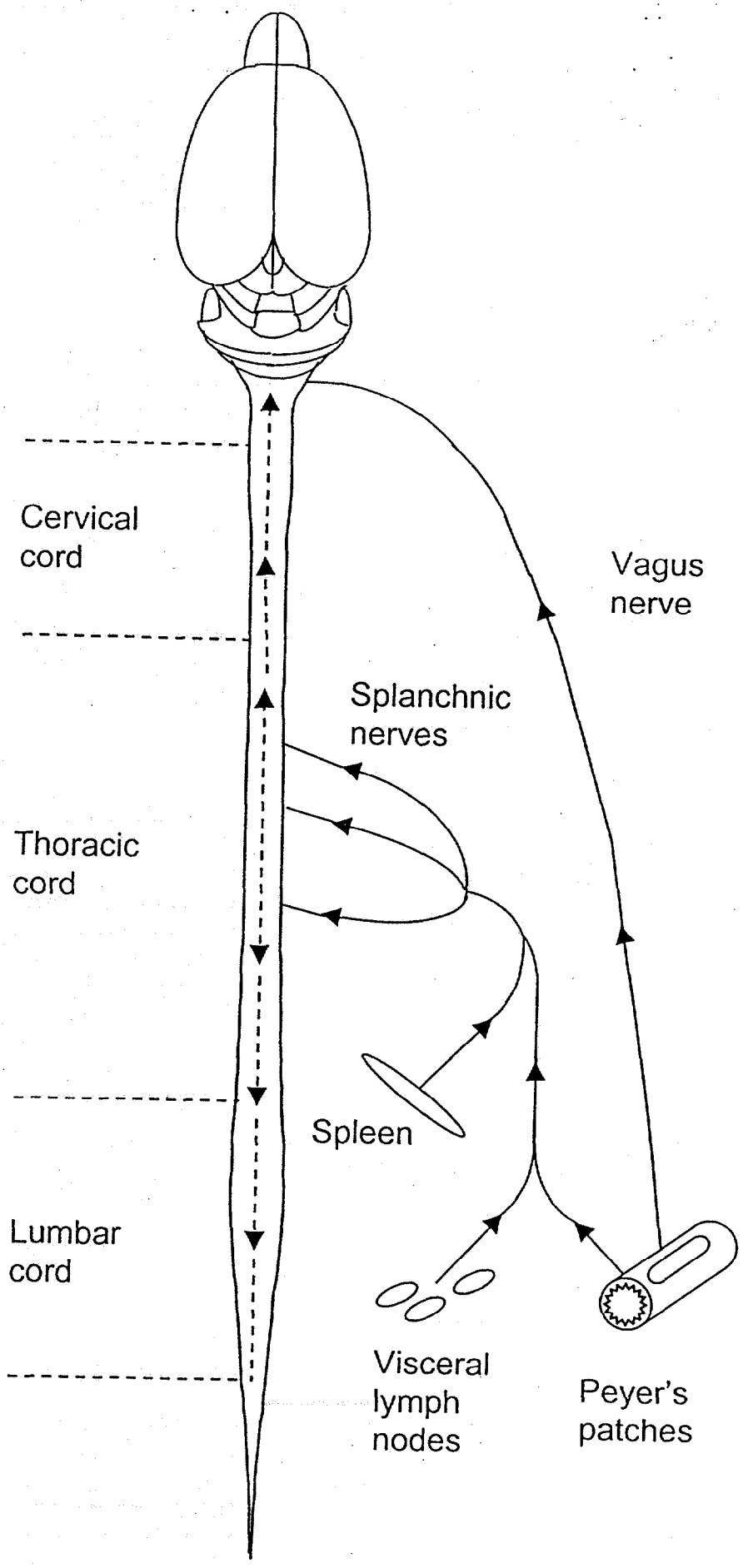
Veterinary Laboratories Agency

Studies of BSE infectivity in tissues of cattle

Gerald A.H. Wells
Consultant Veterinary Pathologist

Veterinary Laboratories Agency
New Haw
Addlestone
Surrey


Cervical
cord
Vagus
nerve
Splanchnic
nerves
Thoracic
cord
Spleen
Lumbar
cord
Visceral
lymph
nodes
Peyer's
patches

Relative Scrapie infectivity titres in tissues and body fluids from naturally infected Suffolk sheep and goats with clinical Scrapie

Category I		**High infectivity**
		- brain, spinal cord
Category II		**Medium infectivity**
		- spleen, tonsil, lymph nodes - ileum, proximal colon
Category III		
	(a)	**Some infectivity**
		- sciatic Nerve, pituitary, adrenal - distal colon, nasal mucosa
	(b)	**Minimal infectivity**
		- cerebrospinal fluid, thymus, bone marrow - liver, lung, pancreas
Category IV		**No detectable infectivity**
		- skeletal muscle, heart - mammary gland, colostrum, milk - blood clot, serum, faeces - kidney, thyroid, salivary gland, saliva - ovary, uterus, testis, seminal vesicle

Based on the data by Hadlow W.J. et al. 1980, 1982, obtained by bioassays of infectivity

Bioassay (RIII mice) of Infectivity in Tissues from Naturally Infected Cattle Clinically Affected with BSE[1]

Infectivity: Brain, cervical spinal cord, terminal spinal cord, retina

No Infectivity: 51 tissues (approx. total assays = 100)[2]

---

1. From: Fraser & Foster (1994) and unpublished data. Also MAFF (1998)
2. Number of animals sampled per tissue is variable and limited

Chronology of Recognition of "New" Scrapie-like Spongiform Encephalopathies in Animals

HOST	DISEASE	INITIAL CLINICAL RECOGNITION	REFERENCE
Cattle (*Bos taurus*)	Bovine spongiform encephalopathy	1985	Wells *et al* (1987)
Nyala (*Tragelaphus angasi*)	Scrapie-like encephalopathy	1986	Jeffrey & Wells (1988)
Gemsbok (*Oryx gazella*)		1987	
Eland (*Taurotragus oryx*)		1989	Fleetwood & Furley (1990
Arabian oryx (*Oryx leucoryx*)		1989	Kirkwood *et al* (1990)
Greater kudu (*Tragelaphus strepsiceros*)		1989	
Scimitar-horned oryx (*Oryx dammah*)		1992	Kirkwood & Cunningham (1994)
Bison (*Bison bison*)		1996	MAFF (1997)
Cat (*Felis domesticus*)	Feline spongiform encephalopathy	1990	Wyatt *et al* (1990) Wyatt *et al* (1991)
Cheetah (*Acinonyx jubatus*)		1991	Peet & Curran (1992)
Puma (*Felis concolor*)		1991	Willoughby *et al* (1992)
Ocelot (*Felis pardalis*)		1994	McCandlish *et al* (pers. communication)
Tiger (*Panthera tigris*)		1995	MAFF (1996)
Lion (*Panthera leo*)		1998	MAFF (1999)

PATHOGENESIS OF EXPERIMENTAL BSE IN CATTLE

Objective:

- To determine the temporal and spatial development of infectivity and pathology following oral exposure of calves to a single 100g dose of affected cattle brain homogenate

Protocol:

- 30 calves dosed orally at 4 months with 100g brain stem
- Groups killed at intervals through to 40 months p.i.

Inoculum:

- Pooled brain stems from 75 cases of BSE (1991)
- Inoculum assayed in RIII mice - incubation period 372.6 ± 7.6 days

Pathogenesis of Experimental BSE in Cattle

Tissues Inoculated into Mice - Neural

Brain frontal cortex, caudal medulla
Pituitary
CSF
Spinal cord: C_2 - C_3, T_{10} - T_{11}, L_3 - L_4
Nodose ganglion
Dorsal root ganglia C_3 - C_6, T_5 - T_8
Trigeminal ganglion
Stellate ganglion
Sciatic nerve
Facial nerve
Triceps muscle
Masseter muscle
Longissimus dorsi muscle
Sternocephalicus muscle

Pathogenesis of Experimental BSE in Cattle

Tissues Inoculated into Mice - Lymphorecticular

Spleen
Thymus
Tonsil
Submandibular lymph node
Retropharyngeal lymph node
Bronchial-mediastinal lymph node
Hepatic lymph node
Mesenteric lymph node
Prescapular lymph node
Popiteal lymph node

Pathogenesis of Experimental BSE in Cattle

Tissues Inoculated into Mice - Alimentary

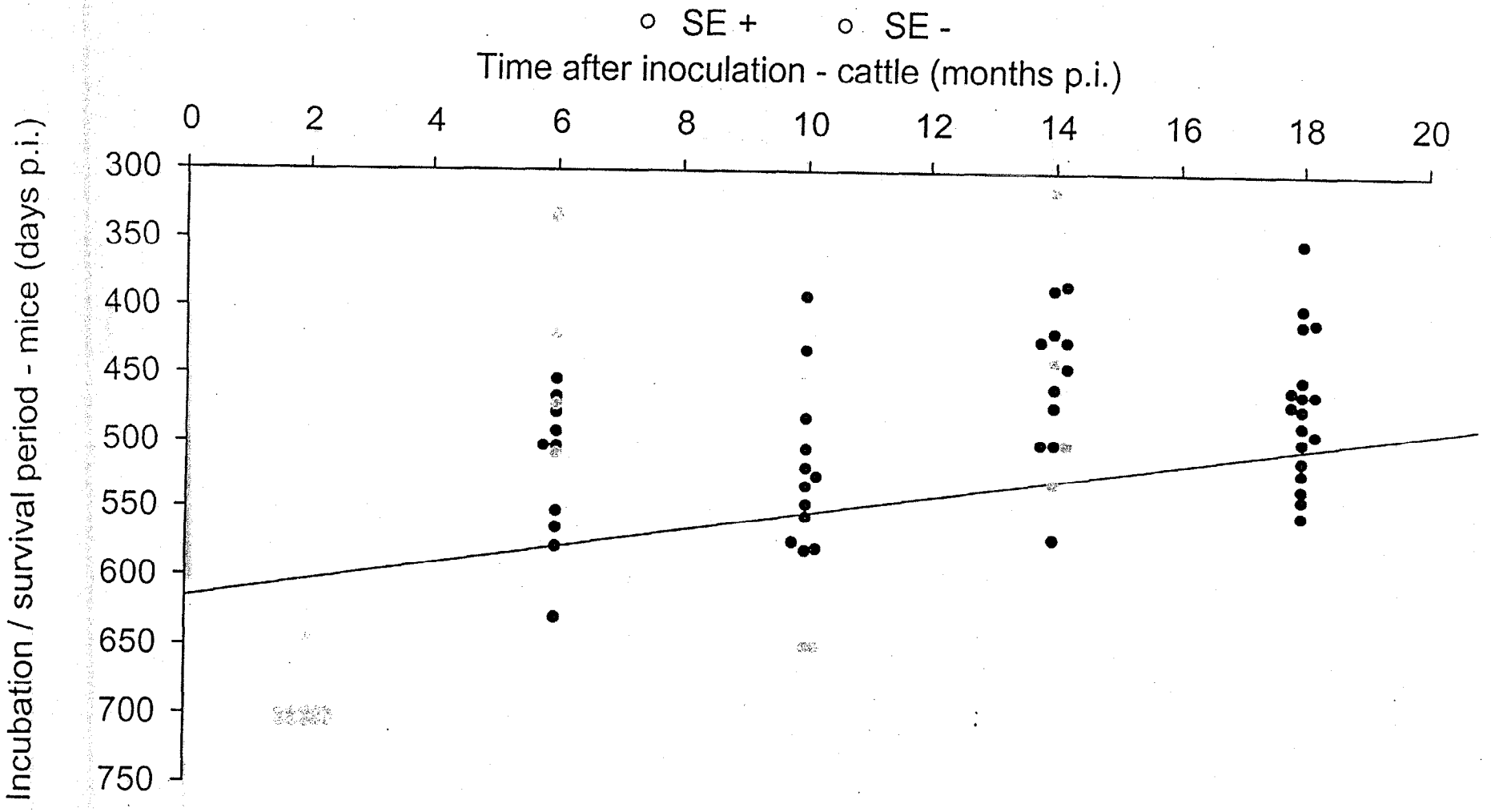
Tongue - (dorsum to include mucosa)
Submandibular salivary gland
Parotid salivary gland
Rumen
Abomasum (pyloric)
Duodenum
Distal ileum (including Peyer's patches)
Spiral Colon
Pancreas
Liver

Pathogenesis of Experimental BSE in Cattle

Tissues Inoculated into Mice - Other

Kidney
Lung (left caudal lobe)
Respiratory epithelium (mid-turbinate)
Heart (left ventricle)
Blood (buffy coat)
Bone marrow (sternum)

Bioassay results of distal ileum (RIII) from cattle killed 2 - 18 months p.i.



Infectivity* in tissues of cattle killed 32 - 40 months p.i.

Animal	Months	Brain cortex	Brain medulla	Spinal cord C_{2-3}	Spinal cord T_{10-11}	Spinal cord L_{3-4}	Dorsal root ganglia C_{3-6}	Dorsal root ganglia T_{5-8}	Trigeminal ganglia	Bone marrow	Di ile
# 254, # 232	32	-	+	+	+	+	+	+	-	-	-
# 299, # 194, # 231	36	-	+	+	+	+	+	+	+	-	+
# 261, # 192, # 300	38	+	+	+	+	+	-	+	+	+	+
# 277, # 296	40	+	+	+	+	+	-	+	+	-	+

* Inoculum for each tissue was derived from a pool of the tissue from the animals of each group killed at a specific time point.

Interim bioassay(C57 Bl) results of distal ileum from cattle killed 36 - 40 months p.i.

Numbers of positive mice / number of mice surviving when first mouse confirmed positive
(mean incubation - days)

Donor Cattle months p.i.	36	38	40
	1/2	4/12	9/19
	(942)	(791)	(663)

Bioassay results (C57Bl) confirming infectivity in bone marrow of cattle 38 months p.i.

Number of positive mice/number of mice surviving when first mouse confirmed positive[1]

Clinical status (incubation: days)	Histopathological examination (survival: days)	PrP immunohistochemistry (survival: days)
2/16 (695, 842)	2[2]/16 (695, 842)	6/16 (653[2], 695, 792, 799, 842, 92

[1] as confirmed by histopathology or PrP IHC

[2] one mouse considered inconclusive with mild spongiform change confined to medulla hypothalamus and lateral thalamus which proved positive on PrP IHC examination.

Summary

- Clinical signs from 35 months post-exposure
- Abnormal PrP in CNS (IHC) from 32 months post-exposure
- Vacuolar changes in CNS from 36 months post-exposure
- Infectivity - distal ileum (6 - 18 and 36 - 40m)
  CNS (32 - 40m)
  DRG (32 - 40m)
  trigeminal ganglion (36 - 40m)
  bone marrow (38m)

Bioassay (RIII) of additional tissues of potential significance to medical procedures

Results: Negative

heart valve
pericardium
aorta
skin
collagen
bone

18m pi & 32m pi

Infectivity Assays in Mink of Tissues from Experimentally Inoculated Mink which Died or were Clinically Affected with TME

	Titre of Infectivity (log_{10} mink LD_{50})		
Tissue	Exp. 1 [1]	Exp. 2 [1]	Exp. 3 [2] (weeks PI)
Brain	$10^{6.5}$	$10^{7.0}$	$10^{6.5}$ (32)
Liver	$10^{4.0}$	$10^{2.0}$	$10^{3.5}$ (32 and 36)
Spleen	$10^{4.0}$	$10^{2.0}$	$10^{4.5}$ (28 and 32)
Kidney	$10^{2.0}$	$10^{2.5}$	$10^{3.0}$ (35)
Muscle (unspecified)	$10^{2.5}$	NT	NT

[1]Marsh *et al* (1969) [2]Hadlow *et al* (1987)

COMPARATIVE EFFICIENCIES OF THE BIOASSAY OF BSE INFECTIVITY IN CATTLE AND MICE

Objectives:

- To obtain a measure of the underestimation of the infectivity titre of BSE tissue when titrated across a species barrier in mice.

- To produce an approximate dose-incubation curve for infectivity of brain from BSE affected cattle by simultaneous titration of a primary inoculum in cattle and in mice.

Protocol:

- 6 x 4 calves inoculated i.c. at 4 months with a single dilution of BSE brain pool using a tenfold dilution range 10^{-3}-10^{-8}.
- Parallel titration in 6 x 20 mice (RIII) inoculated i.c. and i.p. with a dilution range 10^{-1}-10^{-6}.

Status (Nov. 1998):

Cattle - titration incomplete, 70 months p.i.

Inoculum dilution	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Clinically positive histopath. confirmed	4	4	2	2	2	0

Mice - titration complete, $10^{3.3}$ mouse ID_{50}/g of tissue.

Comparative efficiencies of the bioassay of BSE infectivity in cattle and mice

Results

- Karber titres of bovine brain stem pool (5 cases BSE)
  - RIII mice: $10^{3.3}$ $\log_{10}$ mouse (i.c. + i.p.) LD_{50}/g
  - Friesian/Holstein cattle: $10^{6.0}$ $\log_{10}$ cattle (i.c) LD_{50}/g

- Incubation period assay of bovine lymph node pool (4) or spleen (5 cases BSE)
  - RIII mice - negative at end point (700 days p.i)
  - Friesian/Holstein cattle: negative at end point (86 months p.i)

Comparative efficiencies of the bioassay of BSE infectivity in cattle and mice

Conclusions

- Underestimate of infectivity titre of BSE brain tissue titrated across a species barrier in mice: $10^{2.7}$ (500 fold)

- Spleen or lymph node pool from confirmed cases BSE contain $<10^{-1}$ $\log_{10}$ cattle (i.c) LD_{50} /g

Bioassay of BSE Infectivity in Tissues by Intracerebral Inoculation (i.c.) of Cattle

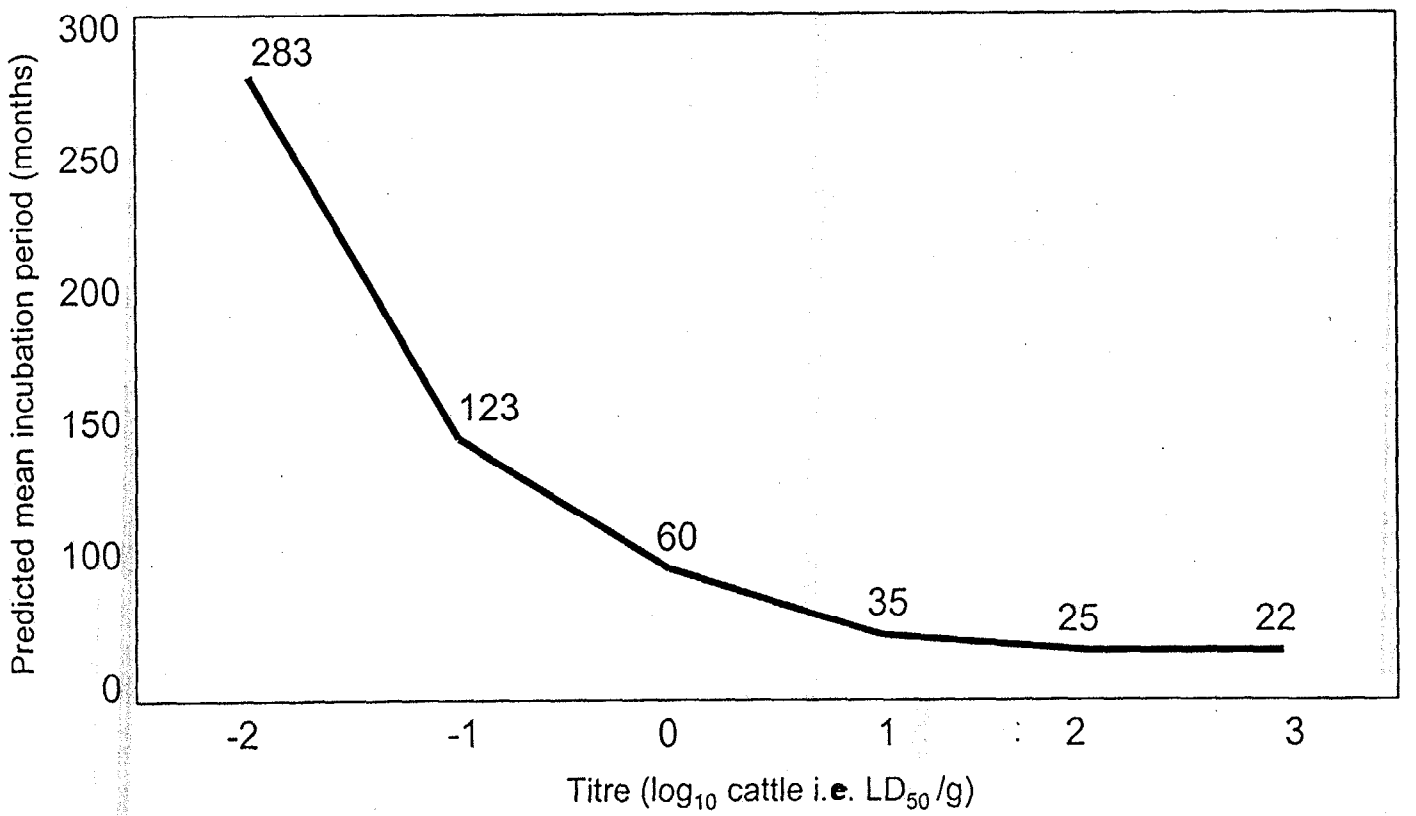
---

Objective

- To determine infectivity in a range of tissues sampled at selected sequential time points from cattle infected orally with 100g of BSE affected brain by within species bioassay

Bioassay of tissues[1] in cattle - interim results:

Tissue (p.i)		Mean incubation - days (± SD)
Distal ileum	(10m)	676 (± 7)
Distal ileum	(18m)	735 (± 23)
Caudal medulla and spinal cord	(32m)	684 (± 10)

1 specified tissue pools from cattle killed at selected time points after oral infection with BSE agent

Estimated dose response curve of bovine brain from cases of BSE after i.c.inoculation into Friesian/Holstein cattle