REPORT ON TSE INFECTIVITY DISTRIBUTION IN RUMINANT TISSUES (STATE OF KNOWLEDGE, DECEMBER 2001)

PREPARED BY THE TSE/BSE *AD HOC* GROUP

Initially finalised at its meeting of 13 December 2001 and updated at its meeting of 10 October 2002 following the submission of (1) a risk assessment by the German Federal Ministry of Consumer Protection, food and Agriculture and (2) new scientific evidence regarding BSE infectivity distribution in tonsils

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REPORT ON TSE INFECTIVITY DISTRIBUTION IN RUMINANT TISSUES (STATE OF KNOWLEDGE, OCTOBER 2001)

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I. MANDATE

The Scientific Steering Committee (SSC) was invited:

(1) To update, on the basis of the most recent scientific data, the sheep tissue infectivity titre table presented in the SSC opinion of 22-23 July 1999 on The Policy of Breeding and Genotyping of Sheep;

(2) To create a similar table for cattle on the basis of all available scientific evidence;

(3) To consider whether any new evidence exists since the adoption of its opinion of 9 December 1997 on the listing of Specified Risk Materials which would indicate that the entire head of cattle, sheep and goats, including skeletal muscle, tongue and associated innervation should be considered as specified risk material.

The SSC invited the TSE/BSE *ad hoc* Group to prepare a scientific report that could serve as the basis for preparing an answer to the above question. A first report was finalised by the TSE/BSE *ad hoc* Group at its meeting of 13 December 2001 it served as the basis for an opinion adopted by the SSC at its meeting of 10-11 January 2002.

On 10 may 2002, the Government of the Federal Republic of Germany requested the European Commission to elicit the opinion of the Scientific Steering Committee on a report of 25 April 2002 prepared by the Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV), which evaluates the possible risks related to harvesting cheek meat and more specifically lists the critical hygiene points in harvesting of bovine heads and cheek meat of cattle. Whilst preparing this opinion, the SSC was informed about a recent outcome of the ongoing UK cattle pathogenesis research, showing very low levels of infectivity in bovine tonsil. In the light of these new data, the TSE/BSE *ad hoc* Group updated its report of 13 December 2001. This update report is follows hereafter. An English translation of the German report is also attached as **Annex 2**, for ease of reference.

<u>Keywords</u>: **Bovine Spongiform Encephalopathy, Transmissible Spongiform Encephalopathy, specified risk material, cattle, small ruminants, sheep, goat , head, tongue, tissue infectivity.**

II   TSE INFECTIVITY LEVELS IN RUMINANT TISSUES

II.1.   PREVIOUS TABULATED DATA

The most recent tabulation of all available data with respect to classification of tissues from clinical cases of scrapie in Suffolk sheep and in goats on the basis of titre of infectivity after assay in mice was given as an annex in the Opinion on The Policy of Breeding and Genotyping of Sheep, Adopted 22-23 July 1999 (EC 1999), and is reproduced here at **Table 1**.

The sheep (Hadlow et al 1982) and sheep and goat data (Hadlow et al 1980) have also been compared previously with preliminary mouse infectivity data on tissues from naturally affected cases of BSE in cattle. This comparison is provided in **Annex**. A list of tissues, from cases of BSE affected cattle, in which no infectivity had at the time of writing been detected by bioassay in mice was also given in Kimberlin (1996) (See **Table 2**). A preliminary table of infectivity categories for tissues from sheep experimentally exposed orally to the BSE agent was given in Annex 3 of the Report attached to the Pre-emptive Risk Assessment should BSE in small ruminants be found under domestic conditions (See **Table 3**).

Table 1: Natural scrapie in sheep and goats: classification of tissues by agent titre in Swiss mice and by age, in pre-clinical and clinical cases of Scrapie in Suffolk sheep and in goats[4] (Unammended from Annex: Opinion on The Policy of Breeding and Genotyping of Sheep, 22-23 July 1999) (EC 1999)

Group	Infectivity Titre (approx.range)	PRE-CLINICAL SHEEP				CLINICAL SHEEP	CLINICAL GOATS
		≤8 months.(0/16)	10-14 months(8/15)[5]	25 months(1/13)	> 25 months(1/6)	34-57 months(9/9)	38-49 months(3/3)
A	HIGH ≥ 4.0					Brain Spinal cord	Brain Spinal cord
B	MEDIUM 3.2 – 4.0		Colon-proximal, Ileum-distal, LN (RP/MP), Spleen	Colon-proximal, Ileum-distal, LN (RP/MP), Tonsil		Colon-proximal, Ileum-distal, Spleen, Tonsil LN (BM), LN (PF, 1/9 negative), LN (PS, 2/9 negative), LN (PR/MP), (rectum-distal+),	Colon-proximal, Ileum-proximal, LN (BM), LN (RP/MP), LN (s.mammary), Pituitary, (Rectum-distal +), Spleen
C	LOW ≤ 3.2 or titre unknown		LN (PS/PF) Tonsil	Brain (medulla/ diencephalon), LN (BM), LN (PS/PF), Spleen		Adrenal, Bone marrow**, Colon-distal, CSF, Liver**, LN (s.mammary x2), Nasal mucosa, Pancreas **, Pituitary, Sciatic nerve, Thymus**,Placenta ** °	Adrenal, Colon-distal, CSF Nasal mucosa, Sciatic nerve, Thymus
D	Undetectable	Ileum, LN (PS/PF) LN (RP/MP), Thymus, Tonsil Spleen	Blood clot, brain (medulla), Colon-distal, Faeces, LN (BM), Serum	Adrenal, Brain (cortex mid-brain), Colon-distal, LN (s. mammary), Nasal mucosa, Salivary glands, Spinal cord, Thymus	Colostrum	Blood clot, Fetus, Heart, Kidney, Lung, Mammary gland, Muscle-skeletal, Ovary, Saliva, Salivary gland, Sem. Vesicle, Testis, Thyroid, Uterus	Blood clot, Bone marrow, Faeces, Kidney, Mammary gland, Milk, Muscle-skeletal, Ovary, Salivary gland, Serum, Uterus

(-/-) (Number positive / number examined)
* = Log_{10} mouse intracerebral LD/50 per 30 mg tissues
+ = Not assayed but high content of lymphoreticular tissue
° = negative in other studies

** = trace or exceptional
PF = Prefemoral
PS = Prescapular
RP = Retropharyngeal

MP = Mesenteric/portal
CSF = Cerebro-spinalfluid
LN = Lymph node
BM = Bronchomediastinal

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4 After Hadlow et al. (1979, 1980, 1982), Pattison *et al.* (1964, 1972), Groschup et al. (1996). Regarding DRG: see text.

5 Techniques for the determination of infectivity become more and more sensitive. The age range may go below 10 months. In individual cases, tonsil infectivity has been detected in lambs of 16 weeks. Placenta has been placed in Group C, but titres are unknown.

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Table 2: Tissues from confirmed cases of BSE in cattle in which no infectivity was detected by bioassay in mice injected both intracerebrally and intraperitoneally (Taken from Kimberlin, 1996)

Nervous tissues	*Lymphoreticular tissues**
Cerebrospinal fluid	Spleen
Cauda equina	Tonsil
Peripheral nerves :	Lymph nodes
- sciaticus	- prefemoral
- tibialis	- mesenteric
- splanchnic	- retropharyngeal
Alimentary tract	*Reproductive tissues*
Oesophagus	Testis
Reticulum	Prostate
Rumen (pillar)	Epididymis
Rumen (oesophageal groove)	Seminal vesicle
Omasum	Semen
Abomasum	Ovary
Proximal small intestine	Uterine caruncle
Distal small intestine	Placental cotyledon
Proximal colon	Placental fluids :
Distal colon	- amniotic fluid
Rectum	- allantoic fluid
	Udder
	Milk
Other tissues	
Blood :	Liver
- buffy coat	Lung
- clotted	Muscle
- foetal calf	- semintendinous
- serum	- diaphragma
Bone marrow	- longissimus
Fat (midrum)	- masseter
Heart	Pancreas
Kidney	Skin
	Trachea

* Tonsil was found positive in the cattle bio-assay (SEAC, 2002) – see elsewhere in this report.

Table 3: Experimental BSE in sheep: Distribution of infectivity by incubation stage and PrP genotype and stage of incubation (Taken from Annex 3 of the Pre-emptive Risk Assessment should BSE in small ruminants be found under domestic conditions, adopted by the SSC on 8-9 February 2001) (EC 2001) and updated from recent experimental results, see II.6.4 of Report

Infectivity titre	Pre-clinical		Clinical	
	ARR/ARR, ARR/ARQ	ARQ/ARQ	ARR/ARR, ARR/ARQ	ARQ/ARQ
High				Brain Spinal cord Spleen
Medium		Spleen Lymph nodes [estimated, not titrated] Tonsil		Lymph nodes Tonsil
Low				
PrP-res detected but infectivity not titrated		Intestine Forestomachs abomasum		Intestine Forestomachs abomasum
Not detectable	Brain, Spinal cord, Spleen, Lymph nodes, Tonsil			

Notes: The summary table is based on the limited research results currently available in this field. Full literature references are provided in the attached report. The table should be used with caution since it relates to experimental, and not natural BSE in sheep, some data are incomplete and some experiments are on-going. Nevertheless it may serve as a guide to the degrees of risk that may exist. The Table should be updated as new results come forward.

No PrP-res has so far been detected in ARQ/ARR or ARR/ARR animals inoculated with BSE up to 2 years post challenge. On the assumption that PrP-res and infectivity are correlated this would suggest that if these animals are infected the titre of infectivity in the years immediately following challenge is lower (or undetectable) in peripheral tissues of these genotypes than in more susceptible genotypes.

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II.2. CONTEXT OF PUBLISHED DATA

The concentration of infectious TSE agent in tissue is determined by bioassay usually using endpoint titration or, sometimes, though regarded less accurate, by incubation time assay. The experimental models that have most often been used for such assays are inbred strains of mice. Whilst the most practical bioassay model, mice are likely to provide an underestimate of the concentration of agent in sheep or cattle tissues because of the effect of the species barrier. With few exceptions, previous titration data have been expressed in the form of $\log_{10}$ ID$_{50}$ units according to Kärber (1931). One mean infective or lethal dose (ID$_{50}$) is defined as the amount of infectivity that will transmit disease to half of a group of inoculated animals. If 1 ml volumes of successive ten fold dilutions of a specimen are inoculated into a total of 20 mice per dilution group (0.05 ml per animal), one ID$_{50}$ would be present in the dilution that transmits disease to 50% (10/20) of the inoculated animals. Titrations often show transmission rates of about 100%,50%, and 0% in successive 10-fold dilutions and providing the last, the limiting dilution, is determined, the survivorship can be used to calculate the units of ID$_{50}$ in the original undiluted inoculum volume. This is usually corrected to express the units of ID$_{50}$ /g of the tissue.

It must be stressed that experience of end point titration and incubation period assays in laboratory rodents suggests that the assays are most reproducible when examining infectivity of a strain of agent adapted or, ideally, cloned in the assay species. The inter-laboratory reproducibility of end point titration assays, at least with ME7 scrapie has been demonstrated in C57Bl mice (Taylor *et al.*, 2000). The recent use of mouse titrations of infectivity for cattle and sheep tissues is in contrast to this since the titrations have been conducted across a species barrier on primary inoculation from cattle or sheep.

There are a number of factors which affect the efficiency of infectivity assays. Route(s) of inoculation affect infectivity titre and dose response curves (Kimberlin and Walker, 1978). The volume(s) of inoculum injected also affects the sensitivity of the assay. In practice, the most efficient route(s) of inoculation are selected to perform the assay. In the assays cited in Tables 1 and 2, the calculated limit of detectability of scrapie infectivity by the intracerebral (i.c.) inoculation of mice is approximately $10^{2.0}$ $\log_{10}$ mouse i.c. ID$_{50}$/g of tissue (Kimberlin 1994) with a volume of inoculum of 30μL. Clearly, the volume of inoculum that can be injected intracerebrally in a mouse is limited. In the mouse assays of infectivity of tissues from cases of naturally affected cattle with BSE (Fraser and Foster 1994 and H. Fraser, personal communication), shown in Table 3, a combination of i.c. and i.p. injections was used with a total volume of inoculum of 120μL, giving a limit of detectability of $10^{1.4}$ ID$_{50}$/g (Kimberlin 1996).

Fraser *et al* (1992) showed that end point titration of BSE on primary pass to RIII and C57Bl inbred mouse strains gave closely similar values for infectivity.

The relationship between incubation period and titre has been questioned by many authors (see Masel and Jansen 2001, for review), casting doubt on the validity of

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estimating titre by incubation period assays. Certain physical and chemical treatments of scrapie affected mouse brain inocula may alter incubation relative to titre, giving a discrepancy between end point titration and incubation period assay of about 10^1-10^2 ID_{50} (Masel and Jansen, 2001). In an analysis of over one hundred scrapie infectivity titrations in mice (McLean and Bostock 2000) a linear rise in mean incubation period with logarithmic decreasing dose was substantiated, but variability in incubation period rose linearly as dose decreased. Thus estimation of titre from dose response curves is less accurate at low doses.

While there are numerous instances of a poor correlation quantitatively between infectivity (depending on how it is measured) and concentration of PrP^{Sc} (Masel and Jansen, 2001), there are also reports demonstrating a relationship between PrP^{Sc} concentration and TSE infectivity (see Lee *et al.* 2001 for review). A "perfect correlation was observed between infectivity and PrP-res detection" (Race *et al.*, 1998) when mouse bioassay (Rocky Mountain Swiss mice) was compared with PrP immunoblotting for assay of brain, spleen, lymph nodes and placenta from scrapie affected Suffolk sheep. However, the nature of this comparison was confined to morbidity data and relative incubation period in the mouse assay (i.e. incubation period was not calibrated against a dose response curve) and presence of PrP-res. Thus in effect the comparison is essentially only qualitative.

Comparisons of the performance of the Bio-Rad version of the CEA Elisa test for rapid detection of PrP^{sc} with mouse titration data for BSE affected bovine brain has indicated a good correlation, providing prospects for estimations of titre from such rapid test results (Deslys et al 2001). This study was confined to brain tissue and rapid PrP tests are not yet available with suitable methodology for other than central nervous system tissue.

Nevertheless, increasing trend toward the use of PrP^{Sc} detection in preference to the time consuming bioassays as a diagnostic marker means that some examination of the measurement of PrP^{Sc} concentration as a proxy for infectivity should be considered where infectivity data *per se* is lacking. In this way for risk assessment purposes it may be possible to provide better estimates from current data rather than basing assessments purely on historical infectivity data. This has not, however, been pursued in the context of the present report and caution should be exercised in quantitation of PrP^{Sc} as a surrogate for infectivity assays. PrP^{Sc} can indeed indicate the presence of infectivity but in same species transmissions infectivity assays are still much more sensitive. Furthermore, the ratio of amounts of PrP^{Sc} to infectivity may vary. Strain of agent (TSE source), PrP genotype, breed of animal, tissue type and stage in the incubation period may all affect the relative amounts of infectivity to PrP^{Sc}.

The use of transgenic mice with modification to enhance the sensitivity of detection of the donor species infectivity may provide comparative data with assays conducted in conventional mice, but no comparative titration results are, as yet, available for such models (Buschmann *et al.*, 2000).

Titration of infectivity in TSE's have been performed largely on central nervous system tissue, notably brain. Recent data on other tissues is confined to a small number of experiments.

II.3. **SCRAPIE IN SHEEP : BIOASSAYS OF SHEEP TISSUES AFTER ORAL OR NATURAL EXPOSURE TO THE AGENT OF SCRAPIE BY INOCULATION OF MICE**

There are no recent titrations or incubation period data on tissues of sheep experimentally infected with scrapie via the oral route. Such data on natural cases of scrapie are confined to titres of brain tissue. A pool of 2867 brains of suspect scrapie cases used in a study of the effects of rendering upon the scrapie agent gave a titre of $10^{4.1}$ mouse (i.c. ID_{50}/g of tissue (Taylor *et al*. 1997) compared to the average titre of infectivity in brains of Suffolk sheep clinically affected with scrapie of 10^5 mouse (i.c.) ID_{50}/g (Hadlow and others, 1979). A pool of scrapie affected sheep brains used for the oral exposure of pigs to the scrapie agent gave widely differing infectivity values when titrated in different strains of mice. Titres were $10^{3.7}$ mouse (i.c. + i.p.) ID_{50}/g in IM mice compared to $10^{2.8}$ mouse (i.c. + i.p.) ID_{50}/g in C57BL mice (S.A.C. Hawkins, personal communication).

II.4. **BSE IN CATTLE: BIOASSAY OF TISSUES FROM CATTLE EXPERIMENTALLY INFECTED WITH BSE AGENT AND KILLED SEQUENTIALLY (VLA PATHOGENESIS STUDY) BY INOCULATION OF MICE.**

The study design has been described previously (Wells *et al*. 1996, Wells et al., 1998). Briefly, forty Friesian/Holstein calves, born in 1991, were assembled from farms with no history of BSE. At four months of age, thirty were each dosed orally with 100g of pooled brain stems from seventy-five cases of BSE. Ten calves received no treatment and served as controls.

Clinical monitoring of cattle was maintained throughout the study to detect the onset of clinical disease.

Starting at six months of age, and then at four month intervals, until 22 months p.i., three challenged calves and one control calf were killed. Thereafter challenged and control cattle were killed at discretionary intervals, with the final kill at 40 months p.i.

Tissues were sampled aseptically for infectivity assays in mice. After each sequential kill, inocula were prepared from 44 tissues, representing principally the lymphoreticular system (LRS), the peripheral nervous system (PNS) and the central nervous system (CNS), alimentary tract, striated muscles and major viscera (see Table 3.1, Wells and others, 1996). All inocula were prepared as ten per cent suspensions in saline, with the inclusion of antibiotics for certain tissues. Single tissue inoculum pools were made from the exposed cattle at each time point. Inocula were similarly prepared but from single tissues of each control animal. Test and control inocula were injected by intracerebral (20μl) and intraperitoneal (100μl) routes into inbred mice for standard qualitative assay of infectivity. Inocula prepared from cattle killed up to 18 months p.i. were injected into RIII mice and/or C57Bl-J6 mice.

Qualitative assays by the i.c. and i.p. inoculation of mice (RIII and/or C57BL) of a large range of tissues from the UK VLA Pathogenesis study of BSE have been completed (Wells *et al.*, 1996, 1998, 1999 and unpublished data). No titration of infectivity in positive tissues has been carried out. For all tissues in which infectivity has not been detected it can be stated that they contain less than $10^{1.4}$ mouse (i.c./i.p.) $\log_{10}$ LD$_{50}$/g. The results are summarised in **Table 4.**

Prospects for further analysis of the data from tissues in which infectivity has been detected to give an approximation to titre, must rely on survival data, dose and incubation period data for RIII and C57BL mice. The analyses of these data are as yet incomplete, particularly with respect to data on RIII mice (G.A.H. Wells and S.A.C. Hawkins, unpublished). Where data on incubation periods for RIII or C57BL mouse assays on tissues from the Pathogenesis Study of BSE is available, approximations to tissue infectivity titres have been estimated and provided in **Table 4.** These are necessarily provisional since most currently available values are from assays conducted in C57 BL mice, for which only a single experiment dose response curve result is available (on brain and after i.c. inoculation only). More values for incubation periods of RIII infectivity assays, on which titres can be estimated from summated data of a series of dose response curves (after i.c. + i.p. inoculations) will become available in the near future.[*GW: I will attempt to see if more information is now available and provide it at our next meeting*] From the available data it is not possible to estimate more accurately than the very low values of estimated infectivity (<10^1 mouse i.c. + i.p. ID$_{50}$/g) for the majority of the tissues of positive assays in the Pathogenesis study (**Table 4**).

A possible explanation for the very low estimates of infectivity in central nervous system may lie in the relatively early clinical status of cattle killed 32-40 months in the Pathogenesis Study. From a range of titrations conducted on brain from clinical or clinical suspect cases of BSE a wide range of titres have been obtained ($10^{2.9} - 10^{5.2}$ mouse i.c. or i.c + i.p) ID$_{50}$/g) (Fraser et al 1992, Taylor et al 1994, Kimberlin 1996, G. A. H. Wells and S.A.C. Hawkins, unpublished). It must be emphasised that this variation has to some extent a basis in sampling in that the highest titres were obtained from hind brain from single cases of terminally affected cattle, whereas the lowest titres were obtained from pools of whole brains from clinically suspect cases of BSE (which could contain ≥10% negative cases)

Table 4.Summary of Results of Infectivity Assays in mice of Tissues from sequentially killed cattle exposed orally to the BSE agent. (S.A.C.Hawkins & G.A.H.Wells, unpublished)

Tissue		Infectivity	Estimate of range of titre of infectivity (mouse i.c./i.p. ID_{50}/g) relative to incubation period (months) of donor cattle
Neural:	Brain: frontal cortex, caudal medulla	+, +	[C57BL] ≤$10^{1.0}$ (32-40m)
	Pituitary	-	
	Cerebrospinal fluid	-	
	Dura	N.D.	
	Spinal cord: C2-C3, T10-T11, L3-L4	+, +, +	[C57BL] ≤$10^{1.0}$ (32-40m)
	Nodose ganglia	-	
	Dorsal root ganglia: C3-C6, T5-T8	+	[C57BL] ≤$10^{1.0}$ (32-40m)
	Trigeminal ganglia	+	[C57BL] ≤$10^{1.0}$ (32-40m)
	Stellate ganglia	-	
	Sciatic nerve	-	
	Facial nerve	-	
	Phrenic nerve	-	
	Radial nerve	N.D.	
	Semitendinosus muscle	N.D.	
	Diaphragmatic muscle	N.D.	
	Triceps muscle	-	
	Masseter muscle	N.D.	
	Sternocephalicus muscle	-	
	Longissimus dorsi muscle	-	
Alimentary:	Tongue (dorsum, include mucosa)	-	
	Submandibular salivary gland	-	
	Parotid salivary gland	-	
	Cranial esophagus	N.D.	
	Rumen	-	
	Omasum	N.D.	
	Abomasum (pyloric)	-	
	Duodenum	-	
	Distal ileum (inc. Peyer's patches)	+	[RIII] <$10^{0.5}$-$10^{1.5}$ (6-14m), $10^{1.2}$ (18m) [C57BL] <10^{1} (36-40m)
	Spiral colon	-	
	Faeces‡	-	
	Pancreas	-	
	Liver	-	
Lymphoreticular:	Spleen	-	
	Thymus (cervical)	-	Note: Tonsil was found positive in the cattle bio-assay (SEAC, 2002) – see elsewhere in this report
	Tonsil	-	
	Submandibular lymph node	-	
	Retropharyngeal lymph node	-	
	Bronchial-mediastinal lymph node	-	
	Hepatic lymph node	-	
	Mesenteric lymph node	-	
	Prescapular lymph node	-	
	Popliteal lymph node	-	
Other:	Kidney	-	
	Urine‡	‡	
	Adrenal	N.D.	
	Lung (left caudal lobe)	-	
	Nasal mucosa (midturbinate)	‡	
	Pericardium‡	-	
	Heart (left ventricle/ septum)	‡	
	Mitral valve‡	‡	
	Aorta‡	-	
	Blood (buffy coat)	-	
	Blood (serum)	N.D.	
	Blood (clot)	N.D.	
	Bone marrow (sternum)	+*	[C57BL] <$10^{1.0}$ (38m)
	Collagen (Achilles tendon)‡	-	
	Skin‡	‡	
	Bone (femoral diaphysis)‡	-	

Key to Table 4:
+   positive
-      negative (i.e. <$10^{1.4}$ mouse (i.c. + i.p.) $\log_{10}$ LD_{50}/g)
N.D.   Not Done (collected and reserved for future study)
‡   Selected tissue assays in RIII mice conducted at only two kill time-points (18 and 32 months after exposure).
*   Very low level of infectivity detected only at one time point (38 months after exposure) which is within the range of onset of clinical signs (end of incubation period) for cattle exposed in the study (Wells et al 1999)

II.5.   BSE IN CATTLE: BIOASSAY OF CATTLE TISSUES BY INOCULATION OF CATTLE

In contrast to the widespread infectivity found in lymphoid tissues of cases of scrapie of sheep, the failure of the mouse bioassay to detect infectivity in tissues outwith the central nervous system of cattle naturally affected with BSE raised the issue of the efficiency of this assay system for the BSE agent. A study was therefore initiated (VLA/CSG SE1821) to provide a measure of the underestimation of the titre of infectivity in tissues across a species barrier in mice and 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. In addition, spleen and lymph node collected from natural cases of BSE were assayed in cattle to provide an order of magnitude estimate of concentration of infectivity in these tissues.

At approximately 4 months of age groups of calves were injected intracerebrally (i.c.) each with a single dilution of inoculum prepared from pooled brain stems from BSE affected cattle using a ten fold dilution range of 10^{-3} to 10^{-8}. Two additional groups of calves were similarly inoculated with a 10^{-1} dilution of a pool of spleen or lymph nodes. All calves were monitored clinically and retained until definite signs of clinical disease developed when they were killed and the brain examined to confirm the morphological phenotype of BSE and the presence of disease specific PrP by immunohistochemistry. A parallel titration in sinc[s7] (RIII) mice was conducted according to standard mouse end point titration protocols over a dilution range of 10^{-1} to 10^{-6}. Mice were inoculated by the i.c. and intraperitoneal (i.p.) routes simultaneously to maximise the efficiency of the assay .

Brain titres of $10^{3.3}$ mouse (i.c. + i.p) ID_{50}/g and $10^{6.0}$ cattle (i.c.) ID_{50}/g were established. The resultant value of the underestimation of the infectivity titre of BSE tissue when titrated across a species barrier in mice is therefore a factor of 500 fold (G.A.H.Wells and S.A.C.Hawkins, unpublished data). Expressed as relative titres, 10^{0} mouse (i.c./i.p.) LD_{50}/g is equivalent to $10^{2.7}$ cattle (i.c.) LD_{50}/g, or the limit of detection of the mouse bioassay (at approximately $10^{1.4}$ mouse [i.c./i.p.] LD_{50}/g) is equivalent to $10^{4.1}$ cattle [i.c.] LD_{50}/g. Additional assays of selected tissues from the original pathogenesis study by intracerebral inoculation of cattle initially confirmed infectivity only in certain tissues which were already found to be positive by the mouse bioassay. However, recent results (SEAC, 2002) show the presence of low levels of infectivity in bovine tonsil from cattle 10 months after experimental oral exposure to BSE infection. So far only 1 of the group of 5 intracerebrally inoculated recipient cattle has developed disease. SEAC (2002), considered that this finding was unlikely to be an experimental artefact or due to residual infectivity in inoculum. An overall review of the results of additional assays of selected tissues, from the pathogenesis study, by intracerebral inoculation of cattle (as of August 2002) is presented in **Table 5** (G.A.H.Wells and S.A.C.Hawkins, unpublished data).

That the relative degree of insensitivity of the mouse bioassay could explain the apparent absence of widespread LRS infectivity in BSE is not supported by the

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results of assays by intracerebral inoculation of cattle with pooled lymph nodes (retropharyngeal, mesenteric and popliteal) or pooled spleens from five terminal clinical cases of BSE. In this study survival data suggested that, if present, the concentration of infectivity in these tissues was, at least, less than one, and possibly less than 0.1 cattle (i.c) LD_{50}/g.