22nd Annual Meeting of the TSE EURL
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Goat PrP-222K polymorphic variant generates silent carriers for classical scrapie prions

Natalia Fernández-Borges, Alba Marín-Moreno, Juan Carlos Espinosa, Sara Canoyra, Nuria Jerez-Garrido, José Luis Pitarch, Irene Prieto, Olivier Andreoletti, and Juan María Torres

CSIC Consejo Superior de Investigaciones Científicas
INIA Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria
CISA Centro de Investigación en Sanidad Animal

INTRODUCTION – Classical Scrapie

CLASSICAL SCRAPIE: Transmissible spongiform encephalopathy affecting the central nervous system of sheep and goats. Known since 1730s and endemic in many countries.

SCRAPIE STRAIN Infectious isolates that exhibit distinct prions disease phenotypes (*incubation time, distribution of prion deposits in the brain, clinical symptoms and PrP^{res} biochemical features*) in the same PrP background.

TRANSMISSION OF CLASSICAL SCRAPIE:

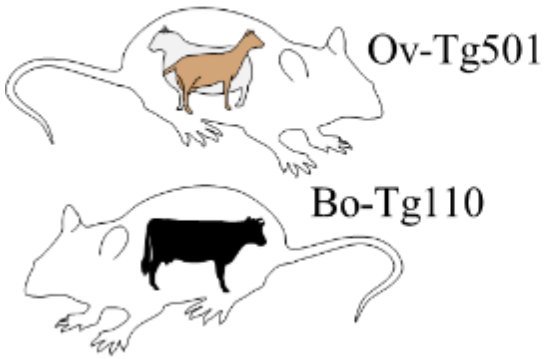
Marín-Moreno et al. Vet Res (2021) 52:57
https://doi.org/10.1186/s13567-021-00929-7

VETERINARY RESEARCH

RESEARCH ARTICLE Open Access

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

Alba Marín-Moreno[1], Patricia Aguilar-Calvo[1,6], Juan Carlos Espinosa[1], María Zamora-Ceballos[1], José Luis Pitarch[1], Lorenzo González[2], Natalia Fernández-Borges[1], Leonor Orge[3], Olivier Andréoletti[4], Romolo Nonno[5] and Juan María Torres[1*]



Category	TgOv (Ov-Tg501)		TgBov (Bo-Tg110)	
	Survival time	PrP^{res}	Survival time	PrP^{res}
I (Italians)	Slow (> 300 dpi)	21 kDa	Fast (< 300 dpi)	21 kDa
II	Fast (< 300 dpi)	21 kDa	Fast (< 300 dpi)	19 kDa
III	Fast (< 300 dpi)	19 kDa	Fast (< 300 dpi)	19 kDa
IV	Slow (> 300 dpi)	21 kDa	Slow (> 300 dpi)	19 kDa

INTRODUCTION – Classical Scrapie

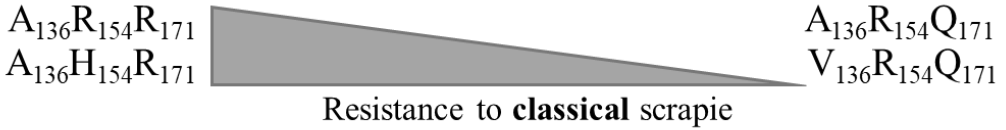
CLASSICAL SCRAPIE: Transmissible spongiform encephalopathy affecting the central nervous system of sheep and goats. Known since 1730s and endemic in many countries.

TRANSMISSION OF CLASSICAL SCRAPIE:

SCRAPIE STRAIN

Infectious isolates that exhibit distinct prions disease phenotypes (*incubation time, distribution of prion deposits in the brain, clinical symptoms and PrP^{res} biochemical features*) in the same PrP background.

HOST GENOTYPE



Goat-TSE-Free"Towards breeding of goats for genetically determined TSEs resistance"

Some studies point to the influence of $I_{142}M$, $N_{146}S$, **$Q_{222}K$** polymorphisms

INTRODUCTION - Q222K polymorhism distribution

OPEN ACCESS
Paper

Prion protein genotype survey confirms low frequency of scrapie-resistant K222 allele in British goat herds

W. Goldmann, E. Marier, P. Stewart, T. Konold, S. Street, J. Langeveld, O. Windl, A. Ortiz-Pelaez

Identification of seven haplotypes of the caprine PrP gene at codons 127, 142, 154, 211, 222 and 240 in French Alpine and Saanen breeds and their association with classical scrapie

F Barillet [1], D Mariat [2], Y Amigues [3], R Faugeras [3], H Caillat [1], K Moazami-Goudarzi [2], R Rupp [1], J M Babilliot [4], C Lacroux [4], S Lugan [4], F Schelcher [4], C Chartier [5], F Corbière [4], O Andréoletti [4], C Perrin-Chauvineau [5]

PLoS One. 2013 Apr 8;8(4):e61118. doi: 10.1371/journal.pone.0061118. Print 2013.

Prion protein gene variability in Spanish goats. Inference through susceptibility to classical scrapie strains and pathogenic distribution of peripheral PrP(sc.)

Cristina Acín [1], Inmaculada Martín-Burriel, Eva Monleón, Jaber Lyahyai, José Luis Pitarch, Carmen Serrano, Marta Monzón, Pilar Zaragoza, Juan José Badiola

Identification of an allelic variant of the goat PrP gene associated with resistance to scrapie

Gabriele Vaccari [1], Michele A Di Bari [1], Luisella Morelli [1], Romolo Nonno [1], Barbara Chiappini [1], Giovanni Antonucci [1], Stefano Marcon [1], Elena Esposito [1], Paola Fazzi [1], Nadia Palazzini [1], Pasquale Troiano [2], Antonio Petrella [2], Giovanni Di Guardo [3], Umberto Agrimi [1]

J Gen Virol. 2006 Apr;87(Pt 4):1029-1033. doi: 10.1099/vir.0.81440-0.

Identification of prion protein gene polymorphisms in goats from Italian scrapie outbreaks

P L Acutis [1], A Bossers [2], J Priem [2], M V Riina [1], S Peletto [1], M Mazza [1], C Casalone [1], G Forloni [3], G Ru [1], M Caramelli [1]

Caprine PRNP polymorphisms at codons 171, 211, 222 and 240 in a Greek herd and their association with classical scrapie

Ilias G Bouzalas [1], Chrysostomos I Dovas, Georgios Banos, Maria Papanastasopoulou, Spyridon Kritas, Anna Oevermann, Dimitra Papakostaki, Chatziandreou Evangelia, Orestis Papadopoulos, Torsten Seuberlich, Georgios Koptopoulos

J Genet. 2017 Jun;96(2):299-305. doi: 10.1007/s12041-017-0763-1.

Prion protein gene polymorphisms in Turkish native goat breeds

Hasan Meydan [1], Erkan Pehlivan, Mustafa Muhip Özkan, Mehmet Ali Yildiz, Wilfred Goldmann

Animals (Basel). 2021 May 31;11(6):1635. doi: 10.3390/ani11061635.

Genetic Variation in the Prion Protein Gene (*PRNP*) of Two Tunisian Goat Populations

Samia Kdidi [1], Mohamed Habib Yahyaoui [1], Michela Conte [2], Barbara Chiappini [2], Mohamed Hammadi [1], Touhami Khorchani [1], Gabriele Vaccari [2]

INTRODUCTION - Q222K polymorhism susceptibility to scrapie?

JVI Journals.ASM.org

Genetic Resistance to Scrapie Infection in Experimentally Challenged Goats

Caroline Lacroux,[a] Cécile Perrin-Chauvineau,[b] Fabien Corbière,[a] Naima Aron,[a] Patricia Aguilar-Calvo,[c] Juan Maria Torres,[c] Pierrette Costes,[a] Isabelle Brémaud,[b] Séverine Lugan,[a] François Schelcher,[a] Francis Barillet,[d] Olivier Andréoletti[a]

Genotype	No. of clinically TSE-affected animals/total no. of animals	Scrapie incubation period (dpi) (mean ± SD)
$I_{142}R_{154}R_{211}Q_{222}$/IRRQ (wild type)	5/5	486 ± 21
M_{142}RQ/IRRQ	5/5	788 ± 99
IH_{154}RQ/IRRQ	5/5	624 ± 148
IRQ_{211}Q/IRRQ	5/5	1,291 ± 325
IRQ_{211}Q/$IRRQ_{211}$Q	10/10	770 ± 139
IRRK_{222}/IRRQ	2/5	1,900, 2,174
IRRK_{222}/IRRK_{222}	1/5	2,101

JVI Journals.ASM.org

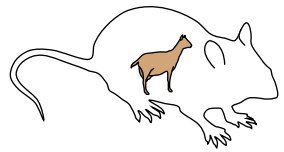
Role of the Goat K_{222}-PrP^C Polymorphic Variant in Prion Infection Resistance

Patricia Aguilar-Calvo,[a] Juan Carlos Espinosa,[a] Belén Pintado,[b] Alfonso Gutiérrez-Adán,[c] Elia Alamillo,[a] Alberto Miranda,[a,c] Irene Prieto,[a] Alex Bossers,[d] Olivier Andreoletti,[a] Juan María Torres[a]

Mean survival time (days) ± SEM $(n/n_0)^a$

Isolate	Q_{222}-Tg501 mice (Q_{222}/−)	K_{222}-Tg516 mice (K_{222}/−)	Tg501 × Tg516 mice (Q/K_{222})
Goat-Sc F10	465 ± 19 (7/7)	>650 (0/5)	>650 (6/6)
Goat-Sc F2	250 ± 36 (4/4)	>650 (0/7)	630 ± 26 (5/6)
Goat-Sc I3	659 ± 10 (5/5)	>650 (0/5)	>650 (0/6)
Goat-Sc I9	600 ± 43 (5/5)	>650 (0/5)	ND
Goat-Sc S2	449 ± 62 (9/9)	>650 (0/6)	>650 (0/6)
Goat-Sc S3	298 ± 22 (6/6)	>650 (0/6)	ND
Healthy goat brain	>650 (0/6)	>650 (0/6)	>650 (0/6)

Prion agent	Animal model
Classical scrapie	Heterozygous QK_{222} goat **(++)**
	Homozygous K_{222} goat **(+)**



?

EXPERIMENTAL DESIGN – Mouse models and isolates

Q_{222}-Tg501

Homozygous

K_{222}-Tg516

Homozygous

Category	Country of origin	Species origin	Code	PrP genotype[a]	PrP^{res}
I (Italians)	Italy	Sheep	198/9	wt	21 kDa
II	Spain	Goat	S2	wt; SP_{240}	21 kDa
	France	Goat	CP060146	wt	21 kDa
II+III	UK	Goat	UKA2	wt; SP_{240}	21 kDa
	France	Goat	F14	wt; $IM_{142}SP_{240}$	19 kDa
IV	France	Goat	F10	wt; SP_{240}	21 kDa
	Cyprus	Goat	C1	wt; PP_{240}	21 kDa
Negative control	France	Goat	Healthy goat brain	wt	-

[a] PrP genotype: wt means $A_{136}R_{154}Q_{171}$

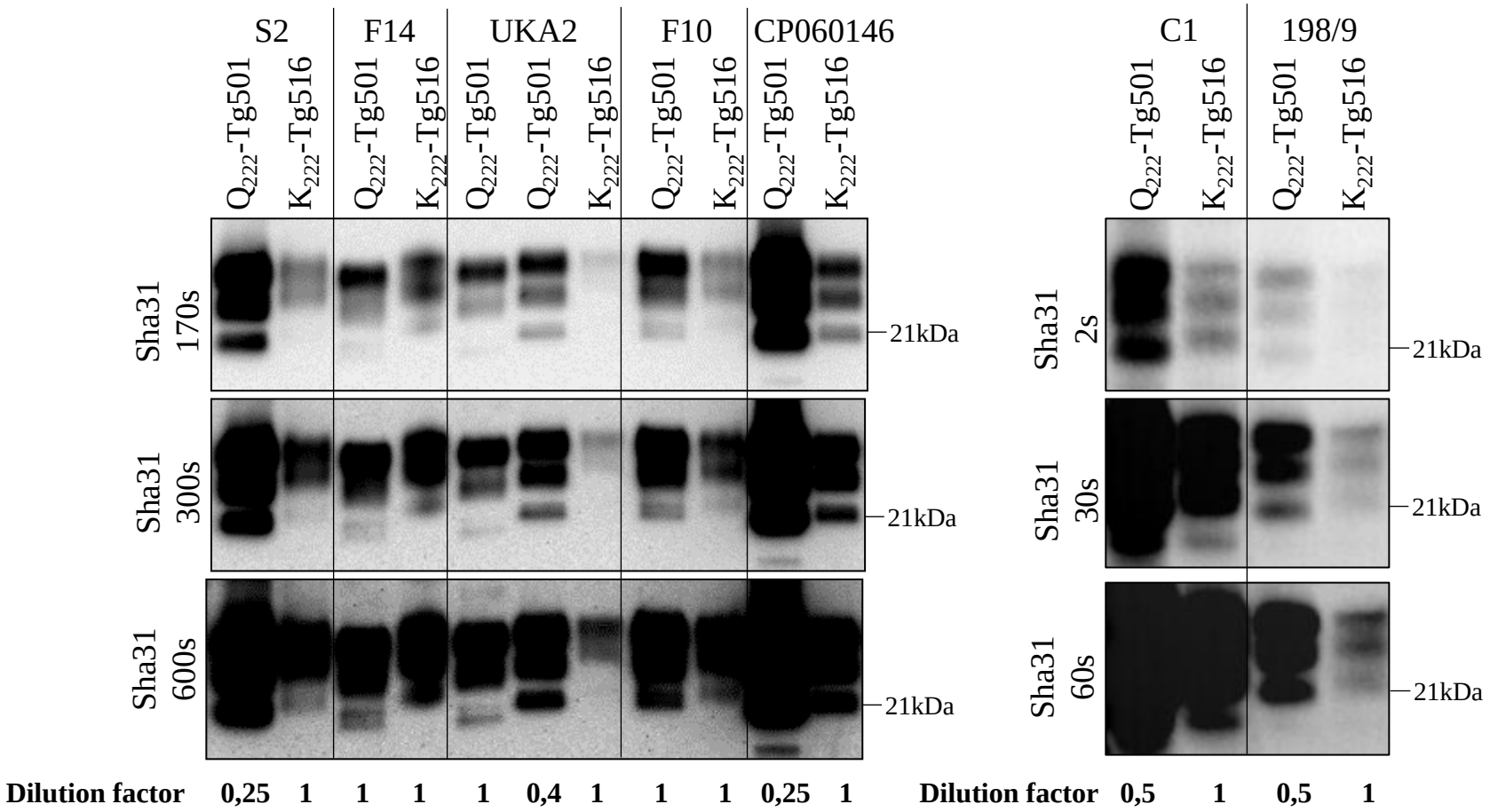
RESULTS– Transmission studies

Category	Isolate	Mean survival time (days) ± SD[a] (n/n0)[b]			
		Q_{222}-Tg501		K_{222}-Tg516	
		1st passage	2nd passage	1st passage	2nd passage
I (Italians)	198/9	592±13 (6/6)	536±46 (5/5)	>650 (1/6)[c]	ND[d]
II	S2	228±15 (6/6)	233±4 (6/6)	>650 (3/4)[c]	>650 (7/7)[c]
	CP060146	379±31 (5/5)	ND[d]	>650 (5/5)[c]	>650 (5/5)[c]
II + III	UKA2	245±36 (5/5)	252±8 (6/6)	>650 (4/4)[c]	>650 (5/5)[c]
	F14	526±46 (4/4)	241±22 (4/4)	>650 (4/4)[c]	>650 (5/5)[c]
IV	F10	449±19 (5/5)	372±14 (6/6)	>650 (6/6)[c]	>650 (5/5)[c]
	C1	483±15 (4/4)	301±10 (4/4)	>650 (7/7)[c]	ND[d]
Negative control	Healthy goat brain	>650 (0/6)	>650 (0/6)	>650 (0/6)	>650 (0/6)

Dead/sacrificed at the end of lifespan
No signs of prion disease

[a] Mean survival time indicated in days ± standard deviation.
[b] n/n_0, number of diseased, PrP^{res}-positive animals/number of inoculated animals.
[c] Animals found dead or sacrificed at the end of their lifespan without showing clinical signs.
[d] Not done.

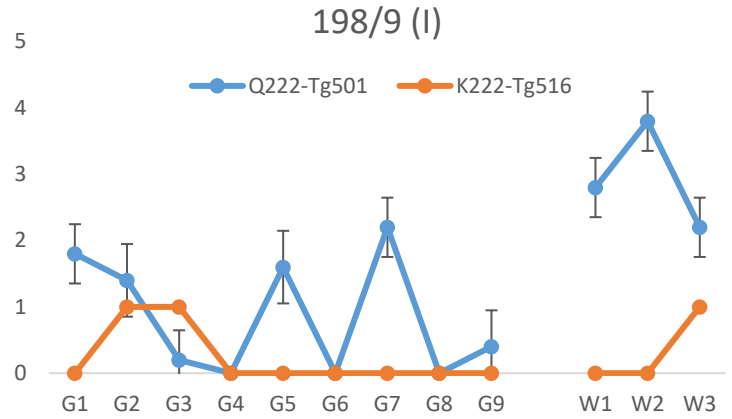
RESULTS– Biochemical patterns

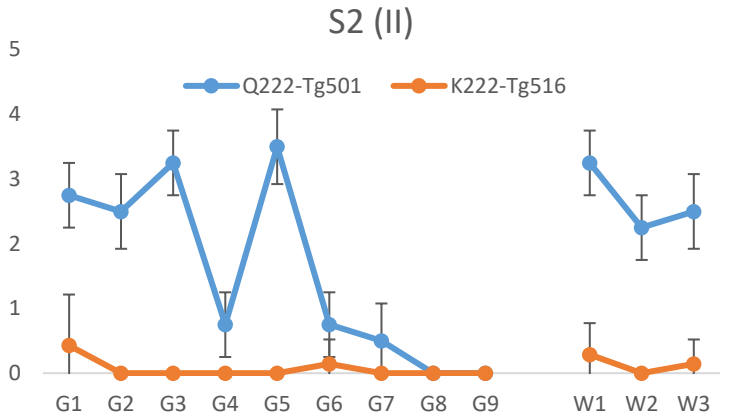


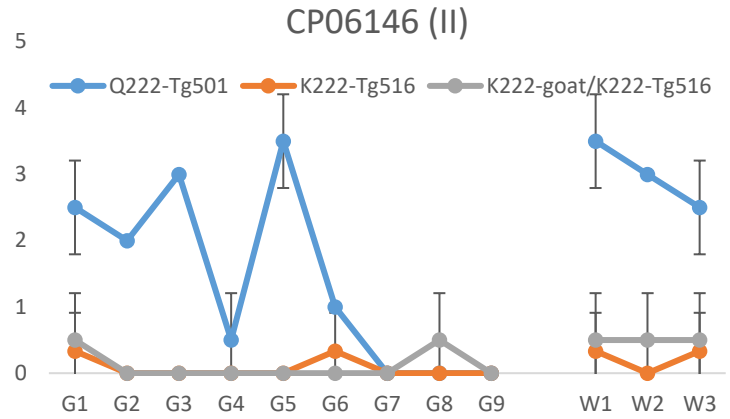
Slight **higher molecular mass (independent** on the **prion strain)**

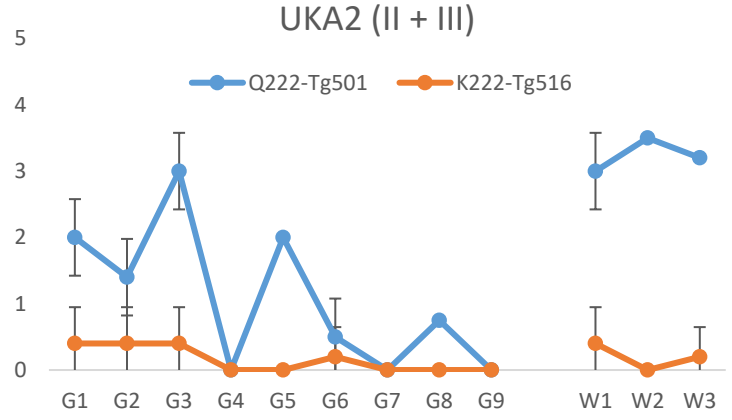
Lower PrP^{res} accumulation in K_{222}-Tg516

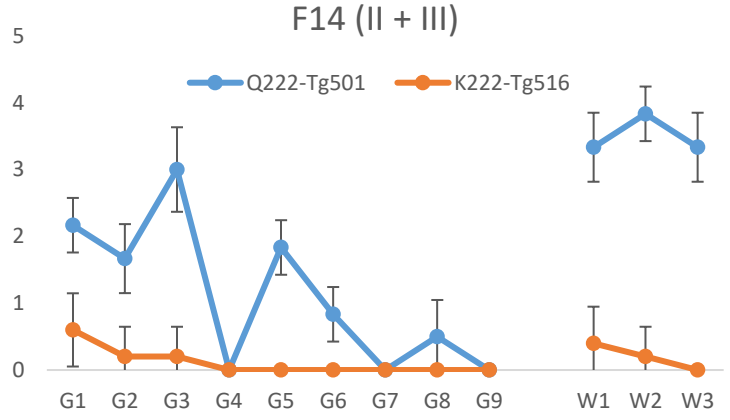
RESULTS– Vacuolation profile of K_{222}-Tg516 (compared to Q_{222}-Tg501)

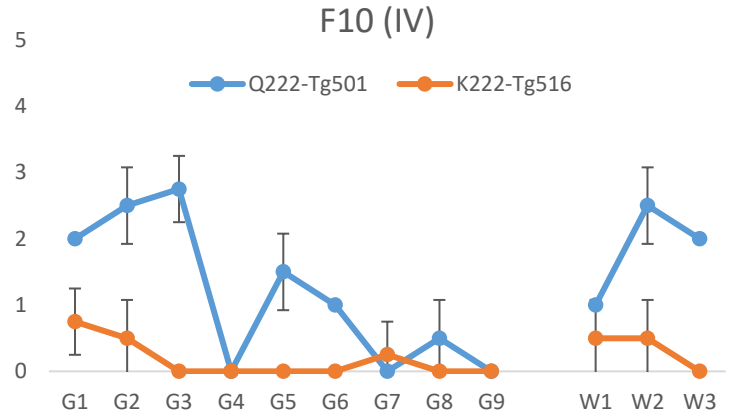


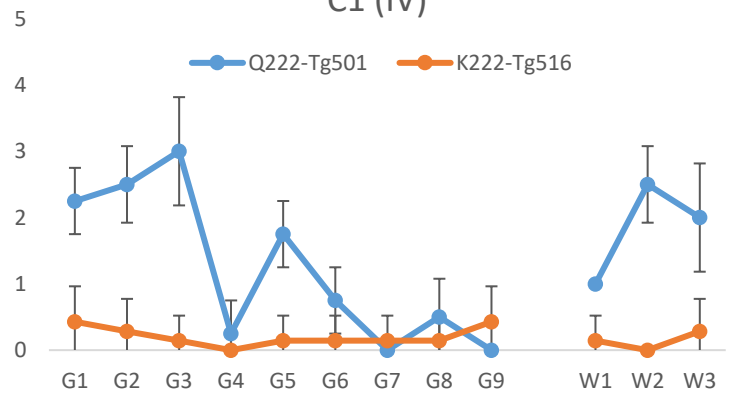






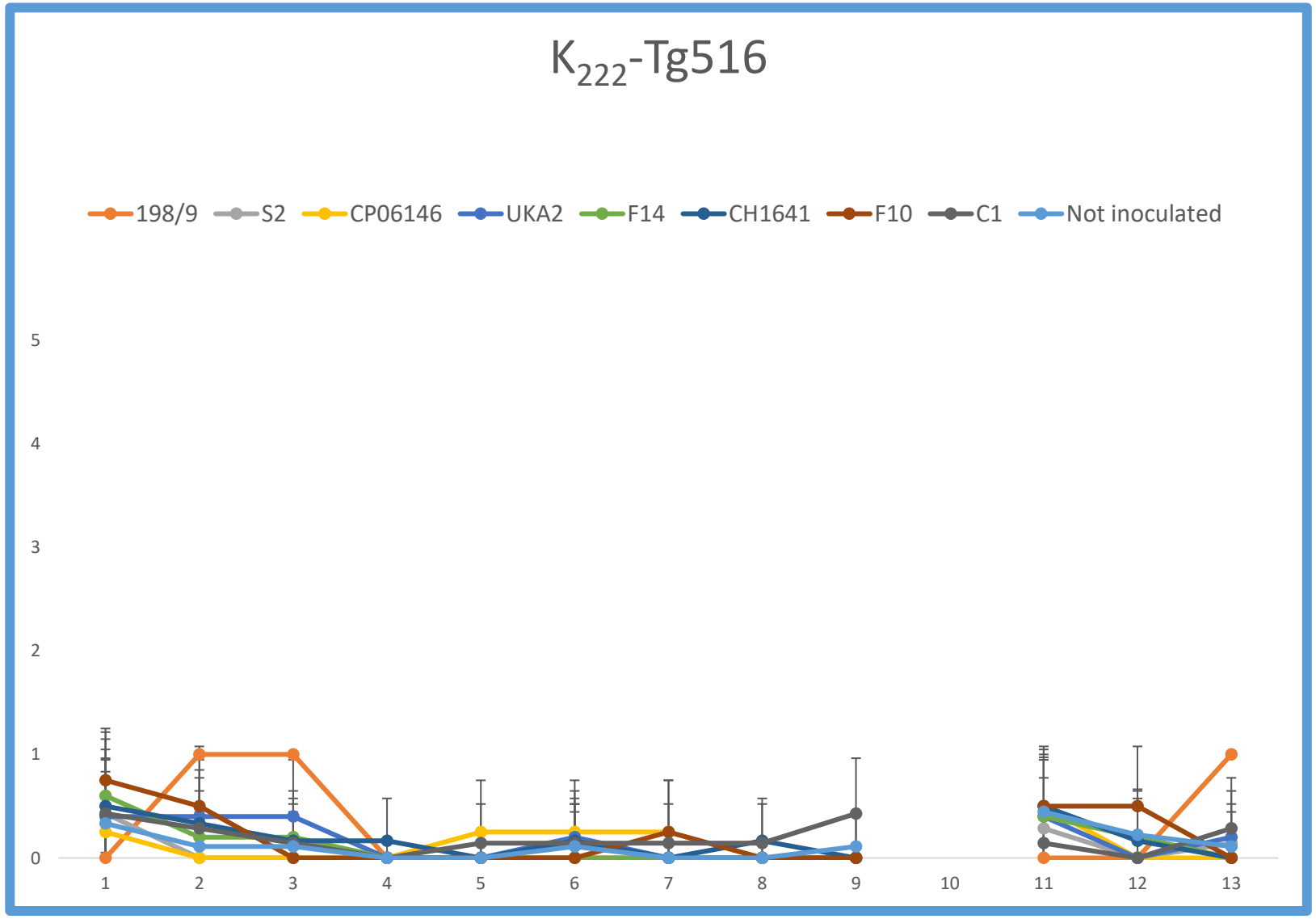






No vacuolation in K_{222}-Tg516

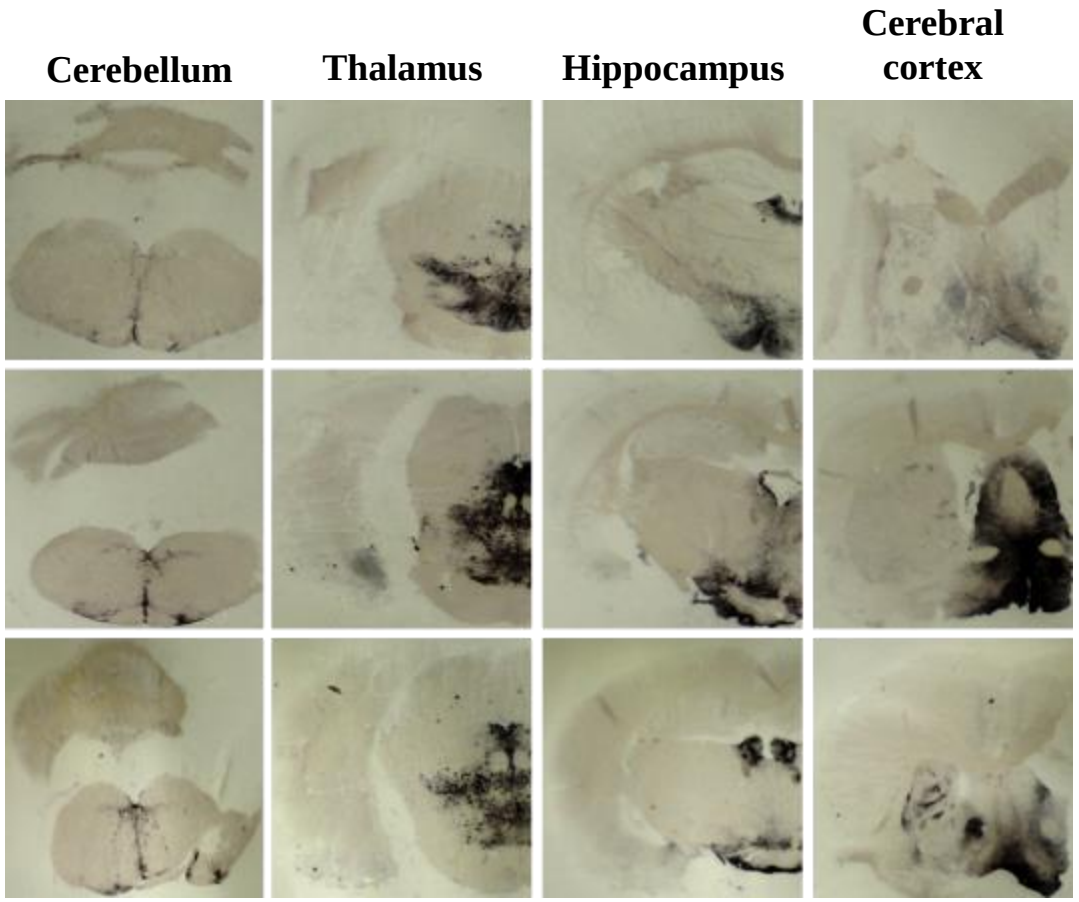
RESULTS– Vacuolation profile of K_{222}-Tg516 (compared to aged mice)



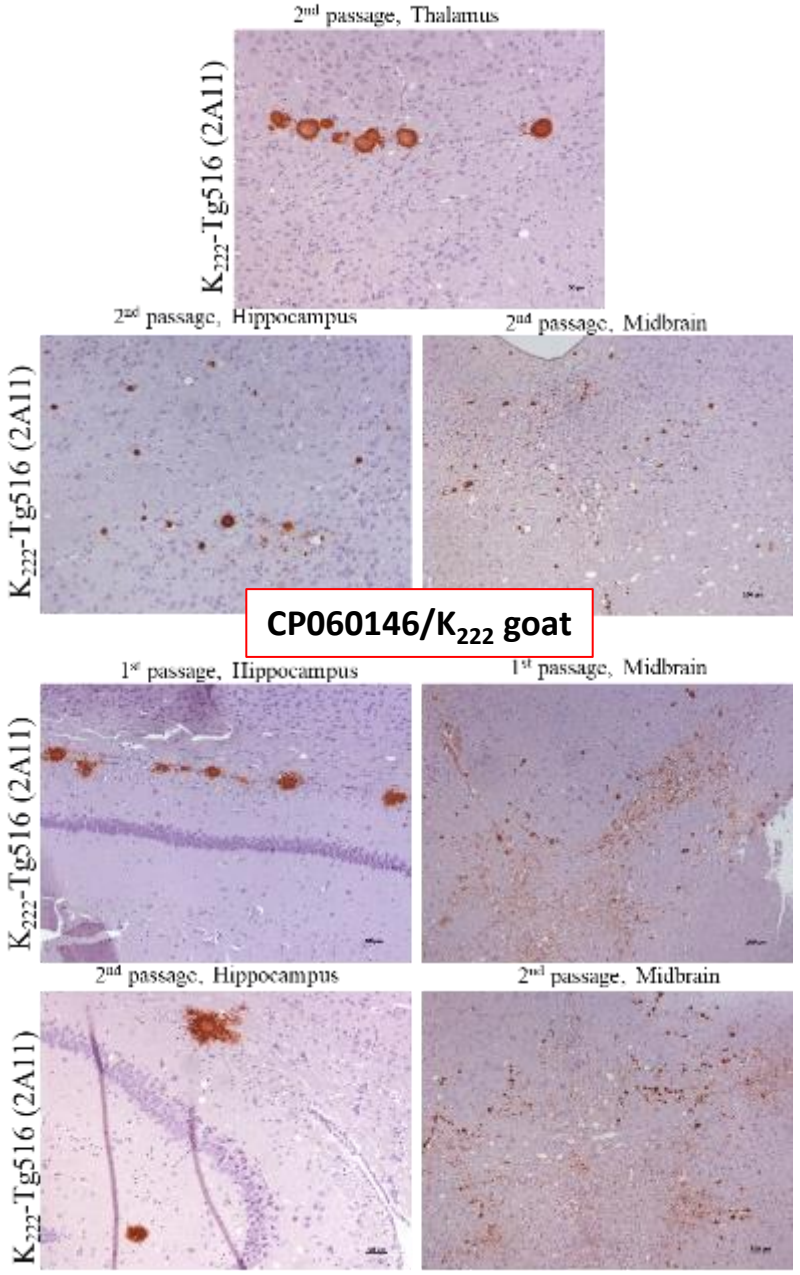
No vacuolation in K_{222}-Tg516
Indistinguishable from **aging**

RESULTS– PrP^{Sc} plaques by immunohistochemistry and Pet blot

CP060146/K_{222}-Tg516

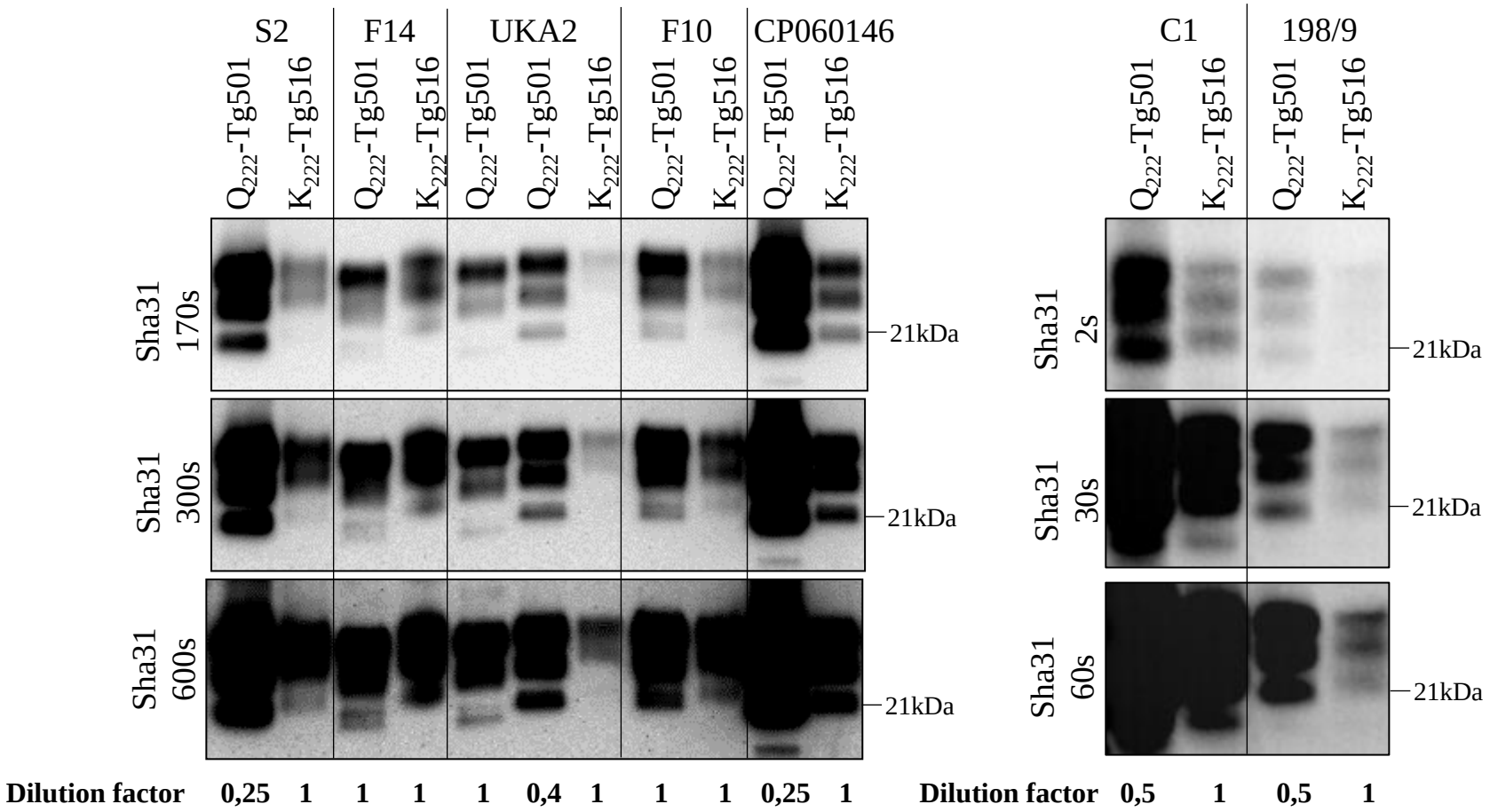


F10/K_{222}-Tg516



PrP^{Sc} deposition in K_{222}-Tg516 **only after further adaptation in the K_{222} environment**

RESULTS– Biochemical patterns



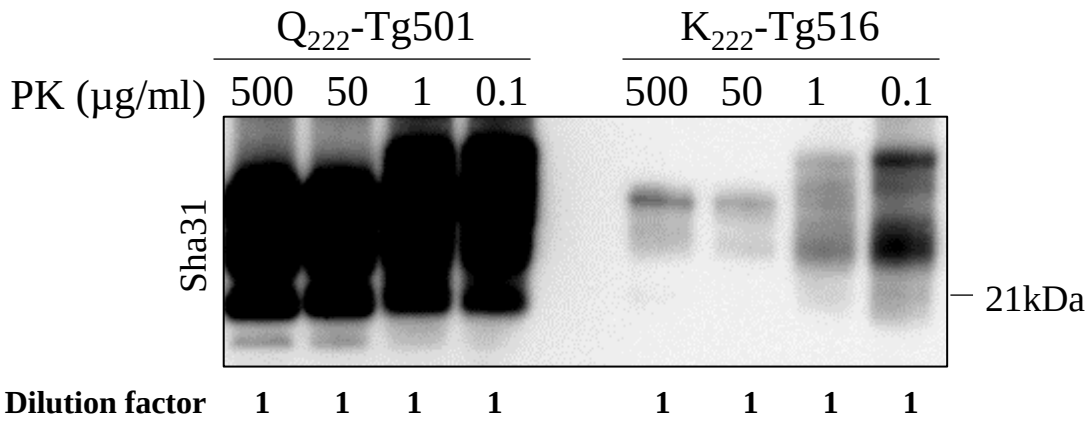
Slight **higher molecular mass (independent** on the **prion strain)**

Lower PrP^{res} accumulation in K_{222}-Tg516

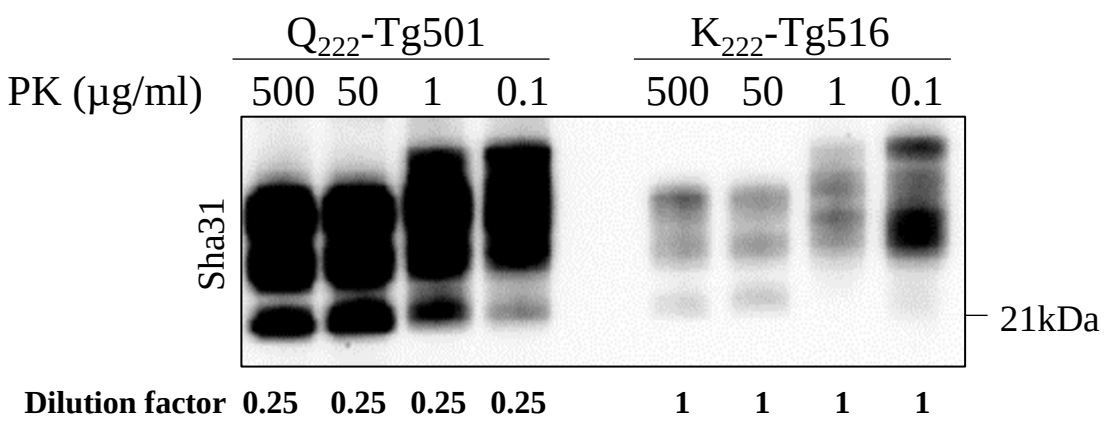
- PK sensitivity
- Less PrP^{res}

RESULTS– Proteinase K resistance analysis

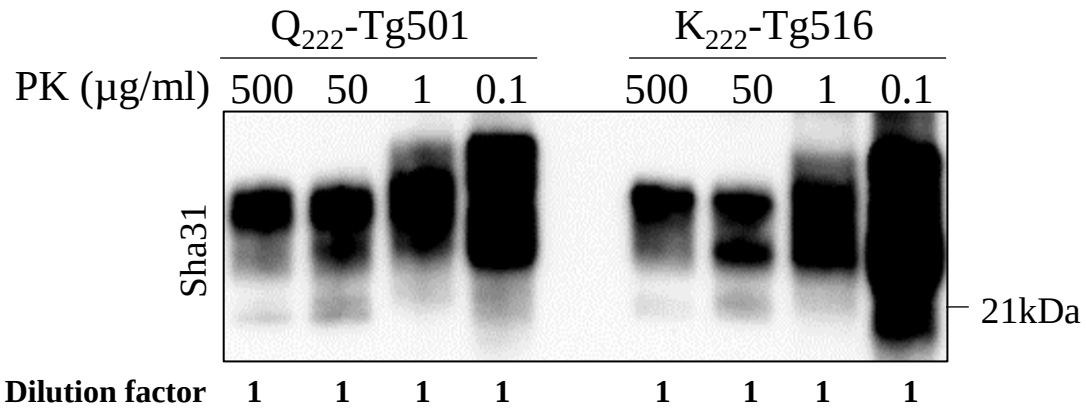
F10/Q_{222}-Tg501



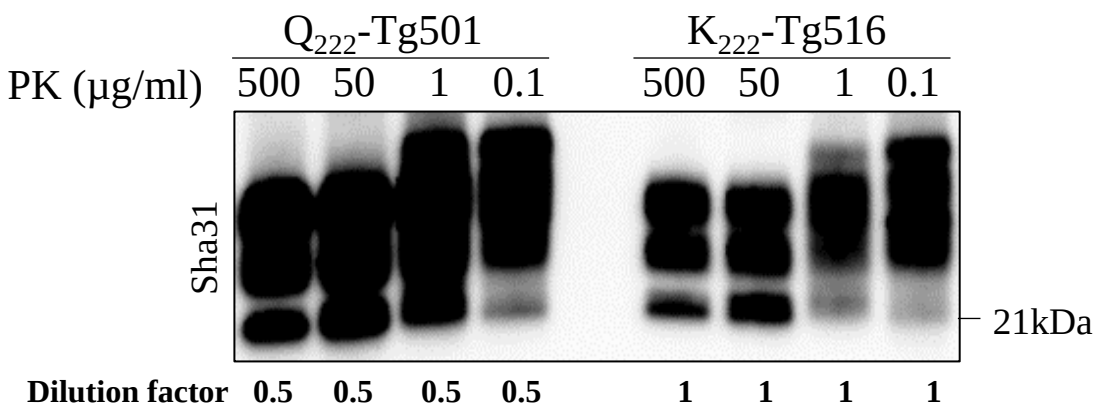
CP060146/Q_{222}-Tg501



F10/K_{222}-Tg516

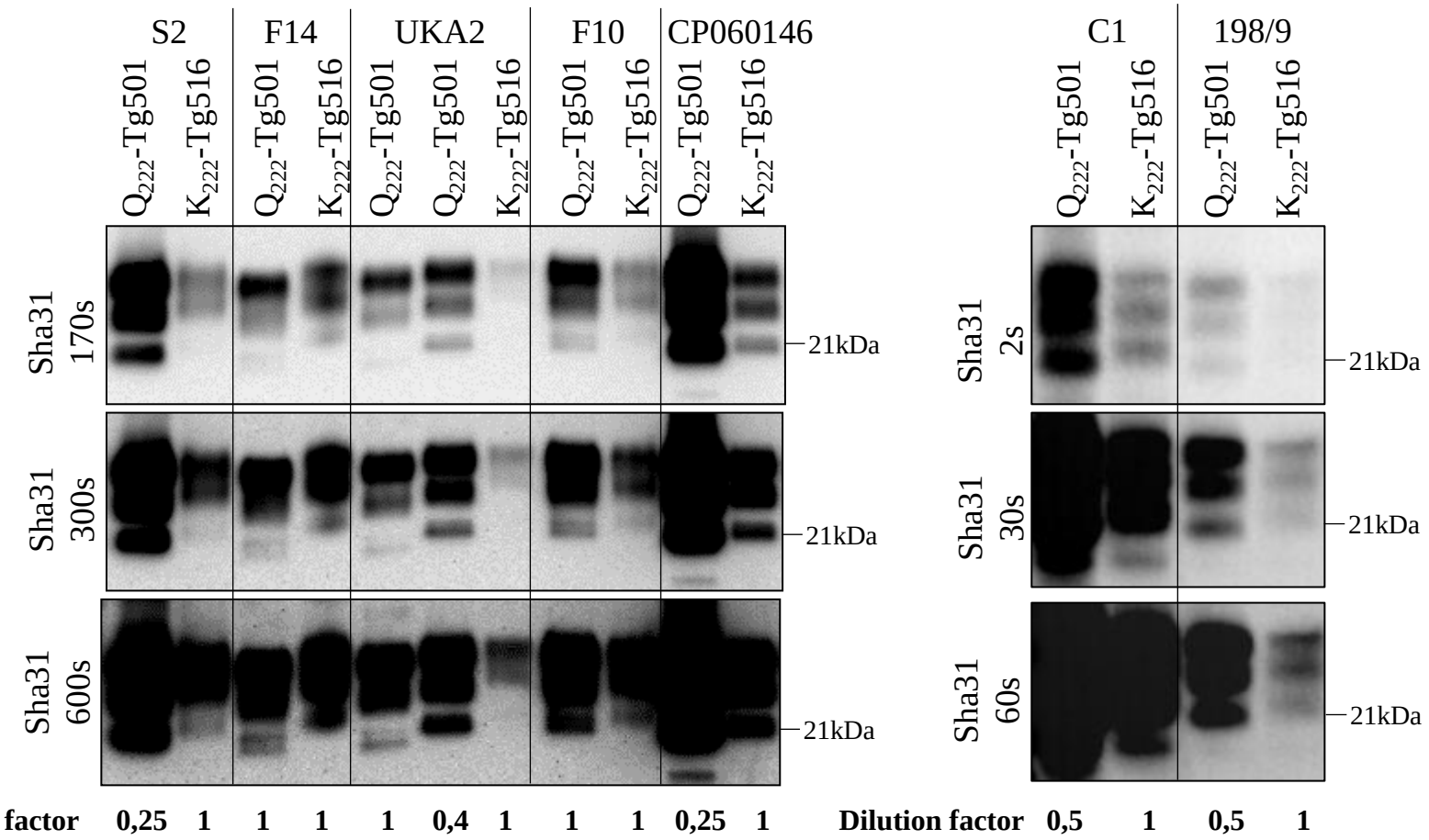


CP060146/K_{222}-Tg516



The isolates when replicating in either Q222 or K222-PrP^C contexts, retain the **same proteinase K sensitivity**.
Real **low brain PrP^{res}** accumulation in **K_{222}-Tg516.**

RESULTS– Biochemical patterns

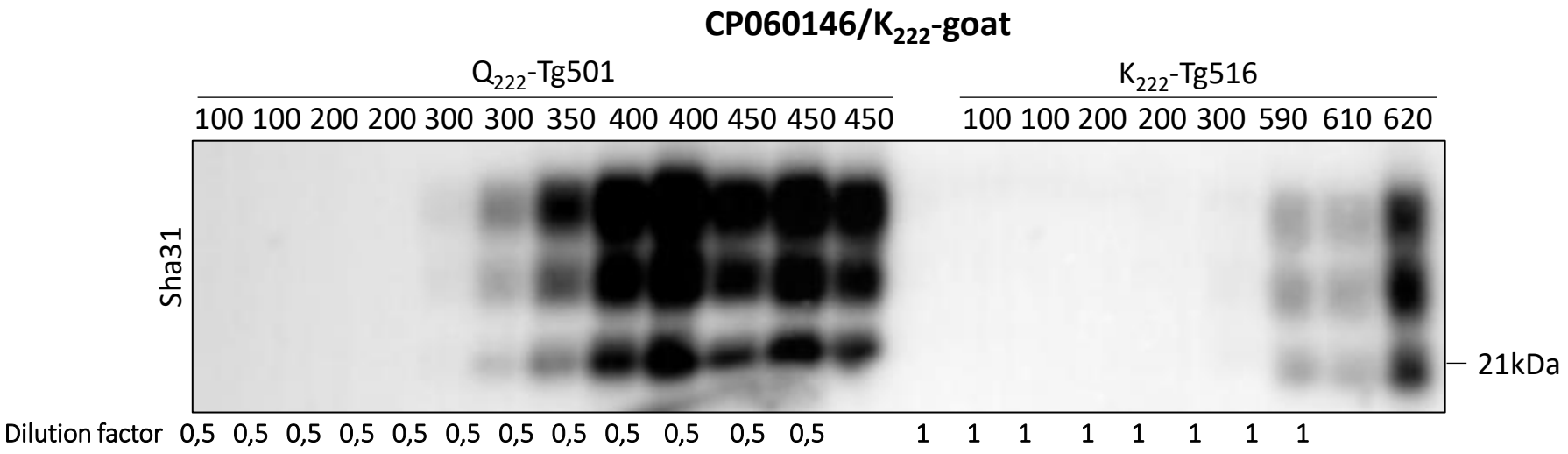


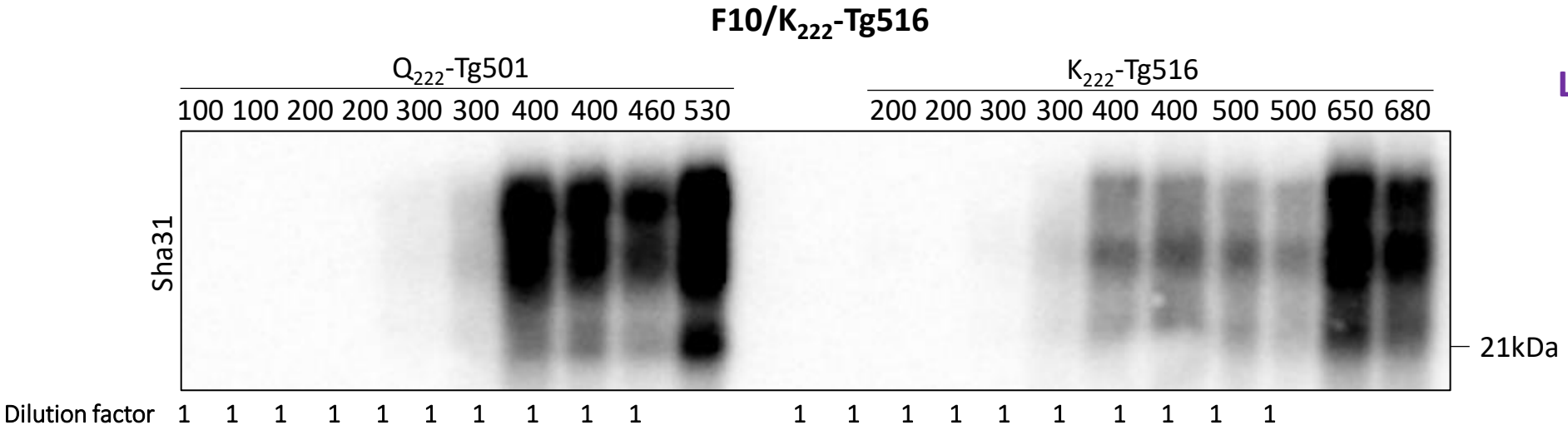
Slight **higher molecular mass (independent** on the **prion strain)**

Lower PrP^{res} accumulation in K_{222}-Tg516

- ~~PK sensitivity~~
- Less PrP^{res}
  - Few accumulation
  - Late accumulation

RESULTS– Kinetics of PrP^{res} accumulation

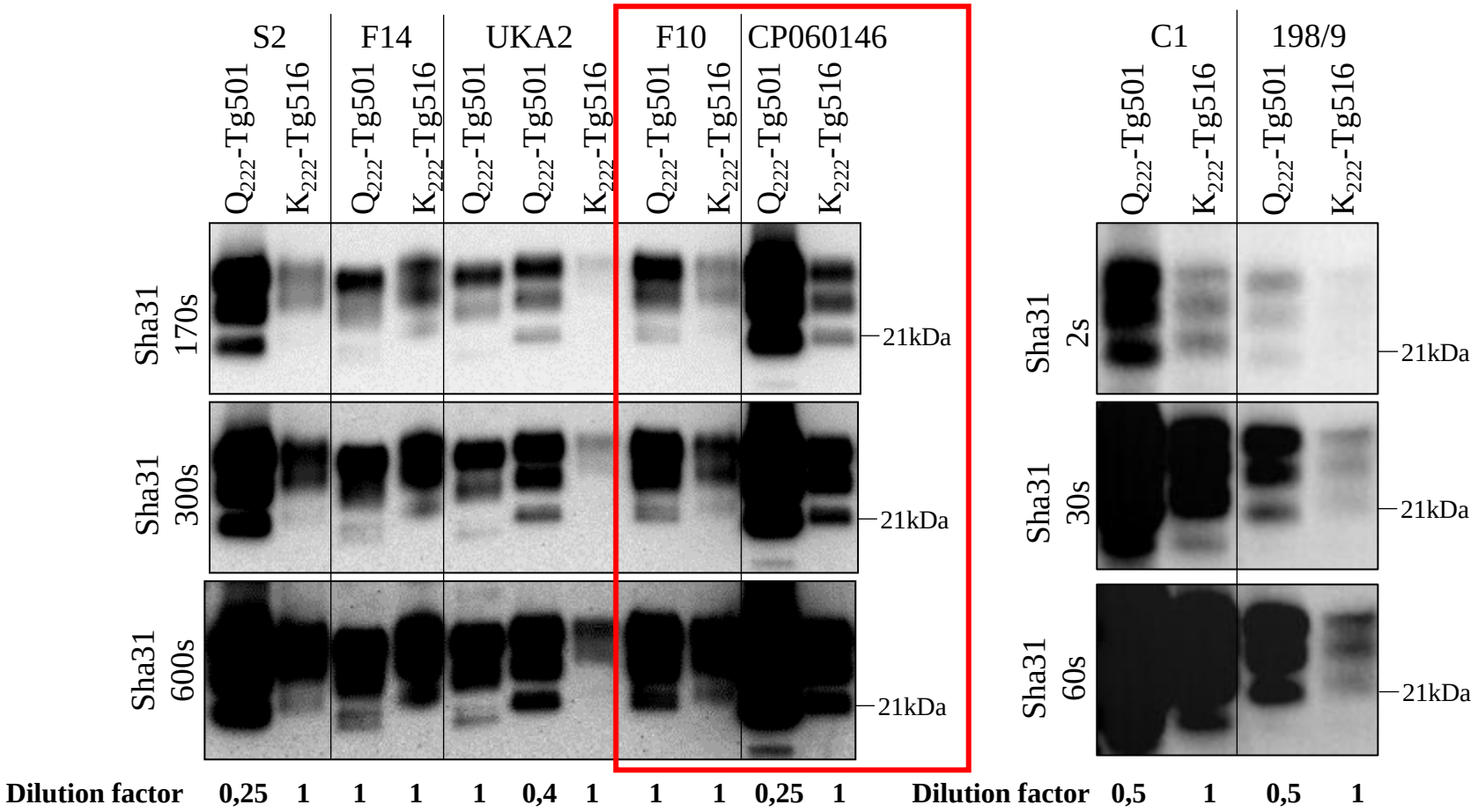




Both K_{222} and Q_{222}-PrP^{res} appeared at **equal levels** by 300 dpi. **Q_{222}-PrP^{res}** accumulation coninued to **increase**, while **K_{222}-PrP^{res}** remained at **low** levels.

Low replication rates in K_{222}-Tg516 mice **and/or** high **efficient clearance** of PrP^{res} aggregates.

RESULTS– Biochemical patterns

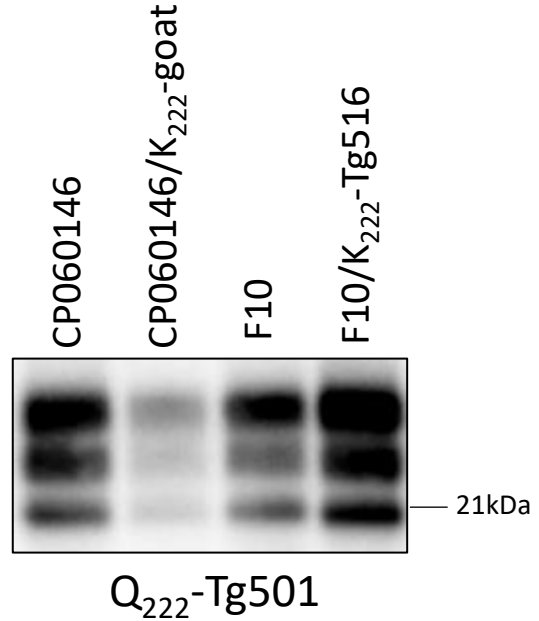


Slight **higher molecular mass (independent** on the **prion strain)**

Lower PrP^{res} accumulation in K_{222}-Tg516

RESULTS– Strain features

Category	Isolate	Mean survival time (days) ± SD[a] (n/n0)[b]			
		Q_{222}-Tg501		K_{222}-Tg516	
		1st passage	2nd passage	1st passage	2nd passage
I (Italians)	198/9	592±13 (6/6)	536±46 (5/5)	>650 (1/6)[c]	ND[d]
II	S2	228±15 (6/6)	233±4 (6/6)	>650 (3/4)[c]	>650 (7/7)[c]
	CP060146	379±31 (5/5)	ND[d]	>650 (5/5)[c]	>650 (5/5)[c]
	CP060146/K_{222} goat	415± 40 (6/6)	ND[d]	>650 (4/4)[c]	>650 (6/6)[c]
II + III	UKA2	245±36 (5/5)	252±8 (6/6)	>650 (4/4)[c]	>650 (5/5)[c]
	F14	526±46 (4/4)	241±22 (4/4)	>650 (4/4)[c]	>650 (5/5)[c]
IV	F10	449±19 (5/5)	372±14 (6/6)	>650 (6/6)[c]	>650 (5/5)[c]
	F10/K_{222}-Tg516	495±26 (3/3)	ND[d]	>650 (5/5)[c]	>650 (6/6)[c]
	C1	483±15 (4/4)	301±10 (4/4)	>650 (7/7)[c]	ND[d]
Negative control	Healthy goat brain	>650 (0/6)	>650 (0/6)	>650 (0/6)	>650 (0/6)



[a] Mean survival time indicated in days ± standard deviation.

[b] n/n_0, number of diseased, PrP^{res}-positive animals/number of inoculated animals.

[c] Animals found dead or sacrificed at the end of their lifespan without showing clinical signs.

[d] Not done.

High infectivity of K_{222}-PrP^{Sc} derived prions

Non-permanent shifts in prion strains

In conclusion– Take home messages

- The K_{222}-PrP^C variant is capable of **sustaining PrP^{Sc} replication** even in the absence of the Q_{222}-PrP^C variant. However, K_{222}-Tg516 mice inoculated with classical scrapie **did not develop typical prion disease** properties:

  - Biologically: No clinical signs
  - Biochemically: Low PrP^{res} accumulation and changes in strain features
  - Histologically: No histopathological alterations - indistinguishable from aging

  … however this mice are **as infectious as** Q_{222}-Tg501 mice.

- This study suggests that prions propagated under the K_{222} allele can act as **potential silent carriers** of the disease. In this context, K_{222} homozygous goats may act as **reservoirs** for Q/K_{222} and Q_{222} goat infection (implications in breeding selection programs).

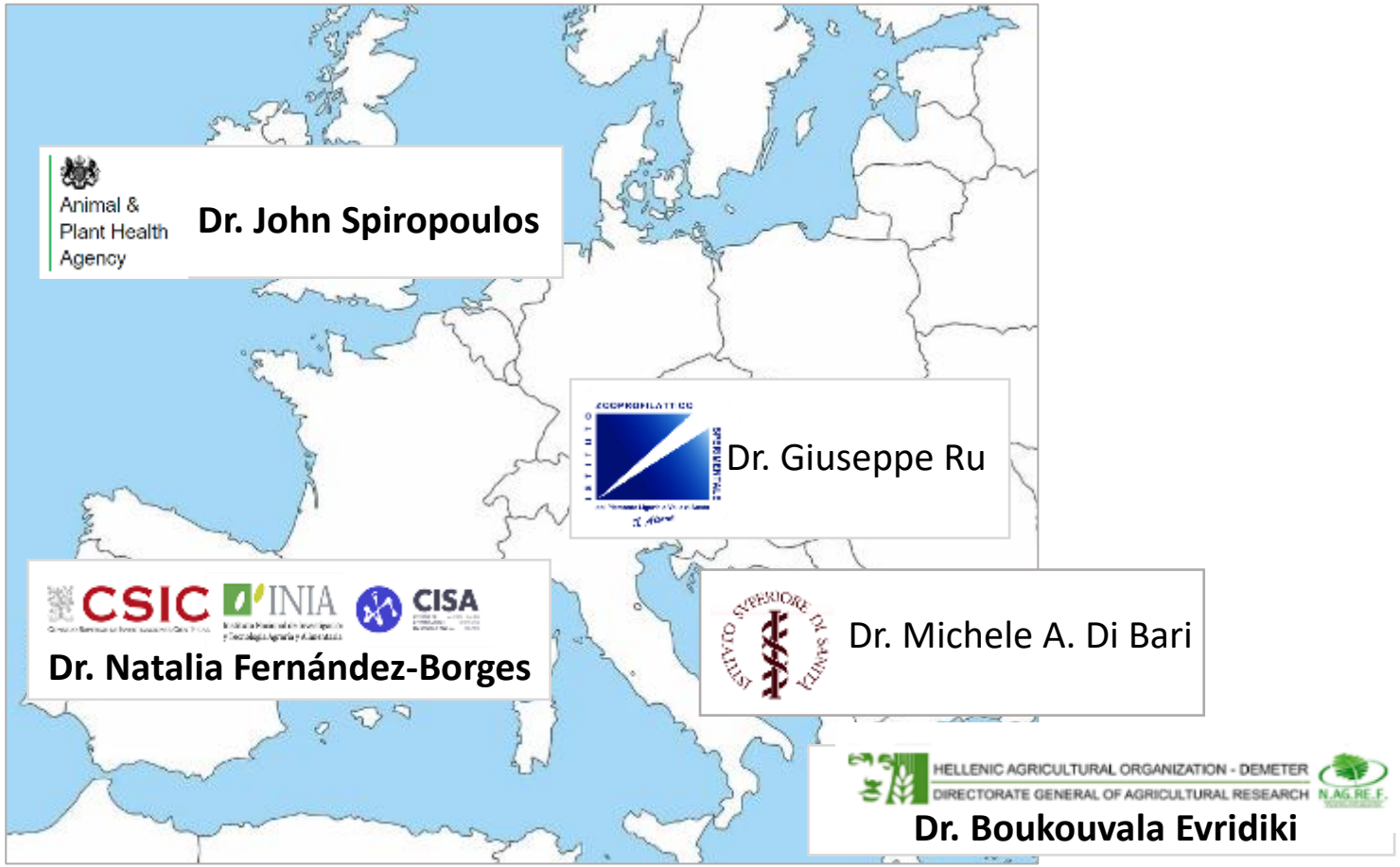
- Understanding whether the presence of the K_{222} allele induces a **change in the biological properties** of the **strains** and their potential **transmission** to other animal species is crucial.

To be continued...

icrad
International coordination of research on infectious animal diseases

Classical scrapie in genetically resistant goats: questioning current concepts and policies

ScResGoats



Transgenic Mouse Models for *In Vivo* Modeling of Protective Effects of *PRNP* Polymorphic Variants

Strain	Wt-Tg501	R_{171}-Tg552	K_{176}-Tg570
Atypical scrapie	++	++	++
Classical scrapie I	++	-	-
Classical scrapie II	++	-	+
Classical scrapie III	++	-	-
Classical scrapie IV	++	-	+
Bovine Spongiform Encephalopathy	++	+	-

Susceptibility	
++	High
+	Limited
-	Low

The Journal of Infectious Diseases

MAJOR ARTICLE

Infectious Diseases Society of America
HIV Medicine Association

OXFORD

Strain-Dependent Susceptibility to Prion Infection Encoded by Arg_{171} and Lys_{176} Sheep Prion Protein Polymorphic Variants

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- The polymorphic variants studied show susceptibility for atypical scrapie.
- Resistance to classical scrapie observed in mice expressing 171R or 176K polymorphic variants. In the case of 176K, the resistance depends on the classical scrapie prion strain inoculated. When infected, the survival times of the mice are prolonged.
- 176K polymorphic variant confers resistance to BSE strain.

Transgenic Mouse Models for *In Vivo* Modeling of Protective Effects of *PRNP* Polymorphic Variants

Strain	Wt-Tg501	R_{171}-Tg552	K_{176}-Tg570	T_{137}-Tg592
Atypical scrapie	++	++	++	?
Classical scrapie I	++	-	-	?
Classical scrapie II	++	-	+	?
Classical scrapie III	++	-	-	?
Classical scrapie IV	++	-	+	?
Bovine Spongiform Encephalopathy	++	+	-	?

Susceptibility	
++	High
+	Limited
-	Low

- Classical scrapie Iceland previosuly selected isolates are being tested in Wt-Tg501 and T_{137}-Tg592.

ICRAD Projec Scice: Classical Scrapie in Iceland, a model for prion diseases worldwide

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