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of BSE infectivity closely similar to that administered to induce disease in the Pathogenesis Study (G. A. H. Wells, unpublished data) suggests a mean incubation of almost 45 months (range 33-55 months).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 remains 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 considerely 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 brain[7] 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.

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[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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