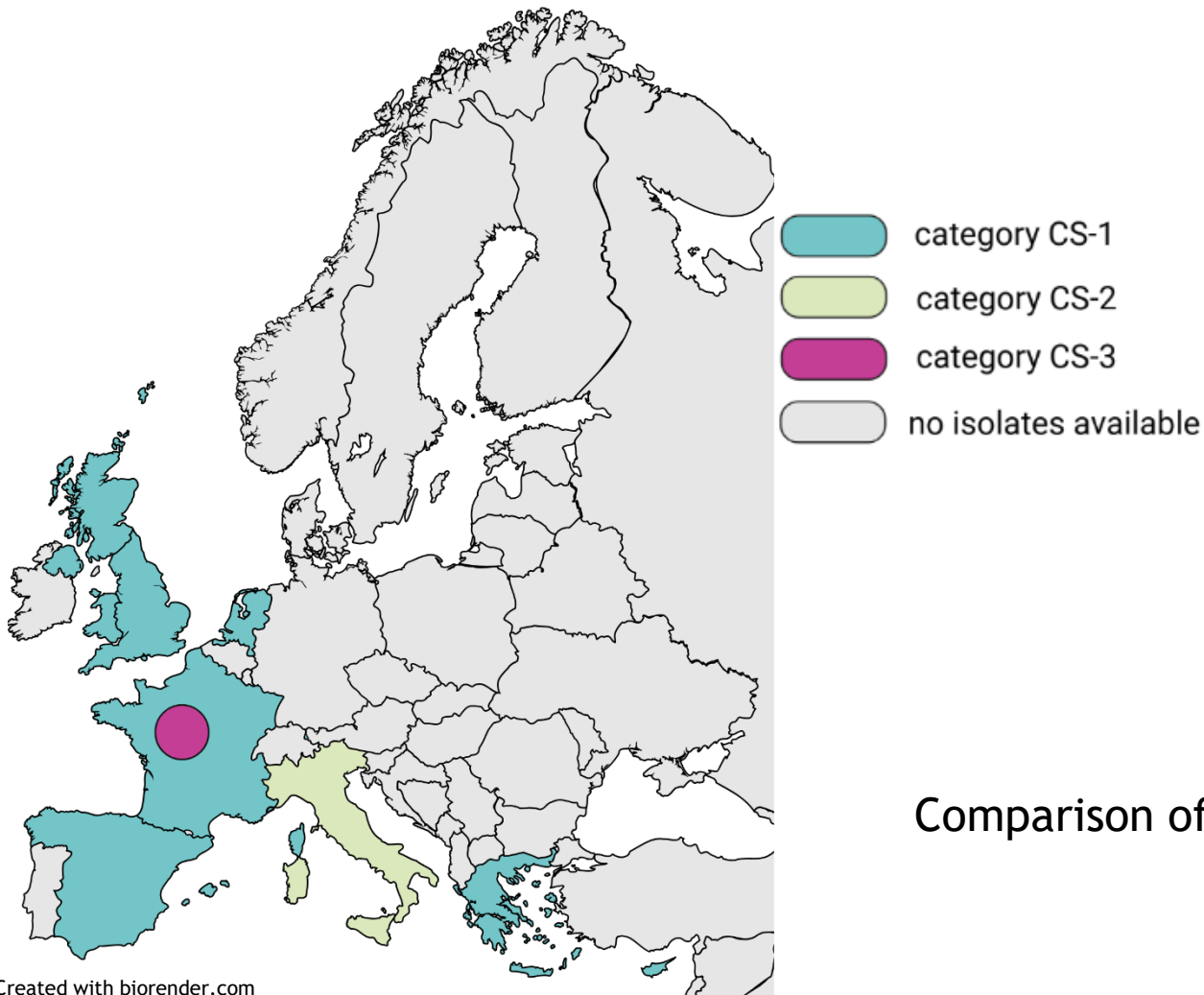
PrP^{Sc} profile Italy



PrP^{Sc} profile France



GOAT SCRAPIE CATEGORIES

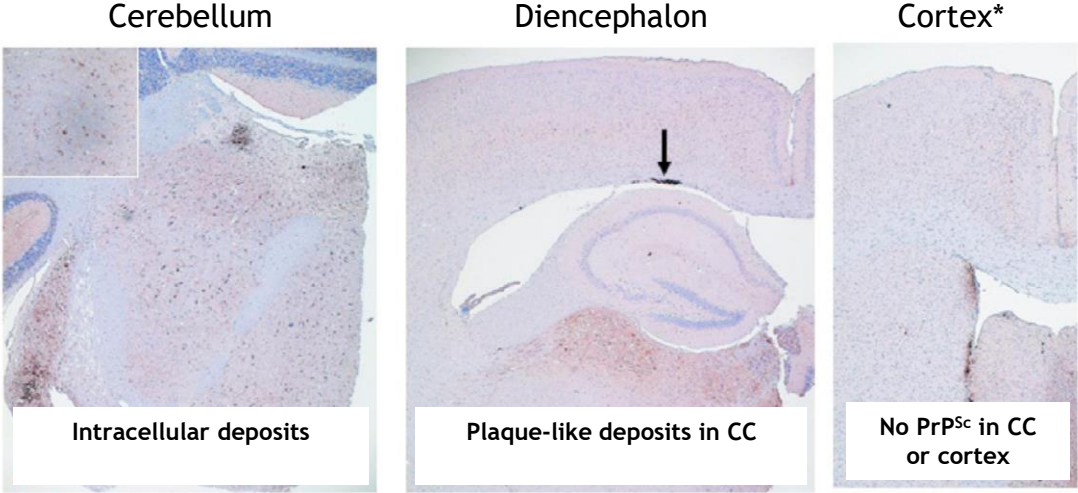
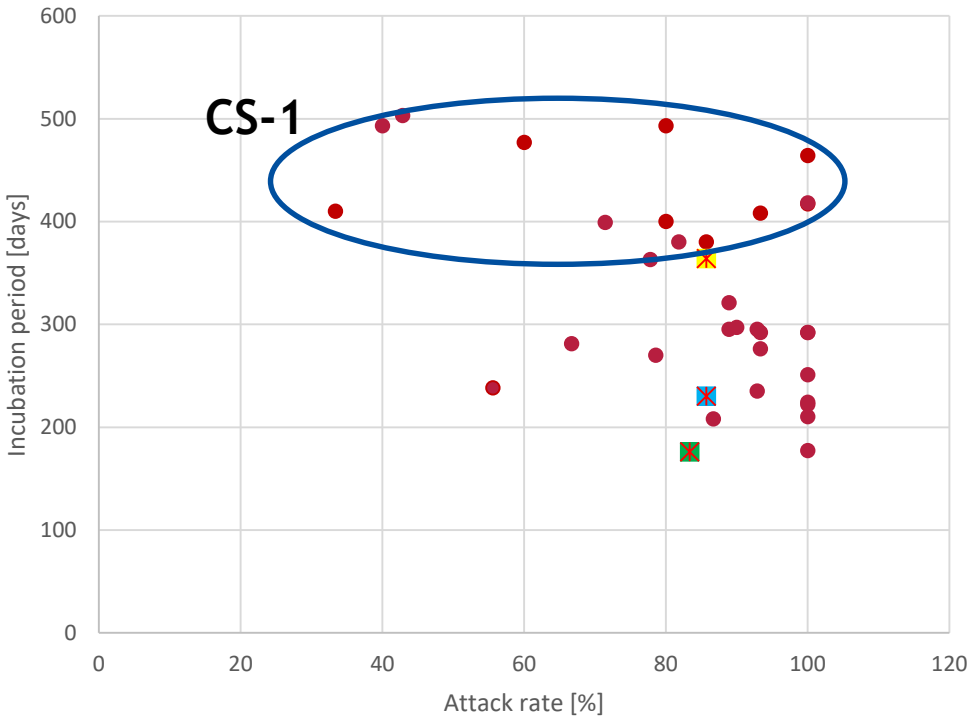


Comparison of mean PrP^{Sc} profiles of all countries → 3 categories:
European mean → CS-1
Italy → CS-2
F16 → CS-3

CHATEGORIZATION OF GOAT ISOLATES

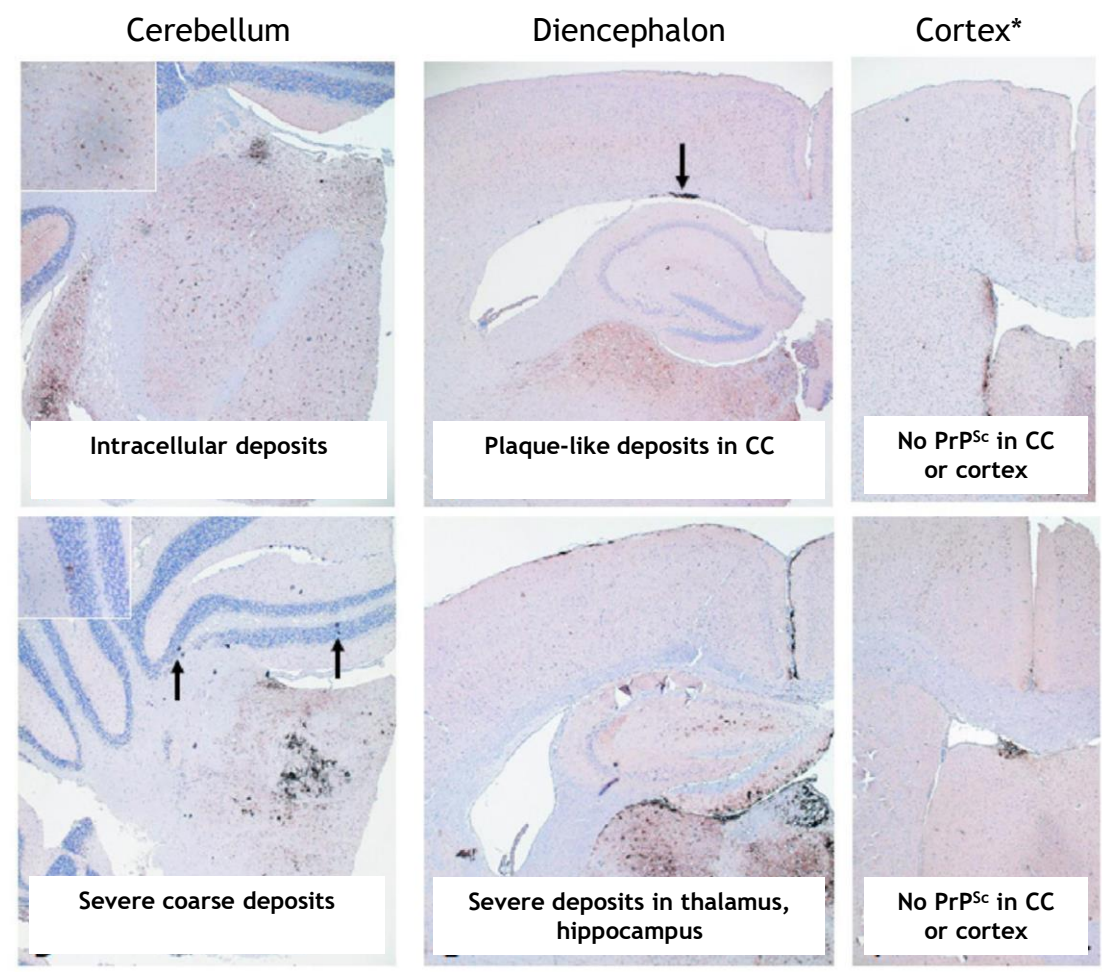
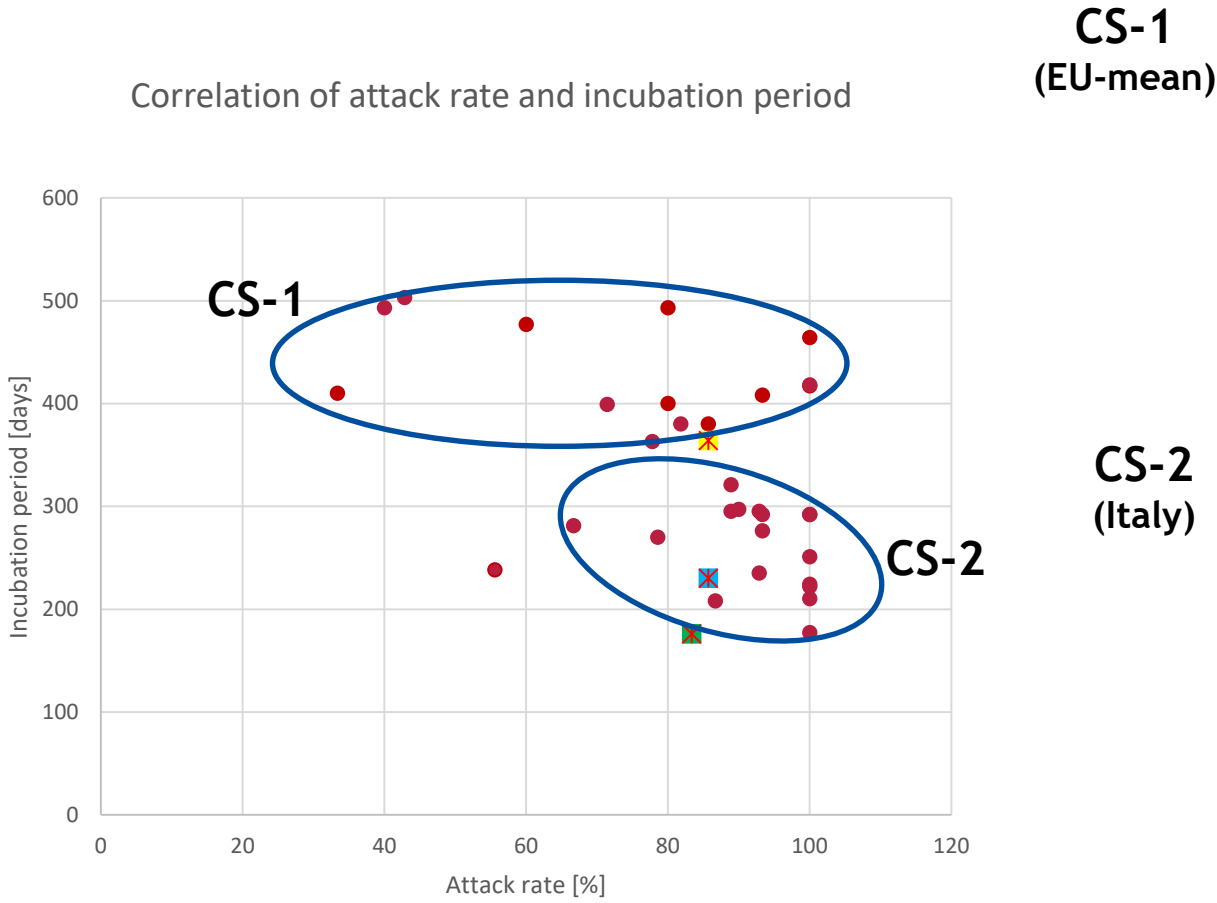
CS-1
(EU-mean)

Correlation of attack rate and incubation period



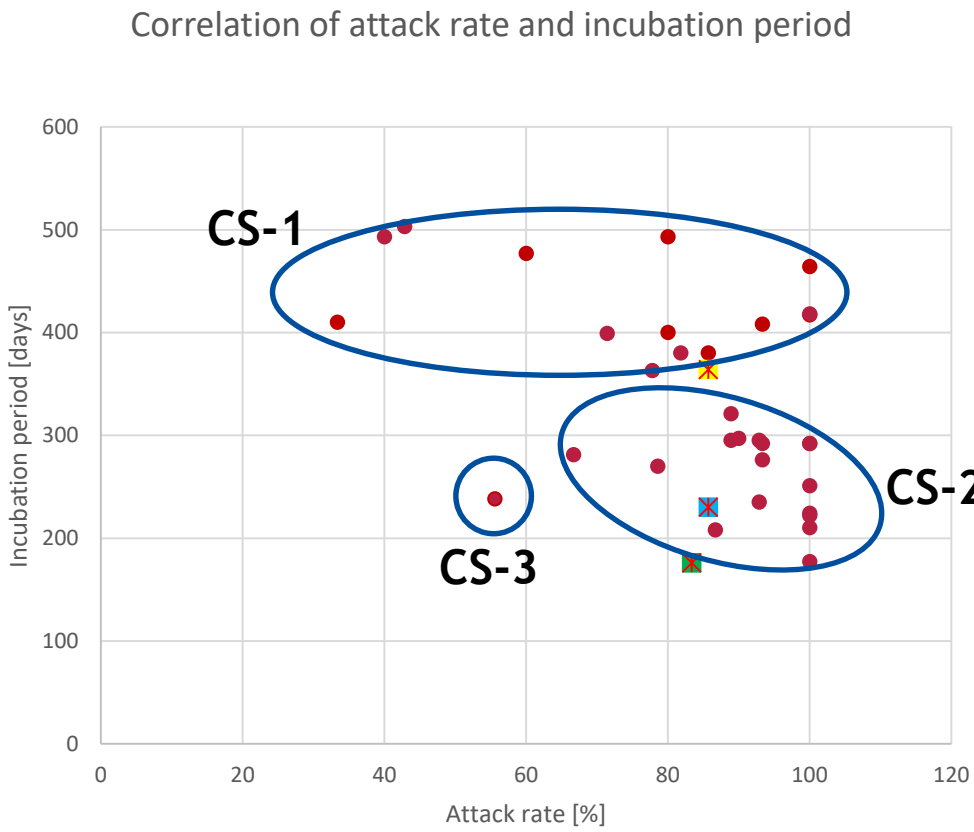
*frontal cortex at the level of the septal nuclei
CC = corpus callosum

CHATEGORIZATION OF GOAT ISOLATES



*frontal cortex at the level of the septal nuclei
CC = corpus callosum

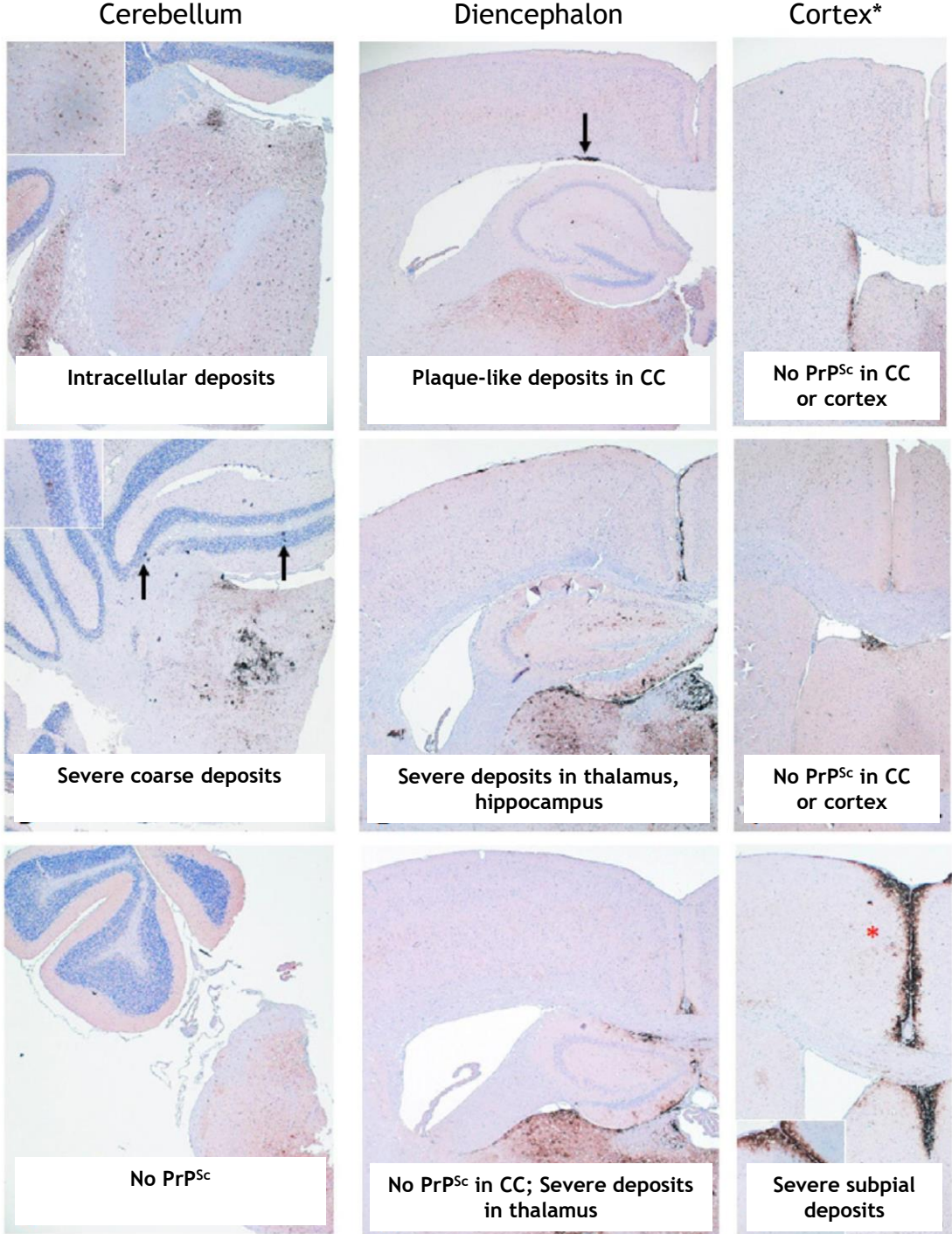
CHATEGORIZATION OF GOAT ISOLATES



CS-1
(EU-mean)

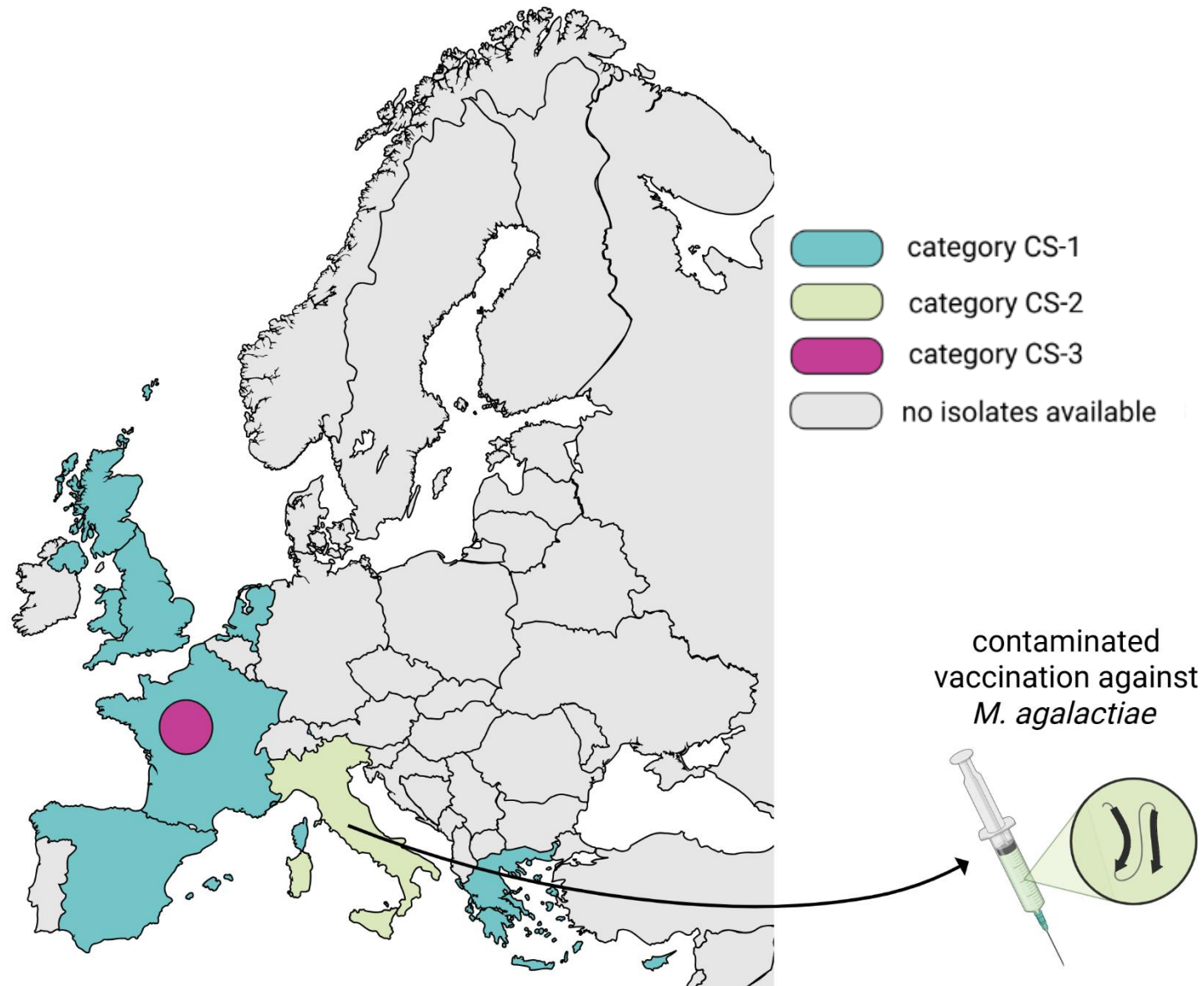
CS-2
(Italy)

CS-3
(F16)

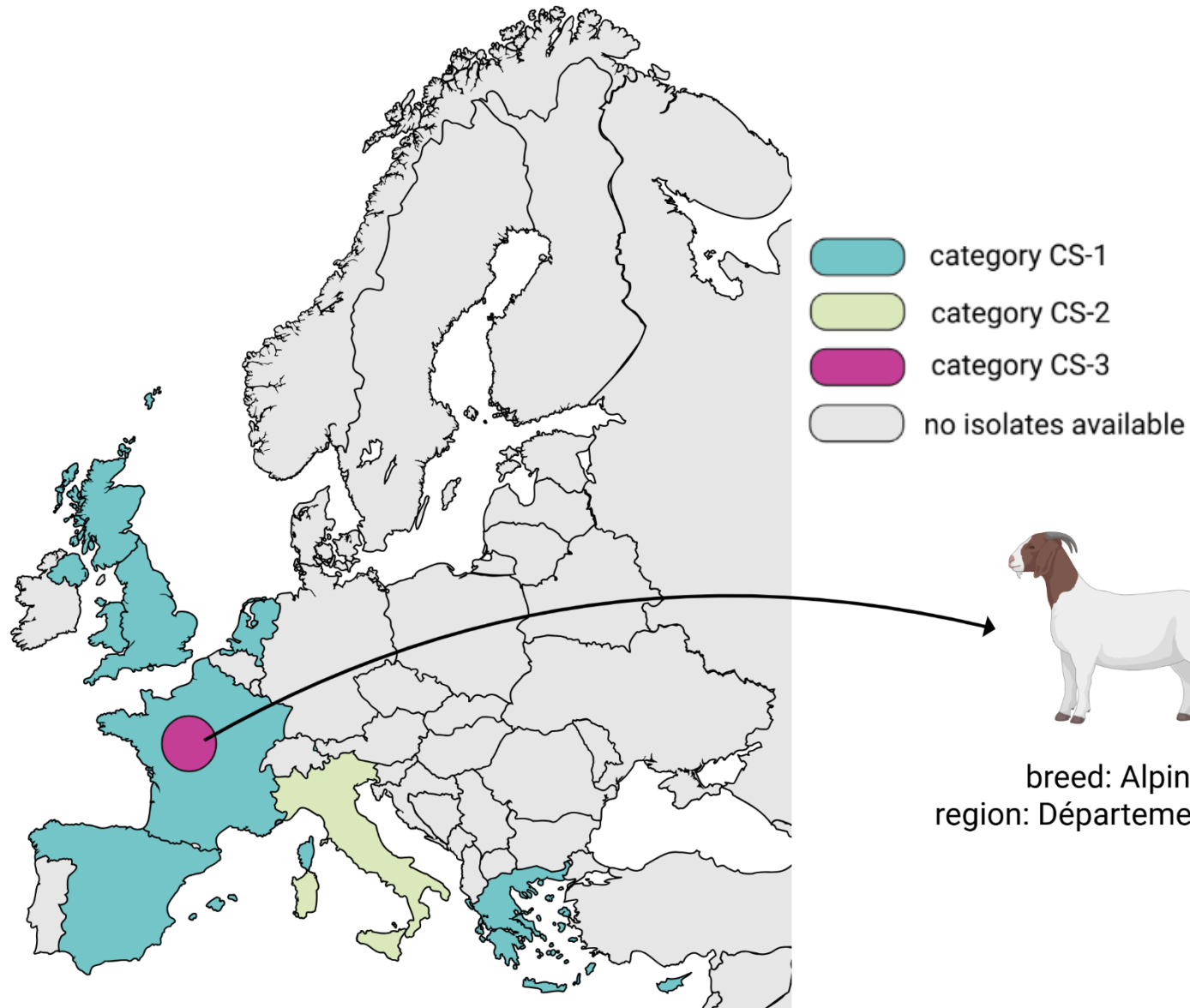


*frontal cortex at the level of the septal nuclei
CC = corpus callosum

INTERPREATION OF STRAIN VARIABILITY

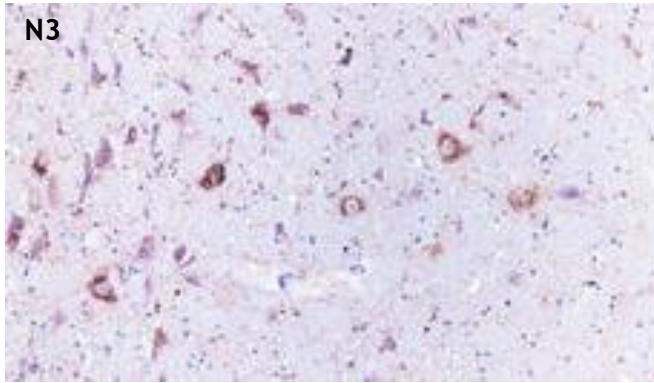
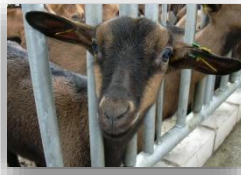


EXPLANATIONS FOR STRAIN VARIABILITY

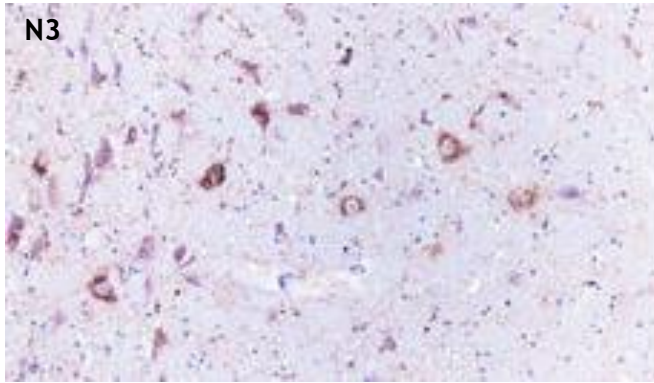


BUT

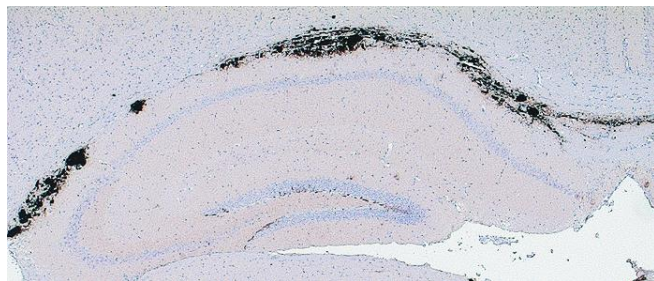
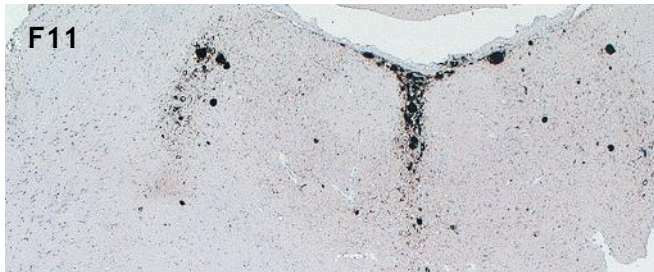
- ✓ The same breed also shows CS-1 strains
- ✓ In Indre CS-1 also circulates
- ✓ No special genotype
- ✓ No knowleged on contaminated vaccines



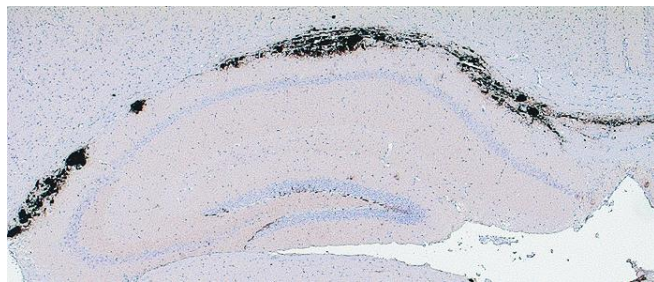
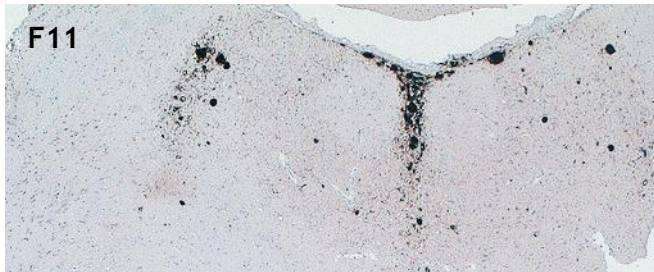
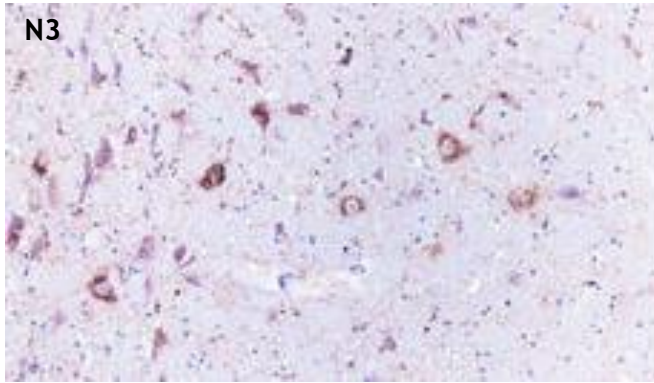
- Predominant CH1641-like intraneuronal deposits with mild fine particulate PrP^{Sc} accumulation in the neuropil
- Found in 7 isolates from Spain (n=2), France (n=1), the Netherlands (n=1) and UK (n=3)
- Biochemical analysis revealed matching results (Nonno et al., 2020)



- Predominant CH1641-like intraneuronal deposits with mild fine particulate PrP^{Sc} accumulation in the neuropil
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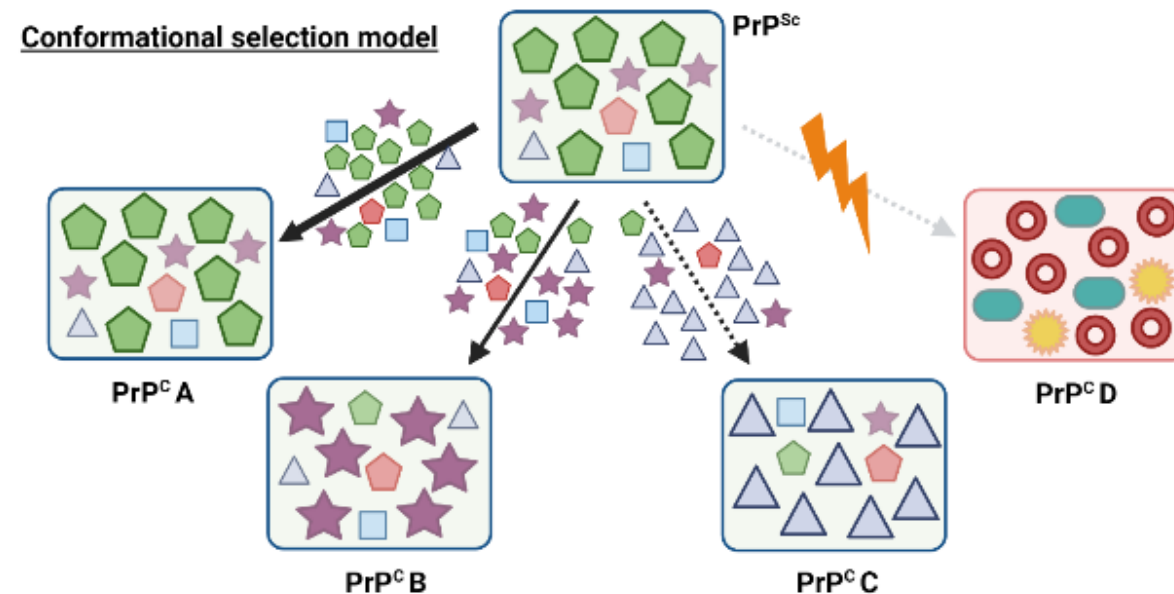
- Predominant BSE-like plaque and plaque-like formations & subpial reaction pattern in the brain stem
- Severe PrP^{Sc} accumulation in the corpus callosum
- Found in one mouse inoculated with the French isolate F11 → *2nd passage initiated (319dpi)*



Strain-mixtures in several isolates with one dominant and several sub-strains



These findings favour the theory of the *conformational selection model*



REVIEW: INVESTIGATION OF EU GOAT FIELD ISOLATES

RESEARCH ARTICLE Open Access

Four types of scrapie in goats differentiated from each other and bovine spongiform encephalopathy by biochemical methods

Jan P. M. Langeveld^{1,2}, Laura Pirisinu², Jorg G. Jacobs¹, Maria Mazza³, Isabelle Lantier⁴, Stéphanie Simon⁵, Olivier Andréoletti⁶, Cristina Acin⁷, Elena Esposito⁸, Christine Fast⁹, Martin Groschup⁸, Wilfred Goldmann⁹, John Spiropoulos¹⁰, Theodoros Sklaviadis¹¹, Frederic Lantier⁴, Loukia Ekateriniadou¹², Penelope Papasavva-Stylianou¹³, Lucien J. M. van Keulen¹, Pier-Luigi Acutis¹, Umberto Agrimi², Alex Bossers¹ and Romolo Nonno^{1,2}

Characterization of goat prions demonstrates geographical variation of scrapie strains in Europe and reveals the composite nature of prion strains

SCIENTIFIC REPORTS
nature research

Romolo Nonno^{1,2}, Alba Marin-Moreno², Juan Carlos Espinosa³, Christine Fast⁴, Lucien Van Keulen⁵, John Spiropoulos⁶, Isabelle Lantier⁷, Olivier Andréoletti⁸, Laura Pirisinu⁹, Michele A. Di Bari¹⁰, Patricia Aguilar-Calvo¹¹, Theodoros Sklaviadis¹², Penelope Papasavva-Stylianou¹³, Pier Luigi Acutis¹⁴, Cristina Acin¹⁵, Alex Bossers¹⁶, Jorge G. Jacobs¹⁷, Gabriele Vaccari¹⁸, Claudia D'Agostino¹⁹, Barbara Chiappini²⁰, Frederic Lantier²¹, Martin H. Groschup²², Umberto Agrimi²³, Juan Maria Torres²⁴ & Jan P. M. Langeveld²⁵

RESEARCH ARTICLE Open Access

Classical scrapie in small ruminants is caused by at least four different prion strains

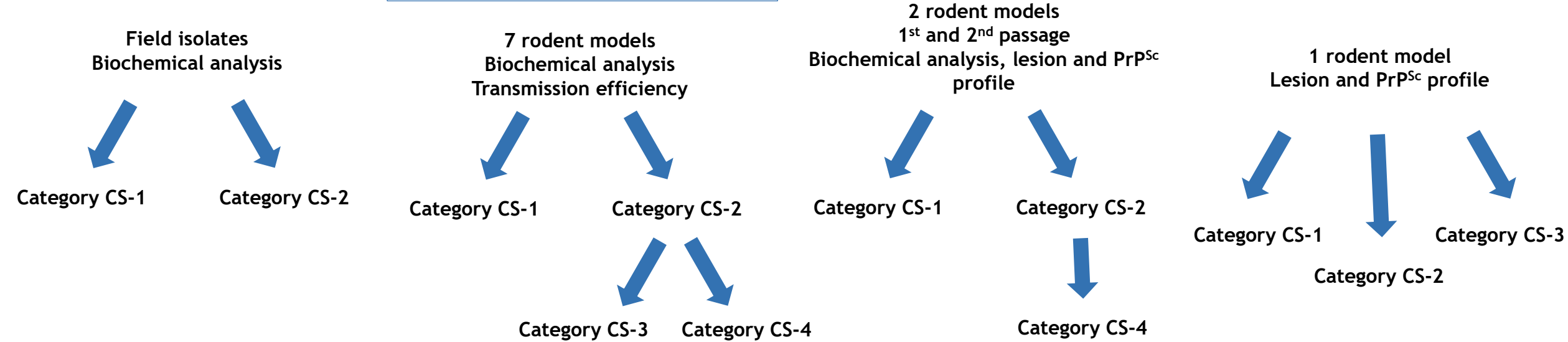
Alba Marin-Moreno¹, Patricia Aguilar-Calvo^{1,6}, Juan Carlos Espinosa¹, Maria Zamora-Ceballos¹, José Luis Pitarich¹, Lorenzo González², Natalia Fernández-Borges¹, Leonor Orge³, Olivier Andréoletti⁴, Romolo Nonno⁵ and Juan Maria Torres¹

pathogens MDPI

Article

Characterisation of European Field Goat Prion Isolates in Ovine PrP Overexpressing Transgenic Mice (Tgshp IX) Reveals Distinct Prion Strains

Sonja Ernst¹, Romolo Nonno², Jan Langeveld³, Olivier Andréoletti⁴, Cristina Acin⁵, Penelope Papasavva-Stylianou⁶, Theodoros Sklaviadis⁷, Pier Luigi Acutis⁸, Lucien van Keulen⁹, John Spiropoulos¹⁰, Markus Keller¹¹, Martin H. Groschup¹² and Christine Fast¹³



Great consistency in the outcome of the different studies

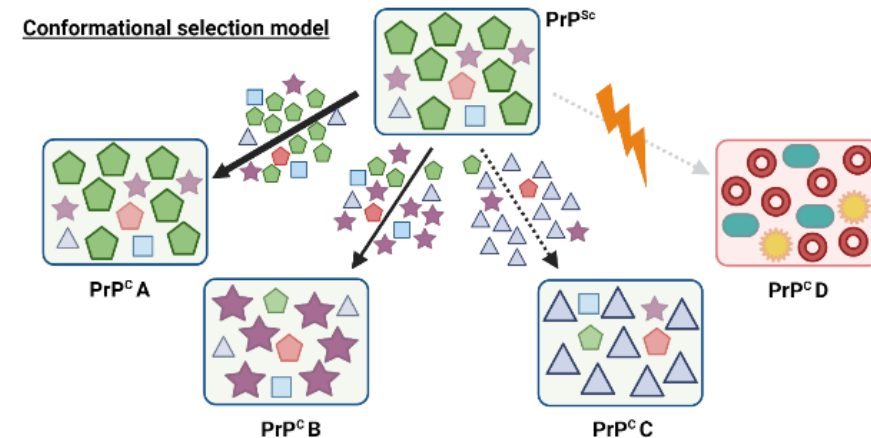
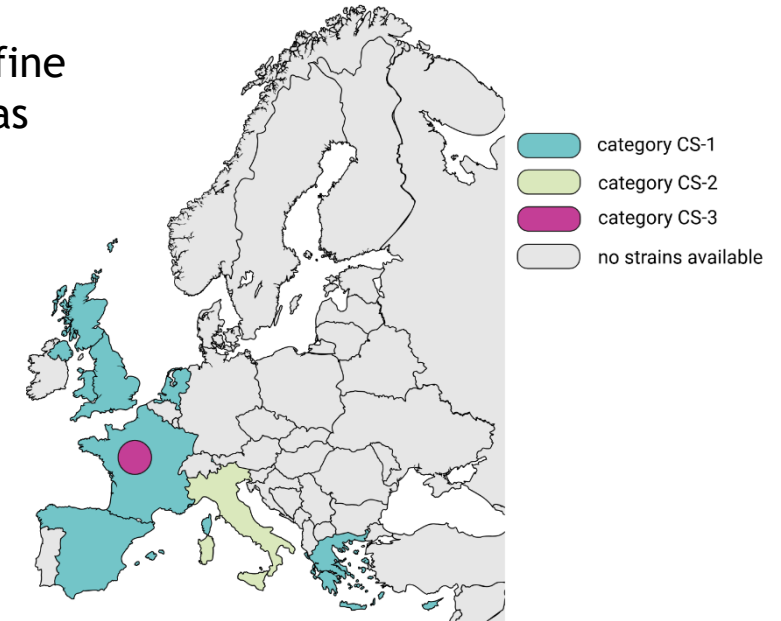
- Successful discrimination of reference strains (gtBSE, atypical scrapie, CH1641) in all studies
- Attack rate and incubation period alone are not sufficient for strain typing but useful in combination with biochemical methods/ IHC
- All studies detected the geographically enclosed Italian (CS-2) goat scrapie strain

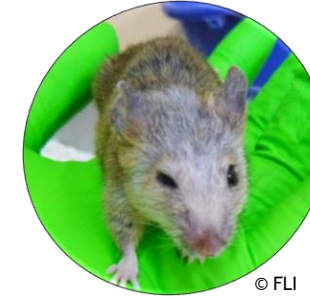
The Tgshp IX mouse model is a reliable tool for prion strain typing and should standardly be included into strain typing of field TSE isolates

High strain variability in goats
→ Increased risk for emergence of novel prion strains

Emergence of novel prion strains could result in interspecies transmission (e.g. between small ruminants and cervids)

- Attack rates highly depends on mouse model/ PrP^{Sc} titre → different parameter define the transmission barrier (i.e. no sheep/goat transmission barrier in Tgshp IX mice, as both share the same genotype)
- In Europe different scrapie strains circulate in goats:
 - CH1641-strain
 - Atypical scrapie
 - Goat BSE
 - ≥ 3 classical scrapie strains
- Several isolates contained sub-strains
 - the conformational selection model seems favorable
 - Goat scrapie prions are highly variable, novel strains could emerge at any time





We want to thank all colleagues for the excellent support during this study!

We thank all Tgshp IX mice that have been sacrificed for this study.

THANK YOU!

Ernst et al., 2024
doi.org/10.3390/pathogens13080629