EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions

OPINION ON

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

ADOPTED BY

THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 10-11 JANUARY 2002

PREAMBLE:

The Scientific Steering Committee received between May and September 2001, several requests for scientific advice on issues related to TSE infectivity distribution in ruminant tissues and the safety of ruminant tissues with regard to TSE risk. The requests relate to (1) TSE infectivity in sheep and cattle tissues and (2) the Safety of ruminant heads.

The requests were made to answer different concerns from different Commission Services[1] (e.g., safety of food or medical devices) and therefore different individual reports were initially prepared. As the answers to each of these requests are based on broadly the same scientific literature and the same range of experiments, they have been integrated into one single report and opinion.

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1 mainly the Health and Consumer Protection and the Enterprise Directorates General.

OPINION

The Scientific Steering Committee (SSC) was invited:

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

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

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

The SSC invited the TSE/BSE *ad hoc* Group to prepare a scientific report that could serve as the basis for preparing an answer to the above question. This report is attached. It was finalised by the TSE/BSE *ad hoc* Group at its meeting of 13 December 2001.

The SSC adopts the following answers to the above questions:

(1) Tissue infectivity tables applicable for small ruminants.

Scrapie in small ruminants. There is no new evidence that became available since February 2001 and the SSC's therefore considers that the table attached to its pre-emptive risk assessment of 8-9 February 2001 remains valid. It is annexed as **Table 1** for ease of reference.

BSE in small ruminants. The SSC considers that, pending more experimental data becoming available, it would be prudent on the latest available evidence to adopt tabulations given at **Table 1** as being probably as representative of BSE as scrapie with regard to distribution and level of infectivity in tissues. *However, 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.*

(2) <u>Tissue infectivity tables related to BSE in cattle.</u> Available 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. The early results of the re-evaluation of such tissues by bioassay in cattle 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 naturally acquired BSE is given at **Table 2** (Tissues with no infectivity from confirmed cases) and **Table 3** (Preliminary estimates of tissue infectivity after experimental and natural exposure).

(3) **Possible consideration as specified risk material of the entire head of cattle, sheep and goats, including skeletal muscle, tongue and associated innervation.**

Regarding ***cattle*** affected by or incubating BSE, the SSC considers that there is no new evidence from tissue infectivity studies that any additional tissues of the head (additional to: brain, eyes, dura mater, pituitary gland and skull) 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. Completed results of mouse bioassays of pituitary, cerebro-spinal fluid (CSF), the cranial cervical ganglion, facial nerve, tongue, salivary glands and lymph nodes of the head from preclinical and clinical stages of experimental BSE in cattle have not revealed infectivity. Furthermore, assay results of trigeminal ganglion 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 remains justified providing contamination by CNS, introduced during slaughter, can be avoided. The head SRMs remain thus appropriate for bovines.

With respect to ***sheep***, there is involvement of lymphoid tissue of the head at an 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 carcass 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.

Furthermore, the practicalities in slaughtering of small ruminants may 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 be present in sheep, the whole or entire head, including the tongue, of all ages of sheep should be included in the list of SRMs irrespective of slaughterhouse practices, until evidence to the contrary becomes available.

Very limited data are available for goats. The conclusions for sheep are therefore considered to be a reasonable approximation also for goats.

Table 1: Natural scrapie in sheep and goats: classification of tissues by agent titre in Swiss mice and by age, in pre-clinical and clinical cases of Scrapie in Suffolk sheep and in goats[2] (Re-edited but unammended from Annex: Opinion on The Policy of Breeding and Genotyping of Sheep, 22-23 July 1999) (EC 1999)

Infectivity titres*: A = high ($\geq 10^{4.0}$)
B = medium ($10^{3.2} - 10^{4.0}$)
C = low ($\leq 10^{3.2}$ or unknown)
D = undetectable

	PRE-CLINICAL				CLINICAL	
Age (months)	**≤ 8**	**10-14**[3]	**25**	**> 25**	**34-37**	**38-39**
Numbers positive / examined	**0/16**	**8/15**	**1/13**	**1/6**	**9/9**	**3/3**
Brain					A	A
Brain (medulla)		D	C			
Brain (medulla / di-encephalon)			C			
Brain (cortex mid-brain)			D			
Pituitary					C	B
Spinal cord			D		A	A
Cerebro-spinal fluid					C	C
Sciatic nerve					C	C
Thymus	D		D		C**	C**
Thyroid					D	
Spleen	D	B	C		B	B
Tonsil	D	C	B		B	
Lymph node (RP/MP)	D	B	B		B	B
Lymph node (BM)		D	C		B	B
Lymph node (PS/PF)	D	C	C			
Lymph node (PF, 1/9 negative)					B	
Lymph node (PS, 2/9 negative)					B	
Lymph node (supra-mammary)			D		C	B
Colon-proximal		B	B		B	B
Colon-distal		D	D		C	C
Ileum	D					
Ileum-distal		B	B		B	
Ileum-proximal						B
Rectum-distal					B+	B
Pancreas					C**	
Adrenal			D		C	C
Nasal mucosa			D		C	C

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

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

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

Infectivity titres*: A = high ($\geq 10^{4.0}$)
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	PRE-CLINICAL				CLINICAL	
Age (months)	**≤ 8**	**10-14**[4]	**25**	**> 25**	**34-37**	**38-39**
Numbers positive / examined	**0/16**	**8/15**	**1/13**	**1/6**	**9/9**	**3/3**
Bone marrow					C**	D
Liver					C**	
Blood clot		D			D	D
Serum		D				D
Salivary glands			D		D	D
Saliva					D	
Muscle- skeletal					D	D
Heart					D	
Kidney					D	D
Lung					D	
Ovary					D	D
Uterus					D	D
Placenta					C**°	
Fetus					D	
Mammary gland					D	D
Colostrum				D		
Milk						D
Semen vesicle					D	
Testis					D	
Faeces		D				D

* = Log_{10} mouse intracerebral LD/50 per 30 mg tissues; (titres given as approximate ranges)
** = trace or exceptional
\+ = Not assayed but high content of lymphoreticular tissue
° = negative in other studies
MP = Mesenteric/portal
PF = Prefemoral
CSF = Cerebro-spinalfluid
PS = Prescapular
LN = Lymph node
RP = Retropharyngeal
BM = Bronchomediastinal

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

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

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

Table 3: Tentative summary of preliminary estimations* on classification of tissues of cattle according to infectivity after experimental oral or natural exposure to the agent of BSE.

Infectivity titres**:

A = high:	$10^{3.0} - 10^{5.0}$ in mouse;	$10^{5.7} - 10^{7.7}$ in cattle ***
B = medium	$10^{1.5} - 10^{3.0}$ in mouse;	$10^{3.3} - 10^{5.6}$ in cattle ***
C = low	$\leq 10^{1.5}$ in mouse;	$\leq 10^{3.2}$ in cattle ***
D = undetectable		
? = data not published		

	EXPERIMENTAL				NATURAL
				clinical	**clinical**
months after exposure	**6-14**	**18**	**32**	**36-40**	**-**
Brain			***B*** / C	C	***A***
Retina					?
Spinal cord			C	C	A
Dorsal root ganglia			C	C	C
Trigeminal ganglion				C	
Ileum-distal	***B*** / C	***C***		C	
Lymph node (retropharyngeal)					***D***
Lymph node (Mesenteric)					***D***
Lymph node (Popliteal)					***D***

For the list of tissues in which no detectable infectivity was found: see tables 1 and 2 of this opinion and table 5 and the Annex of the attached report.

* Refer to the report for further detail

** 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**.

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