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Supplementary Information for

The emergence of classical BSE from atypical/Nor98 scrapie

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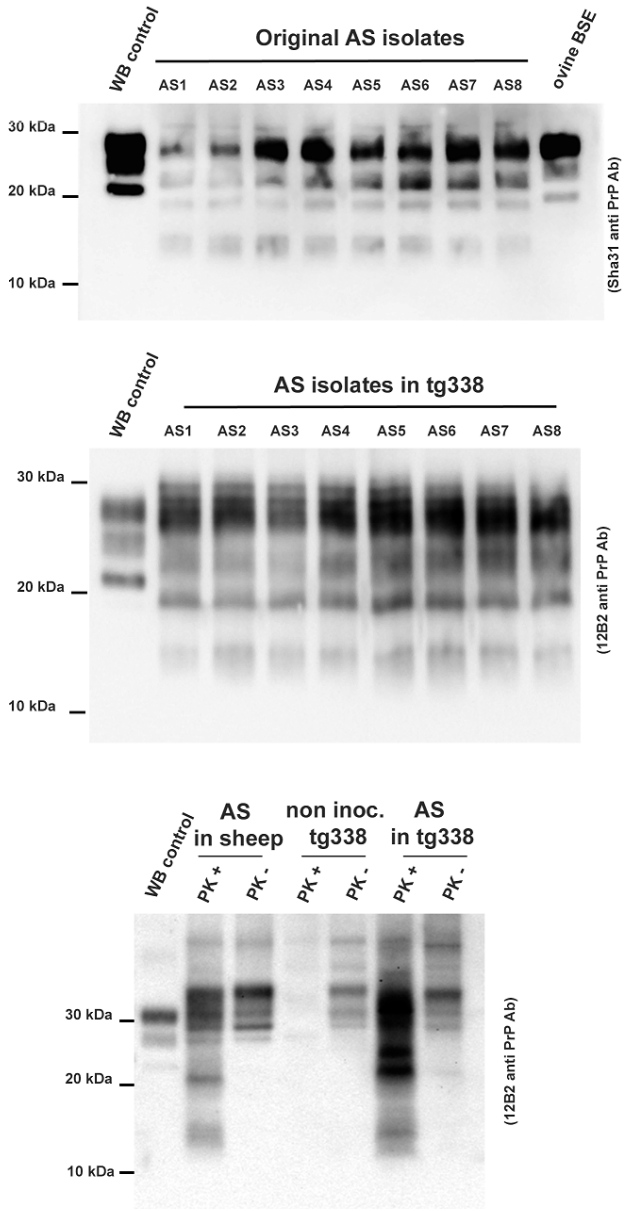

Fig. S1. PrP^{res} western blot profiles in tg338 mice inoculated with atypical/Nor98 scrapie (AS)

Groups of mice (n≥6) that express ovine VRQ PrP (tg338 mice) were intra-cerebrally challenged with atypical scrapie isolates (AS).

The accumulation of PK-resistant PrP (PrP^{res}) in the original AS isolates and in the brain of inoculated mice was established by western blot using anti PrP monoclonal antibodies Sha31 (epitope 145-YEDRYYRE-152) or 12B2 (epitope 89-WGQGG-93). The same western blot PrP^{res} control (classical scrapie isolate) was used on all the gels labelled as WB control.

PrP signal in PK digested / undigested AS isolates and negative control (sheep brain: 2 mg brain tissue equivalent per lane, tg338: 0.2 mg brain tissue equivalent per lane) shows the specificity of Western blot banding pattern observed in AS isolates.

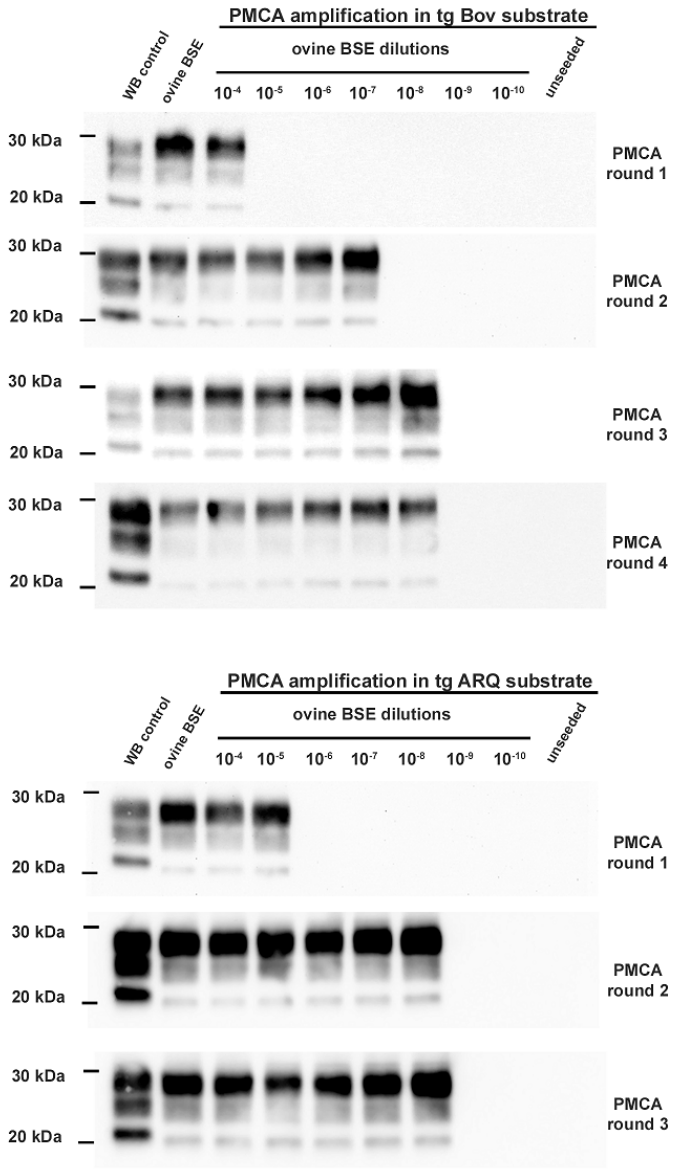

Fig. S2. PMCA amplification of ovine BSE agent

PMCA reactions were seeded with a 1/10 dilution series of a reference ovine BSE brain homogenate (10% weight / volume -10^{-2} to 10^{-10} dilution). This homogenate has been endpoint titrated by bioassay in bovine PrP expressing mice (tgBov, intracerebral route – $10^{7.2}$ LD_{50}/g).

PMCA substrate was prepared using brains from transgenic mice over-expressing the bovine prion protein (tgBov) or the ARQ variant of the sheep prion protein (tgARQ). Unseeded reactions were included as specificity controls. PMCA reactions were then submitted to three to four amplification rounds each comprising 96 cycles (10s sonication-14 minutes and 50 seconds incubation at 39.5°C) in a Qsonica700. After each round, (i) reaction products (1 volume) were mixed with fresh substrate (9 volumes) to seed the following round while (ii) a part of the same product was analysed by Western Blot (WB) for the presence of abnormal PK resistant PrP (PrP^{res} -antibody Sha31 epitope YEDRYYRE). On each gel a scrapie in sheep isolate was used as control (WB control).

Table S1: Inoculation of atypical scrapie isolates in ovine PrP (tg338) expressing mice

Isolates			Tg338					
			1st passage		2nd passage		3rd passage	
Identifiant	Origin	Genotype	Positive mice	Incubation (mean±SD)	Positive mice	Incubation (mean±SD)	Positive mice	Incubation (mean±SD)
AS 1	Fr	ARQ*/ARQ	6/6	250±18	6/6	232±13	6/6	212±9
AS 2	Sp	ARR/ARQ	12/12	243±15	6/6	217±15	ND	
AS 3	Sp	ARQ/ARH	12/12	239±15	12/12	229±12	ND	
AS 4	Nor	ARQ*/ARQ*	5/5	235±12	ND			
AS 5	Sp	ARQ/ARQ	5/5	186±11	5/5	250±16	6/6	217±14
AS 6	Sp	ARQ/ARH	4/4	226±10	ND			
AS 7†	It	ARQ/AHQ	6/6	228±11	ND			
AS 8	Po	ARQ/ARQ	6/6	207±11	ND			
PS42	Fr	VRQ/VRQ	6/6	71±2	6/6	62±1	6/6	61±1
Ovine c-BSE	Fr	ARQ/ARQ	6/6	663±94	6/6	224±36	6/6	134±2
Cattle c-BSE	Fr	-	6/6‡	>700	6/6	682±52	6/6	136±5

Transgenic mice that express the ovine PrP VRQ variant (tg338) were inoculated intra-cerebrally (6 to 12 mice, 20µL per mouse) with 8 sheep or goat (†) atypical scrapie (AS) isolates originating from five different countries; France (Fr), Spain (Sp), Norway (Nor), Italy (It) or Portugal (Po). The AS affected animals displayed a different *PRNP* genotype at codons 136, 154 and 171. Some also displayed a F/L dimorphism at codon 141 (*). Cattle classical BSE (c-BSE), ovine c-BSE (first passage of cattle c-BSE in an ARQ/ARQ sheep by the intracerebral route) and classical scrapie (PS42) isolates were inoculated in the same mouse model. Mice were considered positive when abnormal PrP deposition was detected in the brain. (‡) indicate abnormal PrP positive and found dead animals (without symptoms). Incubation periods (in days) are shown as mean±SD. ND: not done.

Table S2: End point titration of BSE in sheep reference isolate by bioassay in bovine PrP expressing mice (tgBov) and Protein Misfolding Cyclic Amplification

	Bioassay tgBov		PMCA positive reactions	
Sheep passaged c-BSE isolate	**Positive mice**	**Incubation period (days ± SD)**	**TgBov substrate**	**TgARQ Substrate**
Neat	6/6	223±4	ND	ND
10^{-1}	6/6	250±9	ND	ND
10^{-2}	6/6	290±12	12/12	12/12
10^{-3}	6/6	338±18	12/12	12/12
10^{-4}	6/6	386±38	12/12	12/12
10^{-5}	5/6	486±96	12/12	12/12
10^{-6}	1/6	402*	12/12	12/12
10^{-7}	0/6	>700	9/12	10/12
10^{-8}	0/6	>700	6/12	5/12
10^{-9}	ND		0/12	1/12
10^{-10}	ND		0/12	0/12
10^{-11}	ND		0/12	0/12

A 10% w/vol homogenate was prepared using brain stem from a clinically affected sheep (ARQ/ARQ genotype) inoculated with BSE. Groups of 6 tgBov mice were inoculated intra-cerebrally with 20µL of serial ten-fold dilutions of this homogenate. Mice were considered positive when PK resistant PrP (PrP^{res}) deposition was detected in the brain (western blot). Incubation periods (in days) are presented as mean±SD, except for those marked (*) indicating dilutions in which less than half of the mice were scored as positive. The same dilution series was used to seed PMCA reactions (5µL per reaction). Twelve individual replicates of each sample dilution were tested. Two different PMCA substrates were used. The first one was prepared using brains from transgenic mice over-expressing the ARQ variant of the sheep prion protein (tgARQ). The second was prepared using brains from transgenic mice over-expressing the bovine prion protein (tgBov). Reactions were then subjected to 3 amplification rounds. After each round, reaction products (1 volume) were mixed with fresh substrate (9 volumes) to seed the following round. PMCA reaction products (third amplification round) were analysed by western blot for the presence of PrP^{res}. The number of PrP^{res} western blot positive reactions / total number of reactions are reported.

Table S3: Atypical scrapie cases end-point titration in tg338 mice

Dilution	AS 25		AS 26	
	Positive mice	**Incubation period (mean±SD)**	**Positive mice**	**Incubation period (mean±SD)**
Neat	7/7	224±10	6/6	219±4
10^{-1}	ND		ND	
10^{-2}	ND		ND	
10^{-3}	ND		ND	
10^{-4}	6/6	294±41	6/6	272±23
10^{-5}	6/6	329±34	6/6	315±51
10^{-6}	3/6	360, 392, 412*	2/6	368, 451*
10^{-7}	0/6	>650	0/6	>650
10^{-8}	0/6	>650	0/6	>650
Infectious titer (ID_{50} IC tg338/g)	$10^{8.7}$		$10^{8.5}$	

A 10% w/vol homogenate was prepared using brains from two AS affected sheep. Groups of tg338 mice ($n \geq 6$) were inoculated intra-cerebrally with 20µL of serial ten-fold dilutions of these homogenates. Mice were considered positive when PK resistant PrP (PrP^{res}) deposition was detected in the brain by western blot. Incubation periods (in days) are presented as mean±SD, except for those marked (*) indicating dilutions in which less than half of the mice were scored as positive. Infectious titers (ID_{50} / gram of brain tissue) were estimated by the Spearman-Karber's method.