資料４－１

めん羊及び山羊における BSEの感染実験に関する知見

経口投与によるBSE実験感染めん羊の知見

組織におけるPrP^{Sc}の分布と感染性　2,3,4,5,6,7,8

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#595

Transmission of bovine spongiform encephalopathy to sheep and goats

The Veterinary Record, October 2, 1993

J. D. Foster, J. Hope, H. Fraser

TABLE 1: Experimental transmission of BSE to sheep and goats with details of incubation periods and histological and PrP^{Sc} diagnosis

Recipient animal		Route	Signs observed days after challenge	Histological diagnosis	PrP^{Sc} protein
Sheep 'Positive line'	1	ic	724	+	+
	2	ic	880	‡	+
	3	ic	61†	-	-
	4,5	ic	Alive*	-	-
	6	Oral	538	+	+
	7	Oral	994	+	+
	8	Oral	665†	-	-
	9,10,11	Oral	Alive	-	-
'Negative line'	1	ic	440	‡	+
	2	ic	487	+	+
	3	ic	883†	-	-
	4,5,6	ic	Alive*	-	-
	7	Oral	734	+	+
	8	Oral	620†	-	-
	9,10,11,12	Oral	Alive	-	-
Goats	1	ic	506	+	+
	2	ic	509	+	+
	3	ic	570	+	+
	4	Oral	941	+	+
	5	Oral	1501	+	+
	6	Oral	Alive*	-	-

ic Intracerebral
* Up to 1720 days after challenge
† Intercurrent death
‡ Uncertain histology because of autolysis

136VV

136AA

Goat:
ic: 4-6 yrs old
Oral: 2-5 yrs old

Sheep: 6-18 months
ic: 0.5ml of 10% homogenates
oral: 50 ml of 1% homogenates

i.c: 2/5

oral: 2/6

i.c: 2/5

oral:1/6

i.c: 3/3

oral: 2/3

Positive line (VV)とnegative line (AA)でBSEの感受性は変わらない

#597

Detection of BSE infectivity in brain and spleen of experimentally infected sheep

J. D. Foster, M. Bruce, I. McConnell, A. Chree, H. Fraser

Veterinary Record (1996) **138,** 546-548

136AA
171QQ 羊の潜伏期

440 days

734 days

接種経路に関わらず
感染羊の脳、脾に
BSEプリオン蓄積

TABLE 1: Incubation periods (days ± sem) in mice following transmission of brain and spleen from two clinically affected 'negative' line sheep, one of which had been challenged by intracerebral (ic) inoculation and the other by orally drenching with BSE homogenates. These are compared with pooled transmission data from four cows confirmed to have BSE and one sheep with natural scrapie

Source of infection	Mouse strain RIII	C57BL	VM	IM	C57BLxVM
BSE sheep (ic)					
Brain	297 ± 3	408 ± 9	446 ± 10	478 ± 9	662 ± 13
Spleen	334 ± 5	477 ± 13	510 ± 12	ND	695 ± 13
BSE sheep (oral)					
Brain	328 ± 4	431 ± 8	550 ± 10	583 ± 11	771 ± 22
Spleen	355 ± 7	441 ± 9	583 ± 18	ND	732 ± 39
BSE cow*					
Brain	322 ± 2	425 ± 3	497 ± 5	551 ± 4	745 ± 15
Natural scrapie sheep (greyface)					
Brain	386 ± 10	404 ± 5	769 ± 16	815 ± 23	610 ± 8
Spleen	399 ± 10	ND	ND	ND	ND

ND Not done
* Pooled data from four transmissions (BSE 1 to 4 given as individual transmissions in Bruce and others 1994). Pooled homogenate from these brains was used to challenge the sheep in this study

脾臓の潜伏期：やや長い
Lower level of infectivity in spleen

Lesion profile同じ
BSE羊→マウス
BSE→マウスは類似
スクレイピーとは明瞭に異なる

#596

Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission

Journal of General Virology (2001), **82**, 2319–2326.

J. D. Foster, D. W. Parnham, N. Hunter and M. Bruce

Table 1. Details of seven BSE-challenged end-point sheep with PrP genotype, incubation period and clinical signs

The incubation periods are days post-challenge and clinical duration refers to the length of time the animal was observed with ataxia and/or pruritis. Also, details are shown of six BSE-challenged sheep which are still alive.

Animal no.	PrP genotype	*Incubation period (days)*/days post-challenge	Clinical duration (days)	Ataxia	Pruritis
1	AHQ/AHQ	*553*	2	++*	–
2	AHQ/AHQ	*593*	4	++	±
●3	ARQ/ARQ	*596*	2	++	±
4	AHQ/AHQ	*629*	19	++	±
●5	ARQ/ARQ	*761*	1	++*	–
6	AHQ/AHQ	*935*	17	++	–
●7	ARQ/ARQ	*1073*	3†	++	–
8	ARQ/ARQ	1631	Still alive	–	–
9	AHQ/AHQ	1631	Still alive	–	–
10	ARQ/AHQ	1631	Still alive	–	–
11	AXQ/AXQ	1333	Still alive	–	–
12	ARQ/AHQ	1333	Still alive	–	–
13	ARQ/ARQ	1333	Still alive	–	–

*Recumbent within 24 h of clinical signs first being observed.
†Appeared lean 6 weeks prior to culling.

5g brain equivalent (=10^4 mouse ic ID_{50}/g)
7-10 months: 7, 23 months: 2, 46 months: 2,
58 months: 2

Positive sheep: 7/13 (553-1,073 days)
● ARQ/ARQ

投与時期、遺伝子型PrP154R/Hとは関連なし
A number of genetically susceptible, challenged animals appear to have survived.

Table 2. PrP immunostaining in BSE-dosed terminally affected sheep

ND, Not done; –, negative; (+), marginal staining; +, definite staining; ++, strong staining; PP, Peyer's patch.

Animal no.: Sex/incubation period (days):	1 F 553	2 M 593	● 3 F 596	4 F 629	● 5 F 761	6 F 935	● 7 F 1073
Brain	++	++	++	++	++	++	++
Spinal cord							
Cervical	ND	ND	+	+	+	++	++
Thoracic	++	++	++	++	++	ND	++
Lumbar	ND	ND	++	++	++	ND	+/++
Dorsal root ganglia							
Cervical	ND	ND	–	–	–	ND	+
Thoracic	ND	ND	–	–	(+)	ND	–
Lumbar	ND	ND	ND	–	(+)	ND	–
Retina	ND	ND	ND	ND	ND	ND	(+)
Vagus nerve	ND	–	(+)	–	+	–	(+)
Radial nerve	–	–	–	–	–	–	–
Sciatic nerve	–	–	–	–	–	–	–
Coeliac ganglia	ND	ND	ND	+	ND	ND	+
Stellate ganglia	ND	ND	ND	–	ND	ND	–
Nodose ganglia	ND	ND	ND	–	ND	ND	ND
Lung	ND	–	ND	–	–	–	–
Heart	ND	–	–	–	–	–	–
Kidney	–	–	–	–	–	–	(+)
Liver	–	–	ND	–	–	–	–
Spleen	ND	–	(+)	ND	(+)	(+)	+
Thymus	ND	ND	–	–	–	–	–
Nictitating membrane	ND	ND	–	–	(+)	ND	+
Tonsil	++	++	+	++	++	++	++
Peyer's patches	ND	ND	+	+	ND	+	+
Retropharyngeal lymph node	+	+	ND	++	++	++	+
Submandibular lymph node	+	+	+	+	+	++	(+)
Mammary lymph node	ND	ND	(+)	++	(+)	+	+
Bronchial-mediastinal lymph node	+	–	+	+	+	(+)	ND
Prefemoral lymph node	ND	ND	(+)	+	+	+	(+)
Mesenteric lymph node	+	(+)	+	+	+	+	+
Ileo-caecal lymph node	++	+	ND	++	ND	ND	++
Prescapular lymph node	+	(+)	+	+	+	+	(+)
Hepatic lymph node	ND	ND	ND	ND	ND	++	+
Parotid salivary gland	ND	ND	ND	ND	ND	ND	–
Submandibular salivary gland	ND	ND	ND	ND	ND	ND	–
Rumen	–	–	–	–	–	–	–
Omasum	ND	–	–	–	–	–	–
Abomasum	–	–	–	–	–	–	–
Duodenum	(+)PP	–	(+)	–	(+)	+PP	+
Jejenum (35%)	ND	ND	ND	(+)	(+)	(+)	(+)
Jejenum (70%)	ND	ND	ND	(+)	(+)	(+)PP	(+)
Distal ileum	–	–	+PP	(+)	(+)	(+)	+PP
Caecum	ND	ND	ND	ND	ND	+PP	+PP
Pancreas	ND	ND	ND	ND	ND	–	–
Uterus	–	ND	–	–	–	–	–
Ovary	–	ND	–	–	–	–	–
Skin	ND	ND	ND	ND	–	–	–
Semitendinosus muscle	ND	ND	ND	–	ND	ND	–
Longissimus dorsi muscle	ND	ND	ND	–	ND	–	–
Diaphragmatic muscle	ND	ND	ND	–	ND	–	–
Foetal brain/spleen	ND	ND	ND	ND	– (1 mth)	ND	–
Cotyledon	ND	ND	ND	ND	–	ND	–

CNS,末梢神経の一部、ほとんどのリンパ節に蓄積。

#518

Oral Inoculation of Sheep with the Agent of Bovine Spongiform Encephalopathy (BSE). 1. Onset and Distribution of Disease-specific PrP Accumulation in Brain and Viscera

M. Jeffrey, S. Ryder*, S. Martin, S. A. C. Hawkins*, L. Terry*, C. Berthelin-Baker* and S. J. Bellworthy*

J. Comp. Path. 2001, Vol. 124, 280–289
doi:10.1053/jcpa.2001.0465, available online at http://www.idealibrary.com

21 sheep (ARQ/ARQ)
6 months old

Table 1
Clinical onset of disease and accumulation of disease-specific PrP in the central nervous system (CNS) and viscera of inoculated $PrP^{ARQ/ARQ}$ sheep

Sheep no.	*Time (months) between inoculation and*		*Presence of visceral PrP*	*Presence of CNS*	
	necropsy	*clinical onset*		*vacuolation*	*PrP*
1	4	N	+/−	−	−
2	4	N	−	−	−
3	4	N	− 1/4	− 0/4	− 0/4
4	4	N	−	−	−
5	10	N	−	−	−
6	10	N	+/−	−	−
7	10	N	− 1/4	− 0/4	− 0/4
8	10	N	−	−	−
9	16	N	−	−	−
10	16	N	−	−	−
11	16	N	+/− 2/4	− 0/4	+/− 2/4
12	16	N	+	−	+/−
13	22	N	−	−	+/−
14	22	N	−	−	+/−
15	22	21	+ 3/5	+ 2/5	+ 5/5
16	22	20	+	+	+
17	22	N	+	+	+
18	24	24	+	+	+
19	27	27	+ 3/3	+ 3/3	+ 3/3
20	28	28	+	+	+
21*	…	…	…	…	…

ARQ/ARR:21　24mpiまで
ARR/ARR:21　PrP^{Sc}陰性

Table 2
Distribution of disease-specific PrP in lymph nodes, tonsil, spleen and alimentary tract of inoculated $PrP^{ARQ/ARQ}$ sheep

Sheep no.	*Months between inoculation and necropsy*	*Presence of PrP in*										
		lymph nodes						*tonsil* 扁桃	*spleen* 脾臓	*intestine* 小腸	*abomasum* 第4胃	*forestomachs* 前胃
		SM 下顎	*RP* 浅頚	*PS* 膝下	*MES* 腸間膜	*IC* 回盲	*MED* 縦隔					
1–8	4–10	−	−*	−	−	−	−	−	−	−	−	−
9	16	−	−	−	−	−	−	−	−	−	−	−
10	16	−	−	−	−	−	−	−	−	−	−	−
11	16	−	−	−	−	−	−	−	+	+	ND	−
12	16	+	+	+	+	+	+	+	+	+§	ND	−
13	22	−	−	−	−	−	−	−	−	−	−	−
14	22	−	−	−	−	−	−	−	−	+‡	−	−
15	22	+	+	+	+	+	+	+	+	+	−	+
16	22	+	+	+	+	+	+	+	+	+§	+	+
17	22	+	+	+	+	+	+	+	+	+	+	+
18	25	+	+	+	+	+	+	+	+	+	+	+
19	28	+	+	+	+	+	+	+	+	+	+	+
20	29	+	+	+	+	+	+	+	+	+	+	+
21†	…	…	…	…	…	…	…	…	…	…	…	…

Control sheep nos 22, 23, 24 and 25 were killed and examined 4, 10, 16 and 22 months, respectively, after the other 21 animals had been inoculated; the results were entirely negative.
SM, submandibular; RP, retropharyngeal; PS, prescapular, MES, mesenteric; IC, ileocaecal; MED, mediastinal; ND, not done.
* RP lymph nodes positive in sheep nos 1 and 6.
† Sheep 21 was alive but clinically sick 36 months after inoculation.
‡ Immunolabelling confined to a single enteric neuron.
§ Peyer's patches not represented in tissue block.

PrP^{Sc}はリンパ節、消化管に蓄積

Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats

J. D. Foster, D. Parnham, A. Chong, W. Goldmann, N. Hunter

The **Veterinary Record**, February 10, 2001

Incubation period of BSE associate with PrP171

「年齢と伝達率に関連はない」と仮定：羊のageは若くない

141の遺伝子型は考慮されていない

TABLE 1: Transmissions of BSE to Neuropathogenesis Unit Cheviot sheep and goats

	Clinical signs	Pathology	WB/icc for PrP	Incubation period (days)	Not TSE (days after challenge)	Age at challenge (days)	PrP genotype	
Sheep								
IC-1	+	+	+/+	440	–	446	AHQ/AHQ	
IC-2	+	–*	+/+	487	–	462	AHQ/ARQ	short
IC-3	+	+	ND/+	880	–	1220	VRQ/ARQ	medium
IC-4	+	+	ND/+	1874	–	141	VRQ/ARR	
IC-5	?	+	ND/+	1886	–	458	ARQ/ARR	
IC-6	+	+	+/+	1923	–	489	ARQ/ARR	very long
IC-7	?	+*	+/+	2353	–	456	AHQ/ARR	
IC-8	–	–	–/–	–	878	1543	ARQ/ARR	
IC-9	–	–	ND/–	–	2379	807	VRQ/ARR	resistant
OR-1	+	+	ND/+	734	–	486	AHQ/ARQ	
OR-2	?	+*	–/+	1945	–	119	VRQ/ARR	
OR-3	–	–	ND/–	–	640	141	ND	
OR-4	–	–	ND/–	–	756	485	AHQ/ARR	
OR-5	–	–	ND/–	–	2155	300	ARQ/ARR	
OR-6	–	–	ND/–	–	2350	490	ARQ/ARQ	
OR-7	–	–	ND/–	–	2364	485	ARQ/ARQ	
OR-8	–	–	ND/–	–	2970	114	VRQ/ARR	
OR-9	–	–	ND/–	–	3191	481	ARQ/ARR	
OR-10	–	–	ND/–	–	3191	116	ARQ/ARR	
Goats							RPTS 142 143 240	
IC-10	+	+	+/+	506	–	447	5/5 II HH PP	
IC-11	+	+	+/+	509	–	2273	5/5 II HH PS	
IC-12	+	+	+/+	570	–	1210	5/5 II HH PS	
OR-11	+	+	–/–	941	–	1181	5/5 II HH PS	
OR-12	+	+	ND/+	1501	–	1912	5/5 II HH SS	
OR-13	–	–*	–/–	–	1765	853	5/5 II HH PS	

0.05g brain: IC-1 to IC-9 — 7/9

0.5g brain: OR-1 to OR-10 — 2/10

Goats: IC-10 to IC-12 — 3/3; OR-11 to OR-13 — 1/3

* Autolysis-affected tissue, IC Intracerebral, OR Oral, + Positive, ? Uncertain, – Negative, ND Not done, WB Western blot, icc Immunocytochemistry, Not TSE Intercurrent death for reasons other than TSE, RPTS Octapeptide repeat number

Goat
142: I/M, 143: H/R,
240: S/P

Tissue distribution of bovine spongiform encephalopathy infectivity in Romney sheep up to the onset of clinical disease after oral challenge

S. J. Bellworthy, S. A. C. Hawkins, R. B. Green, I. Blamire, G. Dexter, I. Dexter, R. Lockey, M. Jeffrey, S. Ryder, C. Berthelin-Baker, M. M. Simmons

The Veterinary Record, February 12, 2005

Romney sheep
6 months old, ARQ/ARQ, ARQ/ARR, ARR/ARR

mpi	ARQ	ARQ/R	ARR
4	4		
10	4	4	4
16	4		
22	5	4	3
28–38	4*		
34		4	4
46		4	4
120		4	4

* Clinical stage

ARQ/R, ARRの動物からはPrPSc検出されず

LRS(-)でCNS(+)の個体もある

TABLE 3: Summary of the results of immunohistochemistry (IHC) and mouse bioassay for the ARQ/ARQ Romney sheep and the numbers of clinical cases

Interval after dosing (months)	Disease-specific PrP (IHC)	RIII mouse bioassay	Clinical cases
4	Retropharyngeal lymph node + (1/4)	Peyer's patch +	None
10	Retropharyngeal lymph node + (1/4)	Spleen +	None
16	LRS, viscera* and CNS + (2/4)	Spleen, spinal cord, distal ileum, Peyer's patch, liver, prescapular lymph node, mesenteric lymph node, cervical thymus and tonsils all +†	None
22	LRS, viscera and CNS + (2/5) CNS only + (2/5)	Spleen, spinal cord, caudal medulla, distal ileum, Peyer's patch, liver, prescapular lymph node, mesenteric lymph node, coeliac mesenteric ganglion and tonsils all +	Two sheep clinically positive, 661 and 664 dpi
Final group	LRS, viscera and CNS + (4/4)	Spleen, spinal cord, distal ileum, Peyer's patch, liver, prescapular lymph node, mesenteric lymph node all + (data incomplete)	Four sheep clinically positive, 775, 872, 908 and 1162 dpi

* Lymphoreticular system (LRS) and viscera include the retropharyngeal, mesenteric, prescapular, ileocaecal and bronchomediastinal lymph nodes, tonsil, spleen, Peyer's patches and abomasum; the animals indicated were positive for all or most of these tissues
† Not all the tissues were assessed by both histopathology and bioassay
+ Positive, CNS Central nervous system, dpi Days postinfection,

ARQ/ARQ羊はBSEに経口感染する。
PrP^{Sc}の分布、動態はスクレイピーと同じ
PrP^{Sc}の蓄積に先立って感染性が検出される

Liver: low level of infectivity

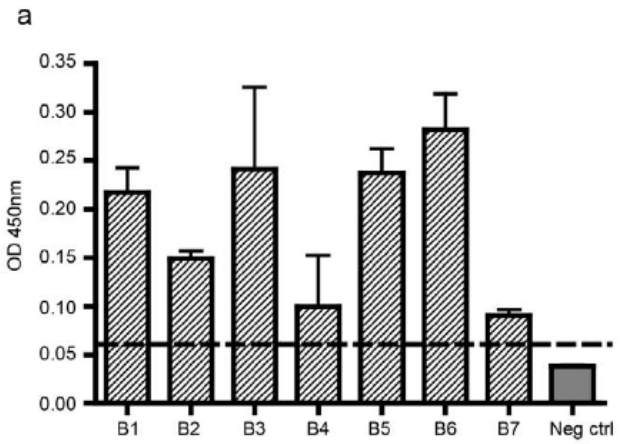
Detection and Localisation of PrP^{Sc} in the Liver of Sheep Infected with Scrapie and Bovine Spongiform Encephalopathy

PLoS ONE | www.plosone.org
May 2011 | Volume 6 | Issue 5 | e19737

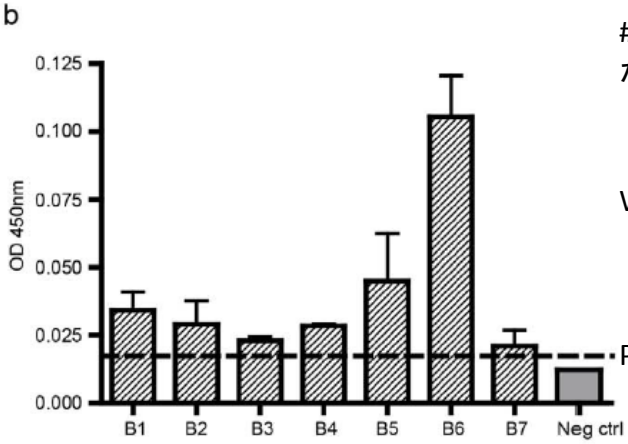
Sally J. Everest[1], Andrew M. Ramsay[1], Melanie J. Chaplin[1], Sharon Everitt[1], Michael J. Stack[1], Michael H. Neale[1]*, Martin Jeffrey[2], S. Jo Moore[1], Susan J. Bellworthy[1], Linda A. Terry[1]

ARQ/ARQ Suffolk

Oral challenge at 6 months of age



IDEXX ELISA kit



Bio-Rad ELISA kit

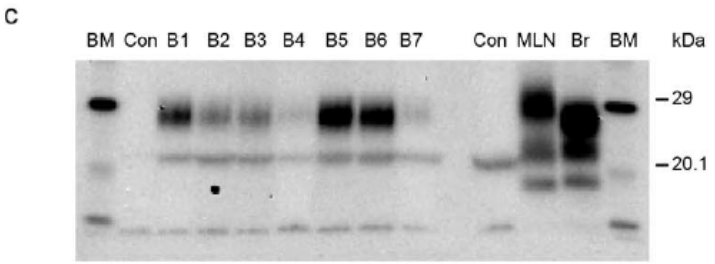
#79で肝臓に感染性が確認された

↓

WBとIHCでPrP^{Sc}検出

↓

PrP^{Sc}はKupffer cellsに蓄積



age (months)	numbers	Clinical signs	Lymphoid tissues
27	3	None observed	positive
29-30	2	Early positive	positive

Figure 2. Detection of PrP^{Sc} in the livers of ARQ/ARQ BSE-challenged ewes. Liver samples from 7 (B1–B7) ARQ/ARQ BSE-challenged ewes and 15 negative control sheep (neg ctrl) were analysed for the presence of PrP^{Sc} using (a) the IDEXX Herdchek assay (b) the Bio-Rad TeSeE ELISA and (c) the Bio-Rad TeSeE sheep and goat Western blot. Values are shown as the mean and standard deviations (n = 4). The cut-off points of the assays are shown (dotted line). M = molecular mass markers (kDa). Mesenteric lymph node (MLN) and brain (Br) from confirmed scrapie positive sheep are shown as positive controls. Liver from a negative sheep (C) is also shown.
doi:10.1371/journal.pone.0019737.g002

Susceptibility of Young Sheep to Oral Infection with Bovine Spongiform Encephalopathy Decreases Significantly after Weaning

Nora Hunter,[a] Fiona Houston,[b] James Foster,[a] Wilfred Goldmann,[a] Dawn Drummond,[a] David Parnham,[a] Iain Kennedy,[a] Andrew Green,[a] Paula Stewart,[a] and Angela Chong[a]

Journal of Virology p. 11856–11862
November 2012 Volume 86 Number 21

TABLE 1 Incubation periods and survival times of sheep orally challenged with BSE at a range of ages

Age at challenge	Dose of BSE (g)	BSE cases/no. challenged	Incubation period (days[a] [SD])	Survival of non-BSE sheep[b] for <2,000 days (no. if >3)	Survival of non-BSE sheep[b] for >2,000 days (no. if >3)
~24 h	0.05	8/9	865 (202)	524	NA[c]
	0.1	4/7	774 (212)	317, 335, 964	NA
	0.5	6/11	1,044 (215)	39–672 ($n = 5$)	NA
	1.0	7/10	685 (164)	9, 135, 150	NA
2–3 wk	0.05	11/12	740 (162)	104	NA
	0.5	11/12	949 (189)	NA	2,263
	1.0	12/12	745 (207)	NA	NA
3 mo	0.05	0/10	NA	8, 193	2,121–2,237 ($n = 8$)
	0.5	0/10	NA	8, 989, 1,890	2,172–2,179 ($n = 7$)
	1.0	0/10	NA	217–1,381 ($n = 4$)	2,143 ($n = 6$)
6 mo	0.05	0/10	NA	519, 1,273	2,021–2,062 ($n = 8$)
	0.5	3/10	729, 742, 932	922	2,021–2,062 ($n = 6$)
	1.0	4/10	821 (183)	682	2,062 ($n = 5$)
15–27 mo	0.05	1/10	2,289	1,214, 1,227	2,039–2,290 ($n = 7$)
	0.5	2/10	594, 849	1,723	2,191–2,304 ($n = 7$)
	1.0	1/10	930	1,010,[d] 1,359	2,290–2,297 ($n = 7$)

[a] If fewer than four cases, individual incubation periods or survival times are presented (days after infection); the survival rates for four or more animals are given as a range.
[b] Animals culled for intercurrent illness/welfare reasons or at the end of the experiment.
[c] NA, not applicable.
[d] One animal with PrP^{Sc}-positive staining in Peyer's patches with no other BSE signs.

High incidence

Doseと潜伏期に関連性なし

感染した羊の結果をプールした

これまでの実験で主に使われていた月齢

すべての結果を141の遺伝子型で分類
↓
PrP141はBSEの潜伏期に関与
↓
LL < FF < LF

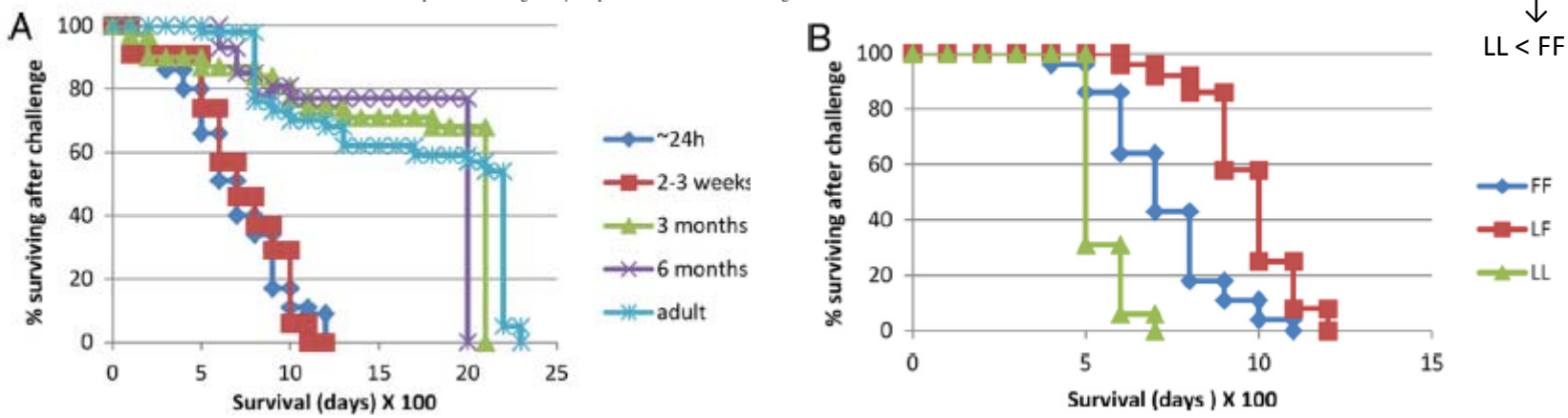


FIG 3 Survival times of sheep challenged with BSE. (A) Sheep of different age groups challenged with BSE; (B) sheep of different *PRNP* genotypes (codon 141) challenged with BSE.

仮説:
PrP^{Sc}の消化管内環境での生存
PrP^{Sc}の消化管からの取り込み
} 幼若＞成熟動物

#800

Maternal transmission studies of BSE in sheep

J. D. Foster, W. Goldmann, C. McKenzie, A. Smith, D. W. Parnham and N. Hunter

Journal of General Virology (2004), **85**, 3159–3163

Progeny surviving to >30 months and born to challenged ewes

	ewes numbers	PrP genotype	incubation periods (months)	Progeny, year of birth					Lamb numbers of each genotype		
				Born 1997	Born 1998	Born 1999	Born 2000	Born 2001	AQ/AQ	AQ/AR	AR/AR
that developed clinical signs of BSE	4	AQ/AQ	18, 31, 36, 58	1	4	5	2	1	9	4	0
that did not develop clinical signs of BSE			Fate, challenged in 1997							n=13	
	4	AQ/AQ	3 alive, 1 culled at 61 mpi	1	2	5	1	1	9	1	0
	6	AQ/AR	5 alive, 1 culled at 51 mpi	7	4	3	5	4	11	11	1
	6	AR/AR	5 alive, 1 culled at 75 mpi	4	5	6	5	6	0	20	6
	Total			12	11	14	11	11	20	32	7
										n=59	
control	4	AQ/AQ	2 alive, 2 culled at 48, 72 mpi	3	5	1	4	1	11	3	0
										n=14	

20頭のCheviot ewes　に5gのBSEを経口感染
4頭の非感染羊

♂AQ/AQまたはAQ/ARと交配して、子羊生産(72頭)

13頭は臨床症状羊の子羊、59頭は未発症羊からの子羊

すべての子羊で30ヶ月齢まで発症は認められなかった

BSEの羊→羊の垂直感染は確認されなかった。

Natural transmission of BSE between sheep within an experimental flock

S. J. Bellworthy, G. Dexter, M. Stack, M. Chaplin, S. A. C. Hawkins, M. M. Simmons, *Veterinary Laboratories Agency (VLA) – Weybridge, Addlestone, Surrey KT15 3NB*
M. Jeffrey, S. Martin, L. Gonzalez, *VLA – Lasswade, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ*
P. Hill, *ADAS DEFRA Drayton, Alcester Road, Stratford-upon-Avon, Warwickshire CV37 9RQ*

The **Veterinary Record**, August 13, 2005

6-months old
ARQ/ARQ ewe lambs (n=30)
Infected with 5g BSE brain (orally)

↓

6 months after
BSE-dosed sheep were mixed with 20 matched animals (same genotype, same age), and kept as a single group.

↓

The ewes were bred from 18 months of age by natural mating (breed/genotype-matched sires from TSE-free flock)

↓

24/30 of dosed sheep reached clinical stage between 655 and 1056 dpi

↓

2 of the lambs born in 2003 also died of BSE

The first clinical disease in the flock occurred in a dosed ewe, the dam of lamb 2, just 73 days after the birth of its lamb, with clinical end point at 655 days after dosing.
The dam of lamb 1 reached clinical end point 198 days after it birth.

It is impossible to determine whether infection was acquired from the dam in utero or during the perinatal period.

This is the first confirmation that BSE can transmit either in utero or perinatally in sheep.

Immunohistochemical Distinction between Preclinical Bovine Spongiform Encephalopathy and Scrapie Infection in Sheep

C. M. A. Thuring, L. J. M. van Keulen*, J. P. M. Langeveld*, M. E. W. Vromans*, F. G. van Zijderveld* and T. Sweeney

J. Comp. Path. 2005, Vol. 132, 59–69

リンパ濾胞が少なくinconclusive

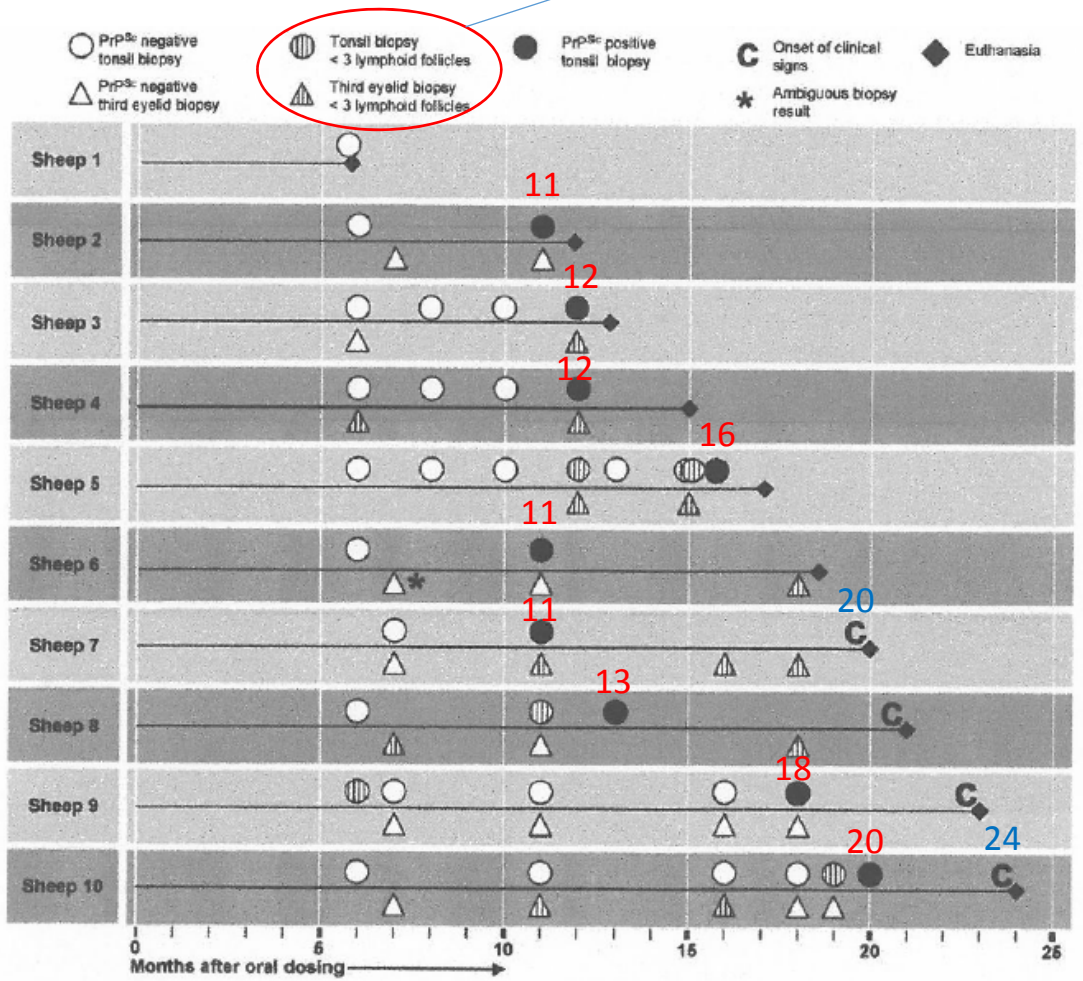
5g BSE orally
ARQ/ARQ: 10
ARR/ARR: 1
(43mpiまで陰性)

4-5 months of age

6ヶ月間隔で扁桃のバイオプシー
↓
11-20mpiで陽性

第三眼瞼:陰性

PrP93-106に対する抗体は扁桃のTBM (tangible body macrophage)に沈着したPrPScの染色性でBSEとスクレイピーを区別できる



1: 6mpiで採材 扁桃陰性

5: preclinical stage 扁桃陽性

4: clinical stage 扁桃陽性

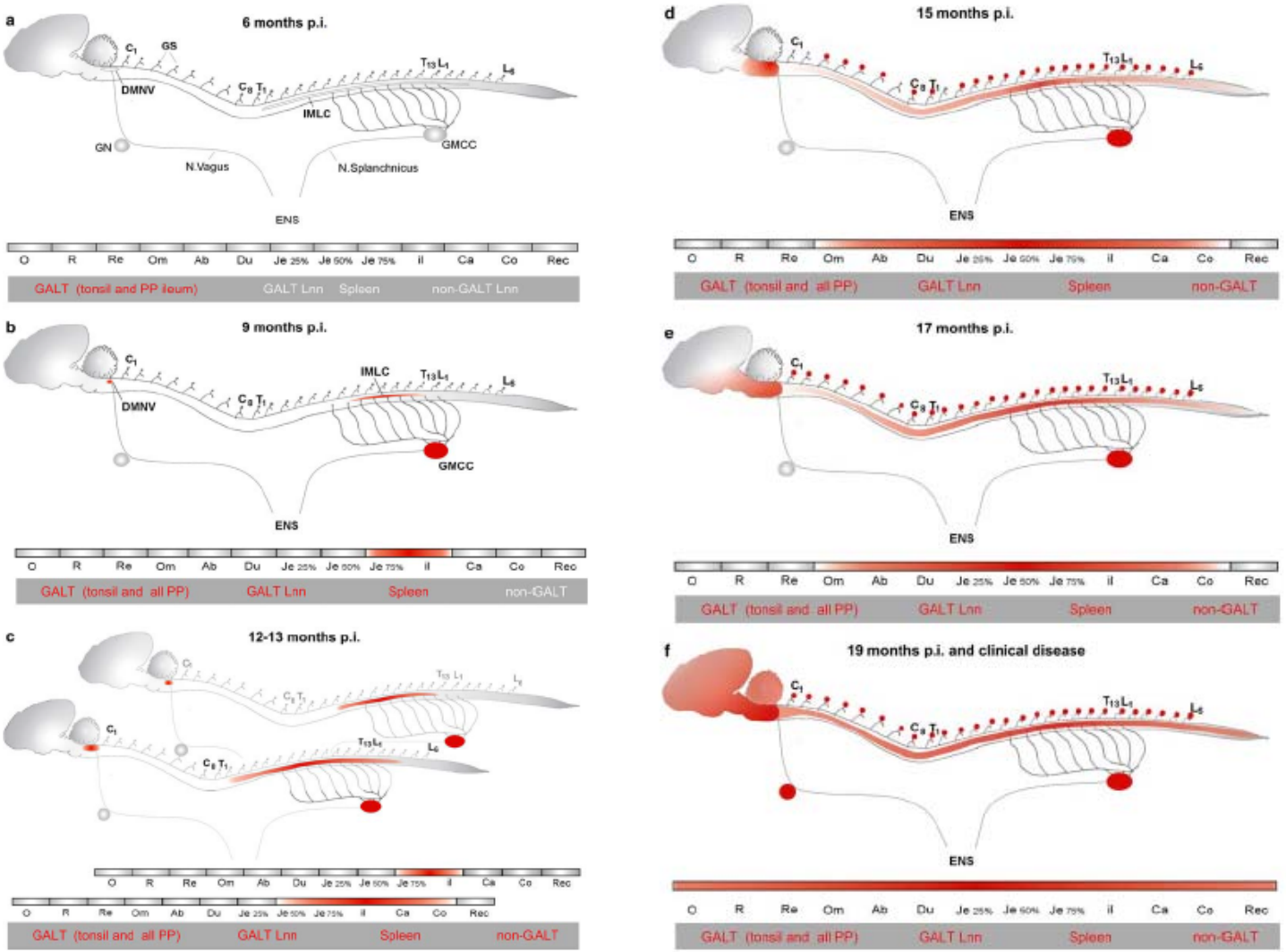
Fig. 2. Detection of PrP^{Sc} in tonsil and third eyelid biopsy samples of homozygous ARQ sheep orally infected with BSE.

Pathogenesis of bovine spongiform encephalopathy in sheep

Arch Virol (2008) 153:445–453

L. J. M. van Keulen · M. E. W. Vromans ·
C. H. Dolstra · A. Bossers · F. G. van Zijderveld

Fig. 1 Schematic representation of PrP^{Sc} accumulation (in *red*) in the lymphoid tissues, ENS and CNS of ARQ/ARQ sheep orally infected with BSE after **a** 6 months **b** 9 months **c** 12–13 months **d** 15 months **e** 17 months and **f** 19 months and after the onset of clinical disease. Abbreviations: *DMNV* dorsal motor nucleus of the vagus; *IMLC* intermediolateral column; *C*, *T* and *L* cervical, thoracic and lumbal segments of the spinal cord; *GN* ganglion nodosus; *GMCC* ganglion mesentericum cranialis/ coeliacum; *GS* ganglia spinalis; *O* oesophagus; *R* rumen; *Re* reticulum; *Om* omasum; *Ab* abomasum; *Du* duodenum; *Je* jejunum; *Il* ileum; *Ca* caecum; *Co* colon; *Rec* rectum; *GALT* gut-associated lymphoid tissues; *GALT Lnn* lymph nodes draining from *GALT* tissues. Only the efferent motor fibers of the autonomic nervous system are shown



ARQ/ARQ:11
ARR/ARR:1 (72mpi)
4-5 months old
5g BSE, oral

Enteric nerves system --> parasympathetic & sympathetic nerves --> medulla oblongata --> spinal cord

#422

Phenotype of disease-associated PrP accumulation in the brain of bovine spongiform encephalopathy experimentally infected sheep

Journal of General Virology (2005), **86**, 827–838

Lorenzo González,[1] Stuart Martin,[1] Fiona E. Houston,[2] Nora Hunter,[3] Hugh W. Reid,[4] Sue J. Bellworthy[5] and Martin Jeffrey[1]

AHQ/AHQに経口投与でBSEが伝達する

ARR/ARRに、脳内接種ではBSEが伝達

Table 1. Animals used for the study, incubation period of the disease and magnitude of total PrP^d accumulation

For description of genotypes, see text.

Group	Breed	Genotype	Age*	Inoculum			Number	Clinical course*	Incubation period*	PrP^d score§
				Source	Type†	Route‡				
1	Cheviot	ARQ/ARQ	736±41	Cattle	Brain[a]	IC	5	9±2·5	609±16	17·6±0·6
2	Poll-Dorset	ARQ/ARQ	768±50	Cattle	Brain[a]	IC	4	2±0·4	499±2	15·6±1·2
3	Suffolk	ARQ/ARQ	1110	Cattle	Brain[a]	IC	5	19±16·4	550±13	15·2±1·1
4	Romney	ARQ/ARQ	365	Sheep	Brain[b]	IC	13‖	14±2	586±32	14·0±0·5
5	Cheviot	VRQ/VRQ	226±32	Cattle	Brain[a]	IC	5	22±7·8	1068±9	16·7±0·7
6	Cheviot	ARR/ARR	680±179	Cattle	Brain[a]	IC	5	68±17	1333±86	6·1±0·6
	Suffolk	ARR/ARR		Cattle	Brain[a]	IC	2			
	Poll-Dorset	ARR/ARR		Cattle	Brain[a]	IC	1			
7	Cheviot	AHQ/AHQ	288±6	Cattle	Brain[c]	OR	3	11±4·7	626±21	7·9±0·9
8	Romney	ARQ/ARQ	182	Cattle	Brain[d]	OR	4	15±5·1	923±75	10·3±1·4
9	Poll-Dorset	ARQ/ARQ	204±22	Sheep	Brain[e]	OR	2	20±5·9	736±20	12·3±1·1
	Suffolk	ARQ/ARQ		Sheep	Brain[e]	OR	3			
	Romney	ARQ/ARQ		Sheep	Brain[e]	OR	1			
10	Cheviot	ARQ/ARQ	273±34	Sheep	Blood[f]	IV	5	14±5·1	566±16	9·8±0·7
11	Cheviot	ARQ/AHQ	397±13	Cattle	Brain[a]	IV	6	56±11·4	715±18	5·8±0·8

*Age at challenge, clinical course (from first signs to cull) and incubation period (from challenge to cull) in days (mean±SEM).

†The different inocula are identified by different superscripts: *a*, *c*, *d*, brain pools of BSE-affected cattle titrated IC/IP in RIII mice (titres were $10^{2\cdot4}$, $10^{3\cdot5}$ and $10^{2\cdot2}$ LD50 g^{-1}, respectively); *b*, brain pool of Cheviot sheep (titre in RIII mice of $10^{4\cdot7}$ LD50 g^{-1}) that developed clinical TSE after oral challenge with cattle BSE inoculum; *e*, brain pool from Romney sheep (titration pending) that developed clinical disease after being dosed orally with inoculum *d*; *f*, non-titrated individual blood aliquots (either 400–450 ml whole blood or buffy coat extracted from 50 ml whole blood) from sheep of group 7.

‡IC, Intracerebral; IV, intravenous; OR, oral (5 g inoculum as a 10 % homogenate).

§Mean±SEM of individual sheep scores (see text).

‖This group of sheep comprised five, five and three animals challenged with 10^{-3}, 10^{-4} and 10^{-5} dilutions of inoculum *b*, respectively.

Bovine spongiform encephalopathy agent in spleen from an ARR/ARR orally exposed sheep

Journal of General Virology (2006), **87**, 1043–1046

Olivier Andréoletti,[1] Nathalie Morel,[2] Caroline Lacroux,[1] Virginie Rouillon,[1] Céline Barc,[3] Guillaume Tabouret,[1] Pierre Sarradin,[3] Patricia Berthon,[3] Philippe Bernardet,[3] Jacinthe Mathey,[1] Séverine Lugan,[1] Pierrette Costes,[1] Fabien Corbière,[1] Juan-Carlos Espinosa,[4] Juan Maria Torres,[4] Jacques Grassi,[2] François Schelcher[1] and Frédéric Lantier[3]

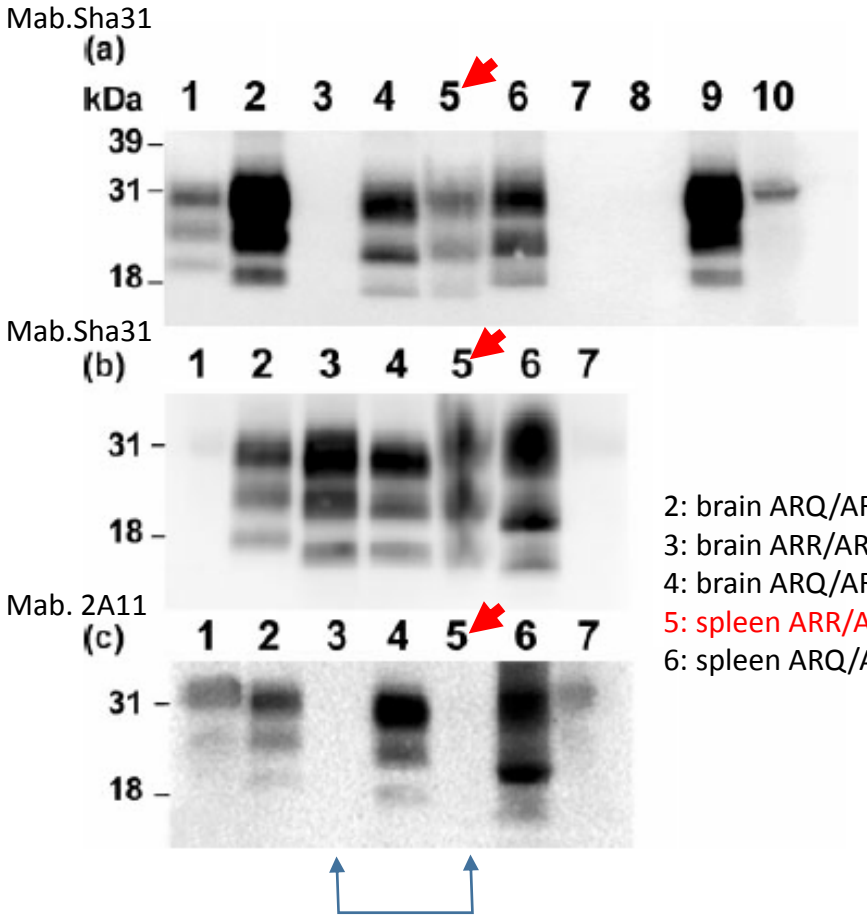
BSEはicでARR/ARR羊に感染するが、これまでoralによる伝達は確認されていない
* n=14と少数例であった
* 6ヶ月齢の羊を使用していた

ARQ/ARQ (n=8)
ARR/ARR (n=10)

24 hr-old, and 14 days of age

2.5g BSE sheep (ARQ/ARQ) brain (oral)

mpi	ARQ/ARQ	ARR/ARR
4	3	3
10	3	3
19	2 (clinical)	
29		4 (healthy)



1: brain ARQ/ARQ, scrapie
2: brain ARQ/ARQ BSE
3: brain ARQ/ARQ, negative
4: spleen ARQ/ARQ BSE (oral)
5: spleen ARR/ARR BSE (oral)
6: spleen ARQ/ARQ, scrapie
7: spleen ARQ/ARQ negative
9: brain ARQ/ARQ BSE

2: brain ARQ/ARQ, scrapie
3: brain ARR/ARR BSE (ic)
4: brain ARQ/ARQ, BSE (ic)
5: spleen ARR/ARR BSE (oral) (10 mpi)
6: spleen ARQ/ARQ BSE (oral)

10mpiの1頭の脾臓のみ。他の組織は陰性。
他のARR羊9頭は、すべて陰性

PrP^{Sc}: ARR allelic variant product of prion replication

#415

Protective effect of the T112 PrP variant in sheep challenged with bovine spongiform encephalopathy

G. C. Saunders,[1] I. Lantier,[2] S. Cawthraw,[1] P. Berthon,[2] S. J. Moore,[3] M. E. Arnold,[4] O. Windl,[1] M. M. Simmons,[3] O. Andréoletti,[5] S. Bellworthy[3] and F. Lantier[2]

Journal of General Virology (2009), 90, 2569–2574

oral: 12-30 weeks, 0-260 weeks
ic: 22-35 weeks, 39-91 weeks

ARQ/ARQはBSEに高感受性（oral, ic)
しかし、いくつかの実験ではBSEにresistantの個体もみられた

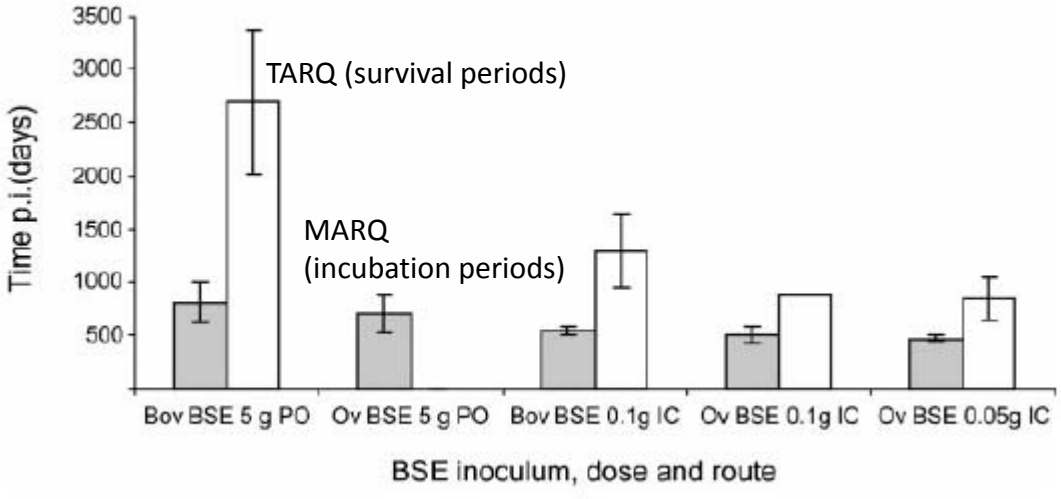


Fig. 1. Mean incubation periods for BSE-positive and clinically affected MARQ/MARQ Suffolk sheep (shaded bars) and survival periods (as at March 2009) of BSE-dosed TARQ-carrying sheep (empty bars). Error bars represent ± 1SD (n=17, 7, 2, 3, 3 for BSE-positive and n=3, 0, 3, 1, 6 sheep for survivors in the respective challenge groups from left to right).

MARQ/MRQ	MARQ/TARQ	TARQ/TARQ	Total
47/49	0/17	0/3	47/69

T112はBSE感染に対してresistantに働く
これまでの実験の中でARQ/ARQで感染しない個体が認められた理由の一つか？

#708

Effect of Q_{211} and K_{222} *PRNP* Polymorphic Variants in the Susceptibility of Goats to Oral Infection With Goat Bovine Spongiform Encephalopathy

J Infect Dis 2015 Aug 26;212(4):664-72. Epub 2015 Feb 26.

Patricia Aguilar-Calvo, Christine Fast, Kerstin Tauscher, Juan-Carlos Espinosa, Martin H. Groschup, Muhammad Nadeem, Wilfred Goldmann, Jan Langeveld, Alex Bossers, Olivier Andreoletti, and Juan-María Torres

Transmission of goat BSE is genotype dependent.
K222: protective effect in the oral susceptibility of goat BSE

6-7 months of age
Goat BSE

Wt (R211, Q222)

R/Q211

Clinical sign
Delayed clinical signs in goat

Q/K222

No clinical signs in goat

Table 2. Infectious Titers in Tissues From WT, R/Q_{211}, and Q/K_{222} *PRNP* Goats Orally Inoculated With Goat BSE, as Determined by Mouse Bioassay

PRNP Genotype and Goat Codes for Pooled Tissues	End Point, mo After Inoculation	Goat Tissue	Mouse Bioassay in BoPrP-Tg110 Mice: Survival Time, Mean (SEM)	No. Diseased (PrP^{res} positive)/No. Inoculated	Infectious Titer[a]
WT[b]					
ZG26, ZG32, and ZG35	6	Brain	650	0/6	–
		Psoas major muscle	650	0/6	–
		Popliteal LNs	650	0/6	–
ZG19, ZG24, and ZG30	12	Brain	650	0/6	–
		Psoas major muscle	650	0/6	–
		Popliteal LNs	650	0/6	–
ZG01	24	Brain +	261 (20)	6/6	+++
		Psoas major muscle -	596, 598	2/6	–
		Retractor bulbi muscle -	469 (29)	3/6	–
		Popliteal LNs -	526	1/6	–
R/Q_{211}					
ZG13	24	Brain -	650	0/6	–
ZG28, ZG05, and ZG20	33, 34, and 36	Brain + + +	258 (15)	6/6	+++
		Psoas major muscle - - -	334 (24)	6/6	++
		Retractor bulbi muscle - - -	307 (84)	5/5	++
		Popliteal LNs - - -	406 (50)	2/6	+
Q/K_{222}					
ZG10	24	Brain -	650	0/6	–
ZG25	44	Brain -	650	0/6	–
		Psoas major muscle -	550	1/6	–
		Popliteal LNs -	650	0/6	–
ZG11	45	Brain -	400 (50)	6/6	+
		Psoas major muscle -	522	1/6	–
		Popliteal LNs -	650	0/6	–

Abbreviations: BSE, bovine spongiform encephalopathy; LNs, lymph nodes; PrP^{res}, protease-resistant prion protein; SEM, standard error of the mean; WT, wild-type.

[a] The infectious titer for each goat tissue was calculated as a function of the survival times obtained in BoPrP-Tg110 mice after their inoculation and expressed as infectious dose (ID) per gram of inoculated tissue (see Supplementary data). Infectious titers of the goat tissues were scored as – (negative) when they were lower than the limit of detection of the mouse bioassay as calculated by the Reed–Muench method [27] (6×10^2 ID/g); + when they ranged from 6×10^2 to 6×10^4 ID/g; ++ when they ranged from $>6 \times 10^4$ to 6×10^6 ID/g; and +++ when they were $>6 \times 10^6$ ID/g.

[b] Goat WT *PRNP* genotype: $R_{211}Q_{222}$/RQ.

Very low infectivity was detected in some tissues very long after inoculation (44-45 months)

山羊の組織
PrP^{Sc}: negative
Infectivity: positive

#446

Distribution of Abnormal Prion Protein in a Sheep affected with L-type Bovine Spongiform Encephalopathy

Y. Matsuura, Y. Iwamaru, K. Masujin, M. Imamura, S. Mohri, T. Yokoyama and H. Okada

J. Comp. Path. 2013, Vol. 149, 113–118

3, 4 months old
ARQ/ARQ sheep
ic: L-BSE (BSE/JP24)

Table 1
Immunohistochemical and western blot detection of PrP^{Sc}

Tissue sample	*IHC*	*WB*
Central nervous system		
Olfactory bulb	+	+
Cerebral cortex	+	+
Thalamus	+	+
Obex	+	+
Cerebellum	+	+
Spinal cord	+	+
Optic nerve	+	+
Retina	+	+
Pituitary gland (neurohypophysis)	+	+
Peripheral nervous system		
Dorsal root ganglia	+	+
Trigeminal ganglia	+	+
Sympathetic trunk	–	–
Celiac and mesenteric ganglion complex	–	–
Vagus nerve	–	–
Facial nerve	–	–
Phrenic nerve	–	–
Suprascapular nerve	–	–
Brachial nerve plexus	–	–
Median nerve	–	–
Radial nerve	–	–
Sciatic nerve	–	–
Tibial nerve	–	–
Adrenal gland	+*	+
Lymphoid tissues		
Spleen	–	–
Tonsils (palatine, pharyngeal, lingual)	–	–
Thymus	–	–
Parotid lymph node	–	–
Mandibular lymph node	–	–
Lateral retropharyngeal lymph node	–	–
Superficial cervical lymph node	–	–
Brachiocephalic lymph node	–	–
Axillary lymph node	–	–
Subiliac lymph node	–	–
Popliteal lymph node	–	–
External iliac lymph node	–	–
Mesenteric lymph node	–	–

IHC, immunohistochemistry; WB, western blot.
+, positive for PrP^{Sc}; –, negative for PrP^{Sc}.
*Immunolabelled PrP^{Sc} was detected with the tyramide single amplification system, but not with the conventional method.

PrP^{Sc} was absent in lymphoid tissues.
Distinct tissue tropism of L-BSE in sheep from those of C-BSE?

#799

Acquired transmissibility of sheep-passaged L-type bovine spongiform encephalopathy prion to wild-type mice

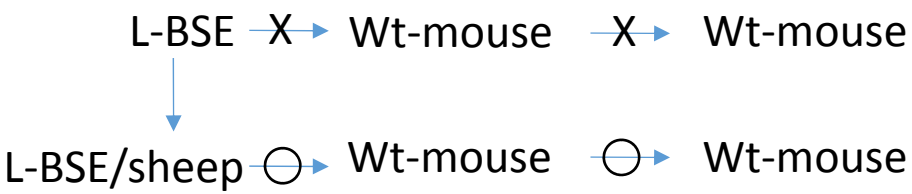
Veterinary Research (2015) 46:81

Hiroyuki Okada*, Kentaro Masujin*, Kohtaro Miyazawa and Takashi Yokoyama

Table 1 Transmission of L-BSE isolates to mice[a]

Hosts	Source	Inoculum	Passage	Survival periods (days)[b]	Range (days)	Proportion of PrP^{Sc} mice[c]	
						Brain	Spleen
ICR [14]	L-BSE/cattle [18]	Brain	1	651 ± 153	322–953	0/35	0/35
ICR	L-BSE/cattle passaged in ICR mice	Brain	2	666 ± 198	386–937	0/10	0/10
C57BL/6[d]	L-BSE/cattle [18]	Brain	1	713 ± 42	673–757	0/5	0/5
ICR	L-BSE/sheep [16]	Brain	1	569 ± 262	172–1012	1/15	9/15
ICR	L-BSE/sheep passaged in ICR mice	Spleen	2	691 ± 61	639–765	6/6	6/6
TgBoPrP	L-BSE/cattle [18]	Brain	1	195 ± 6	187-211	18/18	0/18
TgBoPrP	L-BSE/cattle passaged in TgBoPrP mice	Brain	2	152 ± 2	148-155	24/24	0/24
TgBoPrP	L-BSE/sheep [16]	Brain	1	249 ± 28	234-298	5/5	0/5
TgBoPrP	L-BSE/sheep passaged in TgBoPrP mice	Brain	2	269 ± 17	248-305	13/13	0/13

[a]TgBoPrP, bovine PrP expressing transgenic; L-BSE, L-type bovine spongiform encephalopathy; PrP^{Sc}, disease-associated prion protein.
[b]mean ± standard deviation.
[c]Number of PrP^{Sc} positive tissue samples per number of examined tissue samples. Results from either western blot, immunohistochemistry, or both.
[d]Unpublished data from our laboratory.



#406

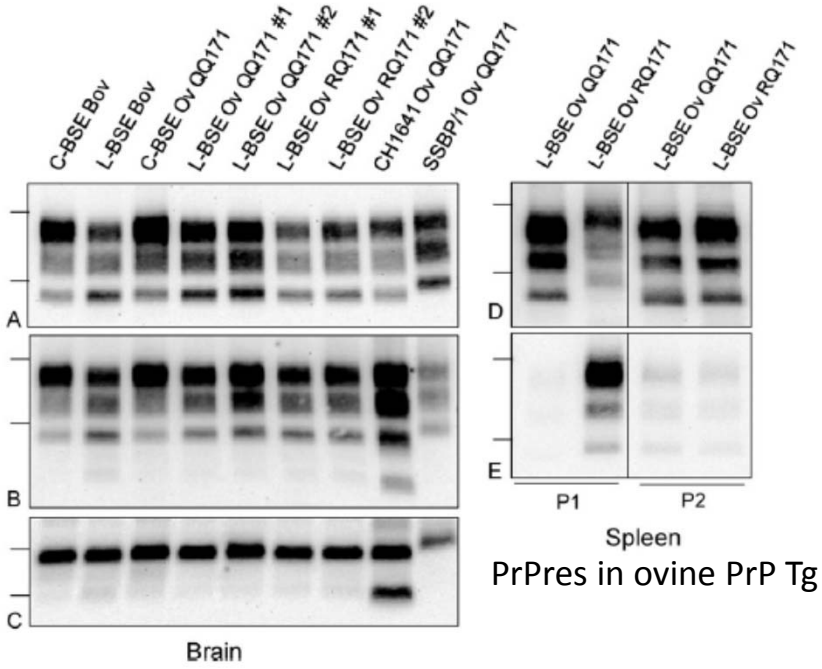
L-Type Bovine Spongiform Encephalopathy in Genetically Susceptible and Resistant Sheep: Changes in Prion Strain or Phenotypic Plasticity of the Disease-Associated Prion Protein?

Biological characteristics of L-BSE after passage by ic route in QQ and QR sheep

Simon Nicot,[1] Anna Bencsik,[1] Sergio Migliore,[2] Dominique Canal,[1] Mikael Leboidre,[1] Umberto Agrimi,[2] Romolo Nonno,[2] and Thierry Baron[1]

Table 2. Summary of Transmission Data in Ovine Prion Protein Transgenic Mice

TSE Inoculum	Passage	Survival Period, d.p.i., mean ± SD	Brain PrPd Positive	Spleen PrPres Positive
From cattle				
C-BSE	P1	421 ± 48	10/10	5/5
L-BSE	P1	627 ± 74	9/10	0/5
C-BSE	P2	354 ± 48	10/10	5/5
L-BSE	P2	202 ± 26	9/9	3/5
C-BSE	P3	414 ± 61	8/8	8/8
L-BSE	P3	220 ± 39	11/11	9/9
Passaged in sheep				
C-BSE QQ171	P1	296 ± 46	19/19	3/4
L-BSE QQ171 #1	P1	183 ± 24	12/12	10/10
L-BSE QQ171 #2	P1	188 ± 29	7/7	5/7
L-BSE RQ171 #1	P1	221 ± 26	5/6	6/6
L-BSE RQ171 #2	P1	195 ± 27	11/12	10/12
CH1641 QQ171	P1	245 ± 17	12/12	0/7
C-BSE QQ171	P2	365 ± 36	11/12	5/6
L-BSE QQ171 #1	P2	236 ± 19	5/5	2/5
L-BSE QQ171 #2	P2	199 ± 49	11/11	4/11
L-BSE RQ171 #1	P2	208 ± 21	9/9	9/9
L-BSE RQ171 #2	P2	189 ± 20	6/7	4/7
CH1641 QQ171	P2	220 ± 31	11/11	0/5



PrPres in ovine PrP Tg mice

RQ171 sheep was susceptible to L-BSE (ic route).
PrPres molecular features differ between L-BSE in RQ171 and QQ171 sheep.
Features of L-BSE from RQ171 and QQ171 are maintained in the Ovine Tg mice.
Splenic PrPres features at P1 in ovine PrP Tg mice reflect the genotype-dependent PrPres variations.