

Table 5: Bioassay of tissues from cattle exposed orally to BSE agent (Pathogenesis Study) by intracerebral inoculation of cattle (5 per inoculum group): details of inocula, according to sequential kill point of source cattle, inocula and inoculation dates (continued)

Inoculum (months p.i.)	Date of inoculation	Survival time ⁴ (months) up to 29/8/01
Kidney (26m p.i.)	18.2.99	31
Distal ileum (26m p.i.)	19.2.99	31
Peripheral nerve ² (26m p.i.)	22.2.99	31
Buffy coat (26m p.i.)	23.2.99	31
Salivary gland (26m p.i.)	25.2.99	31
Skin (26m p.i.)	1.3.99	30
Mesenteric lymph nodes (26m p.i.)	2.3.99	30
Spleen (26m p.i.)	10.3.99	30
Tonsil (26m .i.)	11.3.99	30
Caudal medulla (26m p.i.)	15.3.99	30
Spinal cord (26m p.i.)	16.3.99	30
Bone marrow (32m p.i.)	18.3.99	30
Bone marrow (22m p.i.)	24.3.99	30
Bone marrow (36m p.i.)	29.3.99	29
Bone marrow (26m p.i.)	31.3.99	29
Urine (18m p.i.)	17.8.99	25
Nictitating membrane (field case material)	13.3.00	18

- ¹ Pool of semitendinosus, longissimus dorsi and masseter muscles
- ² Pool of sciatic and radial nerves
- ³ Pool of prescapular and popliteal lymph nodes
- ⁴ Survival time of animals remaining in the experiment rounded to nearest whole month (see text)
- ⁵ No. cattle developing clinical disease/no. inoculated

From a titration of a pool of BSE affected bovine brain tissue by intracerebral inoculation of cattle a dose/incubation curve has been produced from which it may be possible to obtain an approximation of the titre of an inoculum by reference to incubation period data for that tissue. From the available data to date on the bioassay of Pathogenesis study tissues in cattle, tissues containing infectivity are: distal ileum, 6 m.p.i., 10 m.p.i. and 18 m.p.i. and brain stem/spinal cord, 32 m.p.i. The mean incubation periods for the tissues at these time points, when estimated from the dose/incubation curve for the cattle titration suggest titres of 10^1 - 10^2 (6.m.p.i.), 10^4 (10 m.p.i.), 10^3 (18 m.p.i.)_ and 10^3 - 10^4 (32 m.p.i.) respectively. This corresponds very approximately to RIII mouse incubation period data in as much as by the mouse bioassay a rising titre (reducing mean incubation) was indicated by the results of distal ileum assay from cattle 6 months and 10 months after exposure and a plateau of incubation period in mice inoculated with distal ileum from cattle 18 months after exposure. The estimated values in certain instances do however show up to a 1 log₁₀ discrepancy between the cattle and mouse infectivity data.

If one considers the currently available survival times for inoculated cattle in this cattle assay (Table 5) it becomes clear that, should there be any infectivity in the remaining tissue groups it would already be $<10^2$ cattle i.c. ID₅₀/g for most and considerably lower for some groups. A preliminary summary of infectivity classification for cattle tissues is given in Table 6.

II.6. BSE IN SHEEP: BIOASSAYS OF SHEEP TISSUES AFTER ORAL EXPOSURE TO THE AGENT OF BSE BY INOCULATION OF MICE.

- II.6.1. The report attached to the *Opinion of the SSC on Specified Risk Materials of Small Ruminants, adopted 13-14 April 2000* (EC 2000) states that from early results of the transmission of BSE to sheep studies (Sheep BSE pathogenesis experiment, carried out by the UK Institute for Animal Health -IAH) some ARQ/ARQ infected sheep have widespread PrP^{Sc} demonstrable in the lymphoreticular system tissues from 16 months after exposure, but there are, as yet, no corresponding bioassay results for infectivity. The report also stresses that this does not exclude finding infectivity or PrP^{Sc} at other (including younger) ages. Additional evidence, not cited in that report (Somerville *et al.*, 1997) demonstrated PrP^{Sc} in spleens of some QQ₁₇₁ Cheviot sheep infected with BSE.

The IAH sheep BSE pathogenesis experiment is ongoing. Immunocytochemical studies of tissues animals succumbing to BSE have been published (Foster *et al.*, 2001). The 7 animals that succumbed to BSE (6 are still alive) all showed PrP^{Sc} immunostaining in CNS and LRS tissues but not elsewhere. While the published results provide information only on clinical cases of experimental BSE in ARQ/ARQ Cheviot sheep (mean incubation period approximately 25 months after exposure to 5g oral dose) it is important to note that tissues from most major organs, including heart, lung, liver or thymus, showed no PrP^{Sc} immunostaining. Minimal staining was seen in glomeruli of the kidney. No evidence of PrP^{Sc} was found in any of the skeletal muscles tested, nor in reproductive tissues or skin.

It is of interest also that of the peripheral nerves examined (vagus, radial, sciatic) only the vagus, which has been proposed by many as implicated in the pathogenesis of scrapie after oral exposures, and not the somatic peripheral nerves, showed PrP^{Sc} immunostaining. Infectivity assays on a range of tissues from these animals are in progress. Studies on animals killed at intermediate times throughout the incubation period are not complete. Preliminary data support the findings of Jeffrey *et al.* (2001) which suggest that in some animals evidence of the presence of TSE infectivity (e.g. PrP^{Sc} immunostaining) can be detected in some lymphoid tissues from early on after infection.

- II.6.2. Interim updated results of studies by the VLA, UK of the tissue distribution of PrP^{Sc} (Jeffrey *et al.*, 2001) and/or infectivity (mouse bioassay) in Romney (ARQ/ARQ) and Suffolk (ARQ/ARQ) sheep orally exposed to the BSE agent (5g affected brain homogenate) (S. Bellworthy, unpublished data) have established the earliest evidence of the presence of agent in tissues as follows:

Romneys (current data on incubation period range: 20-37 months)

- Retropharyngeal lymph nodes (LN)	4 months after exposure
- Peyer's patch	4 months after exposure
- Spleen	10 months after exposure
- Mesenteric LN	16 months after exposure
- Ileocaecal LN	16 months after exposure
- Mediastinal LN	16 months after exposure
- Tonsil	16 months after exposure
- Submandibular LN	16 months after exposure
- Distal ileum(excluding Peyer's patches)	16 months after exposure
- Mesenteric LN	16 months after exposure
- Prescapular LN	16 months after exposure
- Broncho-mediastinal LN's	16 months after exposure
- Brain and spinal cord	16 months after exposure
- Liver (low level of infectivity)	16 months after exposure
- Intestine	16 months after exposure
- Vagus nerve	16 months after exposure
- Forestomachs	22 months after exposure
- Abomasum	22 months after exposure
- Coeliaco-mesenteric ganglion (sympathetic)	22 months after exposure

New Zealand Suffolk (current data on incubation period of initial clinical cases: 24 months)

- CNS (including spinal cord)	}	10m
- Retropharyngeal LN	}	
- Submandibular LN	}	
- Prescapular LN	}	
- Spleen	}	
- Mesenteric LN	}	
- Peyer's patch	}	
- Ileo-caecal LN	}	
- Tonsil	}	
- Brain		16m

It must be stressed that there is marked variation in PrP detection results between animals and infectivity bioassay has been conducted on tissue pools from multiple animals. In particular there is no constant pattern of LRS involvement.

This work has also demonstrated PrP^{Sc} immunostaining of neurons in the enteric nervous system (ENS) throughout the alimentary tract (least in forestomachs) in some Romney sheep, but not in sheep that lacked immunostaining in Peyer's patches.

No immunostaining has been detected thus far in thymus, even in clinical cases, nor in somatic peripheral nerve trunks (sciatic, phrenic) or nerve roots of the spinal cord.

There are no new data from this study with regard to possible skeletal muscle infectivity.

Similarly dosed ARQ/ARR (heterozygous for BSE/scrapie susceptibility) Romney sheep are currently approximately four years after dosing and remain healthy.

Sequentially killed animals from this component of the study have not, as yet, shown PrP^{Sc} in any tissues suggesting, at least, that infectivity is extremely low in tissues, certainly up to two years after challenge.

These data suggest that unlike the situation in cattle experimentally infected by the oral route with a relatively large exposure dose of BSE agent, the results in sheep indicate a potentially widespread involvement of lymphoid tissues early in the incubation period at least in ARQ/ARQ scrapie/BSE susceptible sheep. New data are consistent with the previously expressed view that BSE in sheep after oral exposure is pathogenetically closely similar to scrapie, particularly with respect to the tissue distribution of infectivity and/or PrP^{Sc}.

II.6.3. Little in terms of infectivity data can be drawn from the single instance of transmission of BSE by blood transfusion in sheep (Houston *et al*, 2000). The recipient sheep (New Zealand Cheviot ARQ/ARQ) developed clinical disease 610 days after transfusion with 400mL of blood from an infected donor sheep approximately halfway through a closely similar incubation period (629 days). There is insufficient information on the relative efficiencies of routes of infection with BSE in sheep, but one interpretation might be taken from the generally accepted differences between efficiency of routes of inoculation in experimental models. The difference between the efficiency of the oral route and the intracerebral route in cattle is in the range 10^5 to 10^6 (G.A.H. Wells and S.A.C. Hawkins, unpublished). A similar value is frequently cited for the difference in efficiency between such routes in mice. If we assume that the intravenous route is almost as efficient as the intracerebral route, and that this could apply equally to sheep, than in the study cited previously (Jeffrey *et al*, 2001) the oral dose of $10^{4.0} \times 5$ which gave a minimum incubation period of 20 months, the total infectivity contained in 400mL of blood, producing a similar incubation period, could be as low as 1-10 mouse ID₅₀ units. Notwithstanding discrepancies in making such calculations across sheep breeds this would certainly be undetectable by mouse bioassay.

II.6.4. Although no endpoint titration was conducted, incubation period data from primary transmission of infection from brain and spleen of sheep (Cheviot ARQ) infected intracerebrally or orally with BSE agent showed comparable incubation periods in each tissue (Foster *et al.*, 1996). These incubation periods were shorter than those obtained from the original primary transmissions of cattle BSE agent to mice (Fraser *et al.*, 1992) which gave endpoint titration results of at least $10^{5.1}$ (i.c.) LD₅₀/g. Experiments to compare the effects of i.c. and i.p. routes or their combination on incubation period in RIII mice (Bruce *et al.*, 1994) have shown slightly increased efficiency of detection of BSE infection (from cattle) with the combined route. It might be concluded, therefore, that the titre of infectivity in

the BSE affected sheep brain and spleen tested by Foster *et al* (1996) was of the order of 10^5 i.c./i.p. LD₅₀/g. Caution has been urged with regard to interpretation of incubation period assays in different tissues/organs since it has been shown that on a single pass of 263K hamster scrapie there was modification of the dose-response relationship for spleen compared to brain (Robinson *et al.*, 1990).

There are no titration data on tissues from sheep experimentally infected with BSE agent.

Mouse bioassay of tissues from the VLA study of oral exposure of Romney and Suffolk sheep to BSE agent (Jeffrey *et al* 2001) are incomplete but for some tissues of exposed Romney (ARQ/ARQ sheep) there is sufficient data on incubation period (S.Bellworthy, personal communication) to attempt approximations of titres of infectivity from RIII mouse dose response curves.

By 16 months after exposure (5g dose of $10^{4.0}$ mouse (i.c. + i.p.) ID₅₀/g) it appears that spleen is approaching a titre of approximately $10^{2.8}$ mouse (i.c. + i.p.) ID₅₀/g, lower at 10 months after exposure and increasing thereafter (data incomplete). Other lymphoid tissues at 16 months after exposure are probably $10^{1.0}$ but increasing thereafter and at 22 months after exposure (still preclinical) central nervous system infectivity is $\geq 10^3$.

No data are available as yet from clinically affected sheep (incubation periods 20-28 months (Jeffrey *et al* 2001)).

The Annex 3 of the Report: Pre-emptive Risk Assessment Should BSE in Small Ruminants be found under domestic conditions, adopted 8-9 February 2001 (EC 2001a), which is based on results of this study is, therefore, still applicable with regard to classification of tissue infectivity for Romney (ARQ/ARQ) sheep experimentally exposed to the BSE agent (Table 3).

II.6.5. In view of this apparent close similarity in the distribution of infection in tissue between experimental BSE in sheep and natural scrapie it would seem that further guidance on the probability and possible levels of infectivity in different tissues should be drawn from previous tabulations of scrapie infectivity in tissues of small ruminants (see Table 1 and Annex).

II.7. CONCLUSIONS

II.7.1. TSES IN SHEEP (AND GOATS)

Scrapie in small (sheep) ruminants

There are no new data from which to update the Table 1 and the Annex for infectivity of tissues of sheep for scrapie. These tables remain therefore valid as far as scrapie infectivity distribution is concerned.

BSE in small (sheep) ruminants

Recent data which would enable updating of sheep tissue infectivity titre tables for infection with the scrapie agent and for infection with the BSE agent are extremely limited. With respect to sheep experimentally exposed to the BSE agent interpretation of data set out above would suggest that infectivity titres in brain and spleen during the clinical disease phase may be comparable. Thus for BSE the possibility has to be considered that spleen (and possibly other lymphoreticular system tissues) may have to be regarded, together with CNS tissues, as containing a High level of infectivity. This is in contrast to previous data (Tables 1 and 2) in which spleen of sheep with scrapie has been assigned Medium infectivity. This clearly has implications for consideration of SRM for sheep where there is a probability of occurrence of BSE in sheep. This accepted, there are no new data from which to update the Tables 1 and 2 for infectivity of tissues of sheep for scrapie or BSE.

With respect to BSE in sheep, it would be prudent on the latest available evidence to adopt tabulations given at **Table 1** and the **Annex** as being probably as representative of BSE as scrapie with regard to distribution and level of infectivity in tissues. The single and important exception is that lymphoreticular tissues in BSE in sheep should provisionally at least, be considered comparable in their level of infectivity with central nervous system tissues.

II.7.2. BSE IN CATTLE:

A basis for producing cattle tissue infectivity tables for infection with BSE is emerging but the data are incomplete and much of the information emanates from a single study of the distribution of infectivity after experimental oral exposure. Available incubation period assay values from the few tissues containing infectivity in experimentally exposed cattle suggests that in most of the infected tissues infectivity is close to the limit of detection of the assay, even in central nervous system (**Table 4**). The early results of the re-evaluation of such tissues by bioassay in cattle (**Table 5**) compliment the mouse data, but such assays will not be completed for at least a further five years. Nevertheless, any further positive results would become available in that period. A tentative summary of available infectivity data for cattle with BSE is given at **Table 6**.

Table 6: Tentative summary of preliminary estimations¹ on classification of tissues of cattle according to infectivity after experimental oral or natural exposure to the agent of BSE.

Infectivity titre ² (approx. range)		Experimental			Natural (Clinical)
		Preclinical (months after exposure)		Clinical (months after exposure)	
Mouse	Cattle ³	(6-14)	(18)	(32)	(36-40)
High (10 ^{3.0} -10 ^{5.0})	High (10 ^{5.7} -10 ^{7.7})				Brain Spinal cord ?Retina (data not published)
Medium (10 ^{1.5} -10 ^{3.0})	Medium 10 ^{3.3} -10 ^{5.6})	Distal ileum (10 months)	Brain		
Low (≤10 ^{1.5})	Low (≤10 ^{3.2})	Distal ileum	Distal ileum	Brain Spinal cord Dorsal root ganglia	Brain Spinal cord Dorsal root ganglia Trigeminal ganglion Distal ileum Bone marrow (38 months)
Undetectable ?($<10^{1.0}$)	?($<10^0$)	For list of tissues see Tables 1, 5 & Annex			Retropharyngeal LN Mesenteric LN Popliteal LN For remaining tissues tested see Table 2 and associated references

1. Refer to **Tables 1, 5 and Annex** for further detail

2. The classification used is preliminary and arbitrary because of a skewed range of infectivity in cattle with BSE compared to sheep with scrapie. It does not correspond to the Groups or Categories used in **Table 1 and Annex**.

3. Values in bold in the table are based on bioassay in cattle.

III. THE SAFETY OF RUMINANT HEADS

Note: Particular note is drawn to previous definitions used in Opinions and Reports for the head and its anatomical parts. For the purpose of the current report, "head" and "entire head" are considered the same and include the whole head, including the tongue. The term "skull" in the bovine context is the head excluding cheek meat (Masseter muscle) and tongue. In small ruminants the term "skull" is the head, excluding skin and tongue.

III.1. INFECTIVITY IN RELATION TO INCUBATION PERIOD

III.1.1. Bovine

In relation to the head in cattle with BSE, infectivity is consistently detected in the central nervous system (CNS) in the clinical disease, both in natural and experimental cases. In the experimental disease in cattle infectivity is detected in the CNS prior to the onset of clinical signs. But, the Pathogenesis Study does not provide interpretable data on the relationship between the earliest detectable infectivity in CNS (or any other tissue) and incubation period after experimental oral infection of cattle with the agent of BSE. In naturally occurring BSE, the age at which brain material may contain infectivity is unknown and it is not possible to predict when a case of BSE will show infectivity in the CNS. In the experimental study of BSE in cattle after oral exposure, in which the lower limit of the incubation period range was 35 months, evidence of infectivity [by conventional mouse bioassay] in the CNS was detected at 32 months, but not at 26 months after dosing (Wells *et al.*, 1998). However, these two observations, of clinical onset and tissue infectivity, cannot be compared directly since (given the sequential kill protocol of the study) the incubation period range of all animals in the study cannot be determined. A preliminary estimate from dose response data of cattle infected orally with a dose of BSE infectivity closely similar to that administered to induce disease in the Pathogenesis Study (G. A. H. Wells, unpublished data) suggests a mean incubation of almost 45 months (range 33-55 months). Because there is no direct experimental data relating infectivity of tissues to incubation period in BSE there is no equation that might be applicable to calculate initial detectability of tissue infectivity in relation to incubation of the natural disease. However, in certain experimental mouse models of scrapie, after peripheral routes of exposure, a constant relationship can be shown between the initial detection of infectivity in CNS and incubation. Within the range of models examined, infectivity was detectable at approximately 54% of the incubation period (Kimberlin and Walker 1988; Kimberlin and Walker 1989). It is not known if such a constant relationship might be applicable to BSE of cattle, but data from naturally occurring sheep scrapie where the approximate incubation period is apparent a similar value of 50% has been suggested (Opinion on SRM of Small Ruminants Adopted 13-14 April 2000). Based therefore, on the overall knowledge gained from natural incidents of TSEs in animals, and on available data, it seems not unreasonable to accept that infectivity may be first *detectable* in the CNS in natural BSE well in advance of clinical onset. This might be as little as 3 months before clinical signs, by conventional mouse bioassay, but theoretically

at least, it could be 30 months, in an animal with an average estimated field case incubation of 60 months. BSE infectivity has been assayed in mice and cattle, providing evidence for a cattle-to-mouse species barrier of about 500 fold ($10^{2.7}$) (G. A. H. Wells, unpublished data) As the cattle-to-human species barrier is yet unknown (E.C., 1999), no calculation of infectivity risk for man from an estimated onset of detectable infectivity in cattle CNS can be made.

As indicated earlier, infectivity in trigeminal ganglia (anatomically located within the base of the skull) in experimentally induced BSE has been detected only in the clinical disease stage and is probably secondary to replication of agent in CNS.

III.1.2. Sheep

There is little new information as yet, but from the VLA's experimental study of BSE in sheep (exposed to a relatively large dose of 5g of infective brain tissue), it appears that after this dose, involvement of the lymph nodes of the head (retropharyngeal), can be as early as 17% (4 months in the specific study) of the incubation period, and CNS involvement may occur from 40-66% (10-16 months in the specific study) of the incubation period. Clearly, with a range of much lower exposures in field situations that might be anticipated in endemic BSE in sheep and possibly different susceptible PrP genotypes in sheep, there may well be proportionally longer incubation periods and correspondingly later involvement of the CNS. However, it must be considered that dissemination of agent to widespread lymphoid sites may be a relatively constant early event in incubation of scrapie and BSE in sheep but could be influenced by their genotype.

III. 2. FACTORS ASSOCIATED WITH AGE

Age-cut-off limits for the skull, central nervous system, eyes and tonsils for bovine, ovine and caprine animals below which age the named tissue is not considered a risk need to be determined on a case-by-case basis which takes into account the criteria of animal species, infectivity in relation to incubation period, factors associated with slaughter protocols and geographical risk level of the source country or region.

There are no new data on the age specific incidence of BSE which would suggest any change in the risk in relation to head tissues of cattle. It has been previously established that the incidence of clinical disease occurrence in cattle below 30 months of age is approximately 0.05%. Experimental data also suggests that after oral exposure of calves to BSE infection, doses of the order of 100g of high titre brain material are required to give an incubation period range with a minimum of approximately 30 months (G. A. H. Wells and S. A. C. Hawkins, unpublished data). There are no further data on tissue infectivity of cattle relative to age which would impact on previous recommendations on listing of SRM pertaining to the head.

The absence of evidence of naturally occurring cases of BSE in sheep or goats and the preliminary nature of information on the pathogenesis of experimentally

induced BSE in sheep prevent clear inferences regarding age factors and the relative infectivity of head tissues. It must be acknowledged that natural exposures to BSE agent via feed or through endemic infection of sheep would probably result in a mean incubation period much like that of naturally occurring scrapie and greater than those resulting from the experimental oral exposures to BSE infection for which there is some data (Foster et al., 1993, and above Bellworthy, personal communication). However, the interactions of dose and host genetics, constituting the variables of effective exposure, do not as yet allow the sort of assessments that have been made in the case of cattle with BSE. Because of this uncertainty and the potential for the involvement of lymphoid tissues of the head at an early stage of incubation in sheep with BSE, there is no basis on which to recommend an age cut-off for the small ruminant head SRM's were BSE to be confirmed in small ruminants. Clearly, this needs also to be considered in relation to the geographical risk of BSE occurring in sheep and, dependent on possible grading of risk, an age cut-off could be applied, as suggested previously [*Opinion and Report from the Working Group: Specified Risk Materials of Small Ruminants, Opinion adopted 13-14 April 2000*] (EC 2000), particularly with respect to certain unprocessed meat products, such as MRM and/or offals (presumed tongue) derived from the head.

III.3. FACTORS ASSOCIATED WITH SLAUGHTER PROTOCOLS

This aspect is discussed in detail in the *Scientific Opinion and Report on Stunning methods and TSE risks* adopted by the SSC on 10-11 January 2002 (E.C., 2002).

The definition of bovine skull (entire head less cheek meat and the tongue) and the related non categorisation of bovine tongue as SRM (see above Table 2) may remain appropriate in relation to certain slaughter procedures. The regulations currently allow removal of tongue provided it is not contaminated (and can be removed within the confines of the abattoir and before contact with heads from other animals might occur). While this remains a reasonable and practical procedure the tongue could nevertheless be at risk from cross contamination with CNS material as a result of leakage from the foramen magnum and notably from the stun hole if a penetrative method of stunning is used.

Furthermore, head meat under hygiene regulations must be removed in a cutting plant designed for the purpose. The movement of large numbers of heads which are often in contact with each other, from an abattoir to the plant increases the risk of cross contamination of the surface of the meat with CNS material. The risk is increased when any penetrative stunning method is used (in the same order of risk as is specified in the report) but is not zero if penetrative stunning is not used because CNS material can still leak from the foramen magnum. It is noted also that all visible nervous and lymphatic tissue must be removed before sale to the consumer and that these tissues (lymph nodes and peripheral nerves) have not revealed detectable infectivity in cattle with natural or experimental BSE.

Thus, there are circumstances where it could be prudent to include the tongue (the entire head) from cattle as SRM. This could be subject to exclusions on the basis

of the use of a non-penetrative stunning method, on an age basis and in relation to the status of the BSE epidemic of a particular country. That is, where evidence can be provided of a declining epidemic and all the necessary measures are consistently enforced (see below), because the incidence of disease (and thereby infection) is low and becoming lower with time in younger animals.

Under normal abattoir procedures there is no contact between gut tissues (the only other tissue known to contain infectivity during the incubation period of experimentally induced BSE) and the head.

The classification of skull as SRM in small ruminants (the head excluding skin and tongue) also necessarily excludes the tongue from the SRM list but because of practicalities of slaughtering it has been suggested that the entire head of small ruminants may be required to be included as SRM at all ages. This would be particularly so in a situation where BSE has been confirmed or is considerably likely to have occurred in a sheep population.

Cross contamination of tongue with CNS from penetrative stunning or from the foramen magnum decapitation is more likely in sheep than in cattle because of skinning of the head. Furthermore, if the CNS is infective then it is highly likely that all lymph nodes of the head, tonsils and possibly peripheral nerves will also contain infectivity.

Without penetrative stunning, the contamination risk is only marginally reduced.

III.4. CONCLUSIONS

There is no new evidence from tissue infectivity studies of cattle affected by or incubating BSE that any additional tissues of the head, other than those already designated, should be regarded as SRM. On the contrary, results of infectivity bioassays in cattle support the view that in the clinical disease stage of BSE, regional lymph nodes, including those of the head have no detectable infectivity at least by mouse bioassay. Completed results of mouse bioassays of pituitary, CSF, the cranial cervical ganglion, facial nerve, tongue, salivary glands and lymph nodes of the head have not revealed infectivity. Furthermore, assay results, of trigeminal ganglia suggest a low titre of infectivity only in the clinical disease stage, probably secondary to CNS involvement.

Results of assays in cattle of certain tissues from cattle taken during the incubation period of BSE after oral exposure, are awaited, but to date have confirmed infectivity only in those tissues in which infectivity had been detected by the mouse bioassay. Thus there is no new infectivity data for cattle to suggest that skeletal muscle, tongue or associated nerves should be considered SRM at any age.

Exclusion from SRM of bovine tongue and cheek meat remain justified providing contamination by CNS, introduced during slaughter, can be avoided.

The head SRMs if a BSE risk exists remain appropriate for bovines.

With respect to sheep, there is involvement of lymphoid tissue of the head at a relatively early stage of incubation in experimental BSE in sheep, consistent with the view that BSE in sheep has a pathogenesis with respect to tissue distribution of infectivity comparable with natural scrapie. Somatic peripheral nerve trunk infectivity, although categorised as “low” in scrapie, may be widespread in the carcase by the clinical disease stage. If, as seems likely, this results from “centrifugal” spread from the CNS and infectivity can be detected in the CNS in experimental BSE of sheep approximately 40-50% through the incubation period, infectivity may be present in somatic peripheral nerve fibres from this stage. These observations make it difficult to recommend an appropriate lower age limit for the exclusion of any head tissues of sheep if BSE were confirmed or considered likely in a given population also because of a possible influence on incubation and tissue distribution by the genotype of the sheep. Furthermore, as stated previously, the practicalities in slaughtering of small ruminants may also necessitate removal of the entire head as SRM at all ages.

Also, the risk of cross-contamination of tongue with tissues with likely infectivity from early in the incubation of BSE, with or without penetrative stunning, in small ruminants, is considered high.

Consequently, if BSE is considered to occur in sheep, the whole or entire head, including the tongue, of all ages of sheep might have to be included in SRM irrespective of slaughterhouse practices. Possible exception to this would require additional risk assessment specifically for the occurrence of endemic BSE in sheep and the application of a geographic BSE (sheep) risk assessment.

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ANNEX: Infectivity titres (bio-assayed in mice) in tissues from up to 9 Suffolk sheep (34-57 months old) and up to 3 goats (38-49 months old), at the clinical stage of natural scrapie compared with the titres in tissues from 1 or more confirmed cases of BSE (Re-edited but unammended from Kimberlin 1994)

Tissues	Titre (mean		SEM of (n) samples) ^a		Titre ^a
	Scrapie, sheep		Scrapie, goats		BSE, cattle
Category I					
Brain	5.6 ± 0.2	(51)	6.5 ± 0.2	(18)	5.3
Spinal cord	5.4 ± 0.3	(9)	6.1 ± 0.2	(6)	+ve
Category II					
Ileum	4.7 ± 0.1	(9)	4.6 ± 0.3	(3)	<2.0
Lymph nodes	4.2 ± 0.1	(45)	4.8 ± 0.1	(3)	<2.0
Proximal colon	4.5 ± 0.2	(9)	4.7 ± 0.2	(3)	<2.0
Spleen	4.5 ± 0.3	(9)	4.5 ± 0.1	(3)	<2.0
Tonsil	4.2 ± 0.4	(9)	5.1 ± 0.1	(3)	<2.0
Category III					
Sciatic nerve	3.1 ± 0.3	(9)	3.6 ± 0.3	(3)	<2.0
Distal colon	<2.7 ± 0.2	(9)	3.3 ± 0.5	(3)	<2.0
Thymus	2.2 ± 0.2	(9)	<2.3 ± 0.2	(3)	not done
Bone marrow	<2.0 ± 0.1	(9)	<2.0	(3)	<2.0
Liver	<2.0 ± 0.1	(9)	--		<2.0
Lung	<2.0	(9)	<2.1 ± 0.1	(2)	<2.0
Pancreas	<2.1 ± 0.1	(9)	--		<2.0
Category IV					
Blood clot	<1.0	(9)	<1.0	(3)	<1.0
Heart muscle	<2.0	(9)	--		<2.0
Kidney	<2.0	(9)	<2.0	(3)	<2.0
Mammary gland	<2.0	(7)	<2.0	(3)	<2.0
Milk*	--		<1.0	(3)	not done*
Serum	--		<1.0	(3)	<1.0
Skeletal muscle	<2.0	(9)	<2.0	(1)	<2.0
Testis	<2.0	(1)	--		<2.0

The data are taken from the following sources: sheep scrapie, Hadlow *et al* (1982); goat scrapie, Hadlow *et al* (1980); BSE, Fraser *et al* (1992); Fraser & Foster (1994), and Kimberlin (1994). The classification of tissues is according to the CPMP Guidelines (EC, 1991). The Table is from Kimberlin (1994) and has been reproduced previously as Table 3 in the SSC Opinion of 9 December 1997 providing a *Listing of Specified Risk Materials (re-edited 23 January 1998)* and in *SEAC Report 1994, (Table 5.2 Amended)*. The only positive bovine tissue (brain), for which a titre is quoted, is from Fraser *et al* (1992). The remaining tabulation for negative tissues of cattle provides the cut off of sensitivity of the assay according to standard calculation of the minimum detectable titre taking into consideration volume of inoculum used. The <1 and <2 entries quoted in the table are in the original paper. The <1 values may relate to the possibility that inoculum used for blood clot and serum was undiluted, but this is not stated in the source paper of the bioassay of tissues from clinical cases of BSE (Fraser and Foster 1994), or (Kimberlin 1994).

^aTitres are expressed as arithmetic means of log₁₀ mouse i/c. LD 50/g or ml of tissue (+ve > 2.0).

+ve = transmission positive but not titrated

NOTE: None of the bovine tissues in categories II and III and no tissues in Category IV had any detectable infectivity. The values shown are maxima based on the limits of detectability of the bioassay in mince (calculated for 30µl of inoculum injected intracerebrally).

* Data on the negative results of bioassay of milk from cattle with BSE were not available in Kimberlin (1994). Subsequently, negative results of bioassay in mice were published and cited by Kimberlin (1996), see Table 2 of Report.