

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(ARQ/ARQ) and Suffolk (ARQ/ARQ) sheep orally exposed to the BSE agent (5g affected brain homogenate) (S. Bellworthy, unpublished data) have established the earliest evidence of the presence of agent in tissues as follows: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
- Forestomaches	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. The data published by Houston *et al* (2000) and Hunter *et al* (2002) and show that a high volume blood transfusion from sheep to sheep can transmit a BSE or scrapie illness within the same species and that infectivity can be transmitted from blood taken during the asymptomatic incubation period of the disease of the donor sheep. Little in terms of infectivity data can be drawn from these results and 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 400 ml 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 1**).

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 1** 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 1** 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 current results of the re-evaluation of such tissues by bioassay in cattle (**Table 5**) compliment the mouse data. They show that low levels of infectivity (below those detectable by the mouse bioassay⁶) may be present in the palatine tonsil, but, to date not in any of the other tissues which gave negative results on mouse bioassay. The currently still ongoing ~~such~~ assays will not be completed for at least a further four 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**.

⁶ The cattle/mouse species barrier is estimated between 500 to 1000; a lack of detection of infectivity by the mouse bioassay may leave infectivity close to 1 oral infectious dose.

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 (6 months) Palatine tonsil (10 months)†	Distal ileum Brain Spinal cord Dorsal root ganglia	Brain Spinal cord Dorsal root ganglia Trigeminal ganglion Distal ileum Bone marrow (38 months)	
Undetectable ?($<10^{1.0}$)	Undetectable ?($<10^0$)	For list of tissues see Tables 1, 5 & Annex 1			Retropharyngeal LN Mesenteric LN Popliteal LN For remaining tissues tested see Table 2 and associated references

¹. Refer to **Tables 1, 5** and **Annex 1** 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 1**. Ranges of comparative values between mice and cattle are based on extrapolation from an anticipated 500 fold increased sensitivity over the mouse bioassay. They are an overestimate according to the cattle i.c. titration dose- response regression curve.

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

† Based on cattle i.c dose-response curve, infectivity is < 10 cattle i.c. ID₅₀/g

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 the initial time of 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.

However, the recent finding (SEAC, 2002) of BSE in 1 of the group of 5 cattle intracerebrally inoculated with palatine tonsil, from donor cattle 10 months after experimental oral exposure to BSE infection, with an incubation period of 45 months, suggests the possibility that BSE infectivity may be present in tonsils in natural cases of BSE from a young age, albeit probably at low levels.

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.

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).

So far, results of infectivity bioassays in cattle have supported the view that in the clinical disease stage of BSE, regional lymph nodes, including those of the head have no detectable infectivity. Furthermore, assay results of trigeminal ganglion suggest a low titre of infectivity only in the clinical disease stage, probably secondary to CNS involvement. However, whereas completed results of mouse bioassays of pituitary, cerebro-spinal fluid (CSF), the cranial cervical ganglion, facial nerve, tongue, salivary glands and several lymph nodes of the head from preclinical and clinical stages of experimental BSE in cattle have not revealed infectivity, there is now evidence from cattle-to-cattle transmission studies that the palatine tonsil may contain very low levels of infectivity at an early stage of the incubation period and that this may affect the safe consumption of tongue if there is a risk of contamination of this tissue.

Sheep and goats:

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).

Bovine skull:

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 not anymore 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). This would remain a reasonable and practical procedure only if contamination either by CNS material or by tonsil tissue is excluded. The tongue could indeed 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) or cross contamination with tonsil as a result of any method of removal of the tongue that did not ensure careful separation of the tongue from all tonsillar tissues.

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. 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.

However, while there are still no new infectivity data from assay of tissues in cattle to suggest that skeletal muscle, tongue or associated nerves should be considered SRM at any age, the hazard identification and risk assessment carried out on behalf of the German authorities (BvGG, 2002) show that the SSC's statement of 10-11 January 2002 that the "*Exclusion from SRM of bovine tongue and cheek meat remains justified providing contamination by CNS, introduced during slaughter, can be avoided*" may not necessarily be appropriate considering the long list of critical points in the process of slaughtering the animal, the removal, storage and transport of the head and harvesting the tongue and cheek meat. The safe harvesting of head tissues would require strict and complex procedures which may not always be realistic under field conditions and which would require major efforts in terms of supervision and control.

Small ruminant skulls:

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.

Avoidance of penetrative stunning only marginally reduces the contamination risk”.

III.4. CONCLUSIONS

There is preliminary new evidence from studies of BSE tissue infectivity by assay of tissues in cattle suggesting a need to review the head tissues of bovine animals which are currently designated as SRM. Concerning the bovine head, Commission Regulation (EC) Number 270/2002 of February 14, 2002 designates the skull including brain⁷ and eyes, dura mater, pituitary gland and the tonsils of animals over 12 months of age, as SRM. For the UK and Portugal (GBR IV) the Regulation designates the entire head (including brain, eyes, trigeminal ganglia and tonsils), excluding tongue, of animals over 6 months of age, as SRM. So far, results of infectivity bioassays in cattle have supported the view that in the clinical disease stage of BSE, regional lymph nodes, including those of the head have no detectable infectivity. Furthermore, mouse assay results of trigeminal ganglion suggest a low titre of infectivity only in the clinical disease stage, probably secondary to CNS involvement. However, whereas completed results of mouse bioassays of pituitary, cerebro-spinal fluid (CSF), the cranial cervical ganglion, facial nerve, tongue, salivary glands and several lymph nodes of the head from preclinical and clinical stages of experimental BSE in cattle have not revealed infectivity, there is now evidence from cattle-to-cattle transmission studies that the palatine tonsil may contain very low levels of infectivity and that this may be present at an early stage of the incubation period. This may affect the safe consumption of tongue if there is a risk of contamination of this tissue. It also implies that tonsil be regarded as SRM from animals of any age.

Furthermore, the SSC’s statement of 10-11 January 2002 that the “*Exclusion from SRM of bovine tongue and cheek meat remains justified providing contamination by CNS, introduced during slaughter, can be avoided*” may not necessarily be appropriate anymore considering the long list of critical points in the process of slaughtering the animal, the removal, storage and transport of the head and harvesting the cheek meat. The safe harvesting would require strict and complex procedures which may not always be realistic under field conditions and which would require major efforts in terms of supervision and control.

⁷ The TSE/BSE *ad hoc* Group considers in this respect the brain and connected tissues as the central nervous system in the skull.

The TSE/BSE *ad hoc* Group therefore considers that:

- (1) the tonsil of a bovine animal of any age should be regarded as posing a risk.
- (2) the tongue of animals certified safe for human consumption does indeed not pose a risk if contamination with CNS and tonsil material is avoided for animals of any age, but this would imply that the harvested section of the tongue is shortened [to the “short tongue”] to avoid, by a cautious margin, removal with the tongue of that part of the root of the tongue containing lingual tonsil.
- (3) cheek meat of animals certified safe for human consumption, which is collected as part of a different process, does not pose a risk if a wide range of precautions to avoid cross-contamination is taken. The feasibility of implementation of these precautions under field conditions may be questioned and would require to be previously verified.

The other 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 1: 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 unamended from Kimberlin 1994)

Tissues	Titre (mean \pm SEM of (n) samples) ^a				Titre ^a
	Scrapie, sheep		Scrapie, goats		
Category I					
Brain	5.6 \pm 0.2	(51)	6.5 \pm 0.2	(18)	5.3
Spinal cord	5.4 \pm 0.3	(9)	6.1 \pm 0.2	(6)	+ve
Category II					
Ileum	4.7 \pm 0.1	(9)	4.6 \pm 0.3	(3)	<2.0
Lymph nodes	4.2 \pm 0.1	(45)	4.8 \pm 0.1	(3)	<2.0
Proximal colon	4.5 \pm 0.2	(9)	4.7 \pm 0.2	(3)	<2.0
Spleen	4.5 \pm 0.3	(9)	4.5 \pm 0.1	(3)	<2.0
Tonsil	4.2 \pm 0.4	(9)	5.1 \pm 0.1	(3)	<2.0
Category III					
Sciatic nerve	3.1 \pm 0.3	(9)	3.6 \pm 0.3	(3)	<2.0
Distal colon	<2.7 \pm 0.2	(9)	3.3 \pm 0.5	(3)	<2.0
Thymus	2.2 \pm 0.2	(9)	<2.3 \pm 0.2	(3)	not done
Bone marrow	<2.0 \pm 0.1	(9)	<2.0	(3)	<2.0
Liver	<2.0 \pm 0.1	(9)	--		<2.0
Lung	<2.0	(9)	<2.1 \pm 0.1	(2)	<2.0
Pancreas	<2.1 \pm 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 mice (calculated for 30 μ l 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.

Annex 2:

HARVESTING OF CHEEK MEAT OF CATTLE
SPECIFICALLY: LIST OF CRITICAL HYGIENE POINTS IN HARVESTING OF
BOVINE HEADS AND CHEEK MEAT OF CATTLE

Report submitted on 25 April 2002 by the German federal Institute for Consumer Health Protection and Veterinary Medicine (BvGG) to the Government of the federal Republic of Germany

Rapporteurs:

Dr L. Ellerbroek

Dr I. Schütt-Abraham

As instructed, we herewith deliver the following opinion, in agreement with the Federal Institute for Meat Research (BAFF).

In the framework of talks between the Federation and the *Länder* on 15 April 2002 at the BMVEL, we discussed how to proceed with the transposition of Regulation (EC) 270/2002 of 14 February 2002 amending Regulation (EC) 999/2001. The amended Regulation provides that the entire head (without tongue) must be removed in the slaughterhouse as specified risk material, unless a Member State permits the harvesting of cheek meat or tongues in cutting plants specifically authorised for this purpose.

Considerable quantities of meat are currently harvested from bovine heads. According to the Federal Statistical Office, a total of 4 114 093 cattle and 485 43 calves were slaughtered in Germany in 1998. According to estimates furnished by the economic operators concerned, half of the harvested bovine heads come from animals aged over 30 months, mainly cows. Depending on age and sex, up to 6 kg meat can be harvested from a bovine head. This includes approximately 2 kg (internal and external) cheek meat, approximately 2 kg neck fat, approximately 1 kg skull meat (i.e. meat attached to the skull), approximately 200 g temple meat and approximately 1 kg snout meat; at present this meat is mainly used in the feedingstuffs industry (as feedingstuffs for animals not intended for human consumption).

Since the derogation in Regulation (EC) 270/2002 applies only to cheek meat of cattle and the harvesting of neck fat, skull meat, temple meat and snout meat will not be permissible in future even under the derogation, the amount of meat harvestable from bovine heads will be reduced to a maximum of 2 kg per head. However, in individual cases it will be impossible to monitor compliance with the restriction prescribing the harvesting of cheek meat only!

In our report of 10 January 2001 (Ref.: BgVV 5222-04-133846) we already indicated a number of critical points where meat can be contaminated with the BSE agent during slaughter. Bearing in mind the usual cattle slaughtering practices in Germany, contamination with BSE risk material cannot be ruled out, particularly when harvesting and processing bovine heads.

In order to prevent contamination in general and contamination with BSE risk material in particular, we consider that comprehensive precautionary measures are of the essence. Notably, care should be taken to avoid contamination of meat with fluids containing brain tissue residue.

The Scientific Steering Committee (SSC) came to the same conclusion and stated in its opinion of 9 December 1997 that the entire head excluding the tongue is to be considered as “organs” with high BSE-infectivity. This is because of the possible risk of contamination with the TSE agent when harvesting heads and subsequent handling. During usual slaughtering practice, contamination of the meat with tissue which may have a very high degree of BSE-infectivity in the case of infected animals is unavoidable. When the head is removed fluid spills out through the foramen magnum and the spinal cord channel; besides, spinal cord tissue is cut. In addition, in the case of captive bolt stunning, blood containing brain material may spill through hole in the forehead, contaminate the skin of the scalp and enter the meat during subsequent handling of the head.

However, depending on the stunning practice and slaughtering technique, the extent of this external contamination with blood, fluid and brain material can vary. In individual cases, brain material may even gush out of the opening as a result of the way stunning is performed. During subsequent skinning of the head this contamination inevitably reaches the meat and may subsequently be spread over the entire surface of the head.

The extent of the potential hazard for consumers as a result of the TSE agent entering the meat following captive bolt stunning depends on the content of infectious prion proteins in the brain and hence on (a) the probability that an animal has been infected and (b) the incubation stage. Reliable estimates of infection probability are not possible given the current number of BSE cases in Germany. However, since most cattle were obviously infected while still calves (Heynkes, 2001: Most BSE cattle were infected as calves, <http://www.heynkes.de/peaks.htm>) and the diffusion of the pathogens from the intestines to the brain along the nerve tract takes a certain time, the risk also depends on the age of the slaughtered animal. This was taken into account by specifying the age limit for the removal of the CNS as SRM (cattle aged over 12 months).

According to the SSC (The risk of dissemination of brain particles into the blood and carcass when applying certain stunning methods; SSC report of 10-11 January 2002, Part II, VII.4), several risk (GBR) levels can be distinguished as regards contamination of meat with the TSE agent following captive bolt stunning. For GBR level III countries (including Germany), the SSC has adopted an age limit of 30 months for cattle. The SSC recognises that the TSE risk is less for cattle aged under 30 months, and higher in the case of cattle aged over 30 months. This estimate is based on the result of infectivity tests in which infection was identified in the brain of cattle infected as calves at the earliest 32 months subsequent to infection (Wells, G.A.H., Hawkins, S.A.C., Green, R.B., Austin A.R., Dexter, I., Spencer, Y.I., Chaplin, M.J., Stack, M.J. and Dawson, M., 1998: Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet. Rec.* 142, S. 103-106) and the results of BSE tests of routinely slaughtered cattle. However, the calves referred to by Wells et al. were not infected until they were four months old. Hence these tests suggest that the brain can be infected already after a 28 month incubation period. However, under practical conditions

infection is possible already in first month of life (cf. Heynkes, 2001). Besides, BSE cases with clinical symptoms were also found in Germany even in animals aged under 30 months. The latest BSE case in Great Britain was even younger. In Great Britain during 1986-2002 there were at least 18 cases of BSE in animals aged under 30 months; the youngest animal was only 20 months old (<http://www.defra.gov.uk/animalh/bse/index.html>). Besides, the currently available BSE tests detect infection only at an advanced stage of incubation, and at the earliest six months before outbreak of the clinical disease. Currently one cannot reliably say how long after a BSE infection BSE pathogens will be detected in the brains of infected animals.

In a further opinion of 13 December 2001 (Report on TSE infectivity distribution in ruminant tissues - state of knowledge, December 2001), the SSC indicates (Section III.3 - Factors associated with slaughter process) in particular the possibility of contamination during the transport of bovine heads.

Besides the possibility of contamination with brain material released through the frontal hole in the skull caused by bolt stunning there is a very high likelihood of the meat being contaminated with a BSE-agent containing material released through the foramen magnum. The current practice of suspending the severed head by the nasal septum in such a way that contamination of the meat with blood, fluid and brain material from the foramen magnum is prevented during skinning is not in our opinion satisfactory. This also applied to heads which have been skinned while still attached to the carcass and which are suspended from the lower jaw symphysis. Since the shot hole and the foramen magnum are not on the same plane but rather at right angles to one another on the head, there is no way of positioning the head in such a way that the spillage or leakage of fluid or CNS tissue fragments can be avoided. The situation is aggravated because the shot hole equalises the pressure so that it is easier for the fluid to escape, whereas if there is only one aperture a certain quantity will first be released but then further release is prevented as a result of the increase in underpressure.

One way of preventing contamination of meat with the BSE agent, which several parties have proposed, is to use a seal (fleece, stopper, plastic foam, etc.) to close both the frontal hole (caused by perforation of the skull during bolt stunning) and the foramen magnum. This procedure might considerably reduce the contamination of cheek meat with brain material. However, the stopper would not only have to be impermeable immediately after insertion into the hole of the unskinned head but also survive the skinning process unscathed. While full external sealing of the hole with a stopper would be effective if bolt stunning is correctly performed - because of the relatively small and circular aperture (the presence of hair on the forehead more or less ruling out other seals) - there is no guarantee that stunning will indeed be performed properly.

Besides, because of inter-animal variations in the anatomical conditions of the foramen magnum it may be quite difficult to position an impermeable and durable stopper. Another problem with bolt stunning is the opening of the frontal sinus, which can connect with the nasal cavity. As mentioned above, the stopper must be able to withstand difficult transport conditions and it must be removable so that BSE samples can be taken in cases when sampling is not performed directly after severing the head.

The attached table provides details on individual critical hygiene points in connection with slaughter, transport, BSE sampling and meat harvesting of bovine heads. The risks associated with current practices are set out and consequences and possible remedies proposed.

Summing up, the procedures presented for reducing contamination of cheek meat by the BSE agent are of a provisional nature and their effectiveness will have to put to the test. Besides, even with the aid of the proposed measures and the restriction of cheek meat extraction to cattle of less than 30 months of age, contamination of meat cannot be fully ruled out.

In our view, the following procedures for reducing contamination of cheek meat of cattle are appropriate as an initial approach:

- harvest only the masseters;
- harvest only meat from animals less than 30 months of age;
- stunning should not be performed with a penetrating bolt (this is probably the most important measure, since opening the cranial cavity not only creates another aperture through which CNS material can escape but also equalises pressure in the cranial cavity, facilitating the release of CNS fragments and fluid);
- removal of head: sever neck musculature and spinal marrow using separate knives (“green”, “red”), store/clean and disinfect the utensils in separate sterilisation basins;
- remove horns without opening the cranial cavity;
- mechanically remove the skin over the head;
- directly move the head to a “screened” cleaning chamber, suspended (nose upwards) without contact with wall;
- take BSE sample immediately after removal of head followed by sealing of the foramen magnum;
- transport and further processing in slaughterhouse with head suspended from the snout or corner of lower jaw;
- handle only in splash-proof head chamber ;
- clean only inside of head (nose, oral cavity) with underpressure;
- avoid all other treatment, including external washing;
- avoid use of water under high pressure when cleaning;
- do not remove eyes;
- avoid all unnecessary contact with head;
- use separate knives (“green”, “red”) when cutting the tongue and removing the tonsillar ring;

- always use fresh knife when inspecting the meat. First apply incisions to the external masseters, then remove tonsils;
- in the case of animals to be tested for BSE: do not use the so-called water or pressurised air method; take samples from head in suspended position; as regards sampling utensils (spoon, “cork borer”) ensure that effective disinfection equipment is present (such as sterilisation basin with hypochlorite solution);
- when storing and transporting heads ensure that they are not suspended over one another and that they do not touch one another; ideally heads should not be transported at all;
- transport heads to cutting plant suspended side by side without touching one another.

In the interests of preventive consumer protection, if these measures should fail to prevent contamination of cheek meat with CNS tissue the derogation should not be availed of and, in the case of cattle aged over 12 months, the entire head without tongue, but with the brain, eyes, trigeminal ganglia and tonsils, should be classified as specified risk material.

Table: Critical stages in the harvesting and processing of bovine heads during slaughter, transport and harvesting of meat

Critical hygiene point	Risks	Consequences/possible remedies
1) <u>Captive bolt stunning</u>	Contamination of adjacent skin and slaughter area Contamination of the environs of the shot hole following removal of skin → Cross contamination hazard	- Avoid penetrative stunning
2) Removal of horns	Opening of the cranial cavity → Cross contamination hazard	- Horn pincer should not be applied lower than the base of the horn; avoid opening cranial cavity - If cranial cavity is opened : no further handling of the head
3) Removal of head Spinal cord is severed with a knife; the same knife is also used on the carcass	Severing of the spinal cord channel with release of fluid and contamination of the exposed areas of meat on the neck with the released fluid and the knives contaminated with spinal cord tissue → Cross contamination hazard	- Sever the vertebral canal as final stage in removal of head after severing the soft parts - Use fresh knife for every carcass and ensure BSE disinfection of the knives used before reuse (ensure that there is a suitable number of single-use knives for the batch) - Remove contaminated meat areas after sealing the foramen magnum (also with single-use knives) - Use separate knife to remove head and for further handling of head - Use separate sterilisation basin for this knife only - Seal perforated cranium (bolt shot hole and foramen magnum) to reduce contamination

Critical hygiene point	Risks	Consequences/possible remedies
4) Suspension of head	Contamination of the exposed areas of meat on the neck as a result of released fluid and spinal cord particles	- Contamination-free transport and suspension of head
<u>5) Processing of head</u>	When working on tables contamination of surfaces with released CNS/fluid is unavoidable → Cross contamination hazard	- Work only on suspended head - Avoid all handling which is not essential for harvesting the cheek meat (meat inspection)
6) Cleaning of head (external washing) The skinned head is washed off externally and internally with a view to cleaning, partly in head-cleaning chambers, partly without screening beside the slaughter line (near the skinned carcasses) After cleaning, the head is placed on the organ conveyor belt (transport to meat inspection point at end of slaughter line)	Dissemination of CNS material from the shot hole to the surface of the head during external washing Splashing water, aerosol formation → contamination of neighbouring carcass surfaces. Cleansing liquid (with blood/brain/spinal cord particles) is not collected separately and removed, but flows over floor → spread of contamination by staff, when high-pressure cleaners are used walls and ceilings are contaminated; during transport on conveyor belt liquid drips off and spills onto the floor During work on tables, contamination of table surfaces with released CNS/fluid unavoidable → Cross contamination hazard	- Avoid external washing (this will require hygienic removal and skinning of head) - Use fully sealed head-cleaning chamber (in EU-approved plants, this has largely been realised); meat inspection of head (incisions to external masseters, etc.) at this place

Critical hygiene point	Risks	Consequences/possible remedies
<p>7) Cleaning of head (internal washing)</p>	<p>When head is shifted, contamination hazard as a result of the release of fluid/CNS material from bolt shot hole and/or foramen magnum</p> <p>Brain particles may flow into the nasal cavity through bolt shot hole (the nasal cavity being connected to the brain via the cranial cavity)</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> - Avoid penetrative stunning - Avoid re-positioning of head
<p>8) Meat inspection</p>	<p>Surface contamination via deep cuts in musculature</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> - Use a fresh knife for each animal: first apply incisions to external masseters, then remove tonsils, fresh knife for each animal
<p>9) BSE-sampling in the slaughterhouse</p> <p>Sampling is performed using sampling instruments (special spoons) or by applying pressure through the hole in the front of the cranium caused by bolt stunning, as a result of which the caudal brain regions are forced out through the foramen magnum</p>	<p>Placing of head on table surface which has been contaminated with spinal cord tissue and fluid following sampling</p> <p>Brain and fluid are deposited on the meat; this applies in particular in the cases of removal techniques in which the brain is forced out of the cranium</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> - Take BSE sample immediately after severing the head and before sealing the foramen magnum - BSE sampling and position should be so designed as to rule out contamination of the meat with brain material - Avoid sampling techniques in which the brain is forced through the cranium - Take BSE sample from suspended head, if possible before final severing of the head

Critical hygiene point	Risks	Consequences/possible remedies
<p>10) Transport of head from slaughterhouse to cutting plant</p> <p>Transport of bovine heads on so-called Christmas trees or in several layers suspended from a hook or in cages</p>	<p>Release of CNS and fluid with brain particles as a result of shaking during transport</p> <p>Contamination of lower heads by upper heads</p> <p>Spinal cord protein is released together with the fluid from the frontal bolt shot hole and from the foramen magnum</p> <p>Incomplete sealing of the frontal bore shot hole and the foramen magnum</p> <p>Transport of heads suspended on so-called Christmas trees or in several layers on a hook or transport in cages (the heads are stacked in several layers) leads to the release of brain material</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> - Check inter alia that the frontal bolt shot hole and the foramen magnum are thoroughly sealed - Remove visibly contaminated bovine heads from the batch - Avoid use of so-called Christmas trees and cages/do not transport heads in several layers suspended from hooks - Transport heads suspended side by side but not touching one another - Place collection basin under the heads
<p>11) Removal of eyes, removal of mouth etc..</p>	<p>Exposure of optic nerves and hence creation of new apertures in cranium</p> <p>Contamination hazard when head is shifted</p> <p>Contamination hazard through contaminated hands</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> - Avoid removal of eyes and all further handling
<p>12) Cutting of head to harvest cheek meat</p> <p>Fixing of head and manual removal of meat from head</p>	<p>Spinal cord protein (possibly containing BSE prions) sticks to the knife; hot water in the sterilisation basin denaturises the protein but does not inactivate the BSE pathogen</p> <p>→ Cross contamination hazard</p>	<ul style="list-style-type: none"> - Thorough (mechanical) cleaning of knife before hot-water treatment - Treatment of heads in batches by age classes (<12 months; 12 to 24 months; > 24 months).

