資料Ｎｏ．３

# ＢＳＥ感染牛の組織別の牛に対する感染性について

せき柱の感染性は、せき柱に残存する背根神経節によるものである。１頭当たりのせき柱約２０kg当たりに対し、背根神経節は約３０ｇである。

## １　実験感染及び自然感染させた牛の組織別感染性の暫定的評価
（ＥＵ科学運営委員会：２００２年５月報告から抜粋）

| | 実験感染 | | | 自然感染 |
|---|---|---|---|---|
| | | | 有症状 | 有症状 |
| 暴露後経過月 | 6-26ヶ月 | 32ヶ月 | 36-40ヶ月 | ― |
| 脳 | ― | 中又は低 | 低 | 高 |
| せき髄 | ― | 低 | 低 | 高 |
| 背根神経節 | ― | 低 | 低 | 低 |

注：「―」は感染性なし

## ２　ＢＳＥ牛１頭中の感染価分布
（ＥＵ科学運営委員会：１９９９年１２月報告から抜粋）

| 組　織 | １グラム当たりの感染価※1 | 重　量（ｋｇ）※2 | １頭当たりの感染価 |
|---|---|---|---|
| 脳 | １０ | ０．５ | ５，０００ |
| せき髄 | １０ | ０．２ | ２，０００ |
| 背根神経節 | １０ | ０．０３ | ３００ |

※１　１感染価：５０%の牛が発症する量（$ID_{50}$）（経口投与）

※２　体重５３７ｋｇの牛の場合

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
**C1 - Follow-up and dissemination of scientific opinions**

# OPINION AND REPORT

# ASSESSMENT OF THE HUMAN BSE RISK POSED BY BOVINE VERTEBRAL COLUMN INCLUDING DORSAL ROOT GANGLIA.

## ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE AT ITS MEETING OF 16 MAY 2002

## OPINION

In the light of (a) the results of the BSE monitoring carried out so far and in particular the age distribution of positive BSE cases and (b) the recent assessment of the possible risk posed by bovine dorsal root ganglia in Ireland, the Scientific Steering Committee (SSC) was asked:

(1) to assess a recent quantitative assessment of risk from possible BSE infectivity in dorsal root ganglia, produced for the Food Safety Authority in Ireland.

(2) to give a quantitative assessment of the BSE risk [for human consumers] posed by bovine vertebral column including dorsal root ganglia.

(3) to address the question of whether evidence can be found to justify an increase of the current age limit of 12 months for treating vertebral column as SRM in bovine animals? If yes, to which extent and under which conditions? If no, what would be the conditions for increasing the age limit?

On the basis of the attached report of the TSE/BSE *ad hoc* Group, the SSC answers the above three questions as follows:

1) *Regarding parts (1) and (2) of the mandate on the quantitative assessment of risk from possible BSE infectivity in dorsal root ganglia*

The SSC considers that the risk assessment produced for the Food Safety Authority in Ireland is scientifically sound but applies only to Ireland. The produced risk estimates cannot be generalised for other countries, because consumption patterns[1] and BSE incidence are different.

Preparing similar assessments for other countries, or for the EU's continental part as a whole, would require the collection of the appropriate information for these countries or for the EU, part of which is not likely to be readily available but would need to be collected by surveys.

An essential element in such risk assessment is the moment into the incubation period as from which the spinal cord and dorsal root ganglia can contain infectivity. Data from a single experiment, mostly referred to as the *cattle pathogenesis study*, has in the past been interpreted as showing that detectable infectivity in the spinal cord is only present in the last months of the incubation period, which would justify the consumption of meat-on-the bone or of vertebral column bones [for gelatine and fat production] up to an age of 12 months before the expected possible appearance of clinical signs. The SSC considers however that the BSE cattle pathogenesis study cannot be exploited to express the time of detectable infectivity in the Central Nervous System tissues as a fraction of the total incubation period and that the limited number of animals used in this study do not allow to conclude that infectivity is absent in the spinal cord until a few months before clinical signs are manifested.

---

[1] Quantities consumed by individuals, parts of the carcass used for the production of meat-on-the-bone, frequency of consumption of meat-on-the-bone and other carcass parts to which dorsal root ganglia may be attached, age distribution of the animals slaughtered, ...

From experiments with other animal species and for which more data are available (e.g., mice, hamster, primates, sheep, ...) it may be concluded that the assumption made by the SSC on 12 January 2001 - i.e., that in general, as a reasonable worst case assumption, the dorsal root ganglia and the spinal cord are considered to pose a higher risk as from the second half of the incubation period - remains valid.

2) *Regarding part (3) of the mandate on evidence to justify an increased age limit above 12 months for treating vertebral column as SRM in bovine animals.*

   a. The SSC considers that neither the available results of the pathogenesis research nor the results of the 2001 rapid BSE testing programme reflecting the exposure situation until early 1998 permit to conclude on the question whether or not an increase of the age limit above 12 months for treating vertebral column as SRM in bovine animals born before the feedban is justified.

   b. For cattle born after the total feed-ban the SSC confirms its opinion of 12 January 2001 that such animals, *if the feedban is properly implemented,* should bear a low risk of being infected. A guidance on proper implementation of feedbans is provided in "*Effective feed ban: Guidance note for third countries, 18 July 2001*".[2]

   c. The SSC recommends that the various Member States assess the human exposure risk before and after the implementation over time of consecutive risk management measures as listed in the opinion of 12 January 2001, including the total feedban.

      Based on such assessments an evaluation for the whole EU will become possible and the SSC will be able to revisit and update its opinion on human BSE risk related to Specified Risk Materials, including dorsal root ganglia.

[2] web-site address: http://europa.eu.int/comm/food/fs/bse/index_en.html.

# REPORT ON THE

# ASSESSMENT OF THE HUMAN BSE RISK POSED BY BOVINE VERTEBRAL COLUMN INCLUDING DORSAL ROOT GANGLIA.

## FINALISED BY THE TSE/BSE AD HOC GROUP MEETING AT ITS MEETING OF 2 MAY 2002

**TABLE OF CONTENTS**

I. BACKGROUND AND MANDATE

II. QUANTITATIVE ASSESSMENT OF THE BSE RISK FOR CONSUMERS POSED BY BOVINE VERTEBRAL COLUMN INCLUDING DORSAL ROOT GANGLIA.

III. INTERPRETATION OF THE BOVINE BSE PATHOGENESIS STUDIES WITH RESPECT TO THE TIME AFTER EXPOSURE AT WHICH INFECTIVITY CAN BE DETECTED IN THE CENTRAL NERVOUS SYSTEM AND SPINAL AND CRANIAL GANGLIA.

IV. ANALYSIS OF THE AGE DISTRIBUTION OF RAPID-TEST BSE-POSITIVES IN EUROPEAN UNION IN SECOND SEMESTER OF 2001.

V. CONCLUSIONS

VI. LITERATURE

ANNEXES

## I. BACKGROUND AND MANDATE

1. In its opinion of 9 December 1997 on Specified Risk Material (SRM), the SSC recommended that the vertebral column should be regarded an SRM because of the close association and possible contamination with the spinal cord and dorsal root ganglia.

In its opinion on Human Exposure of December 1999, the SSC stated that the brain, the spinal cord, the dorsal root ganglia respectively represent 64.1%, 25.6% and 3.8% of the total infective load in a BSE infected animal. It recognised in that opinion that based on quantitative data, the brain, spinal cord, dorsal root ganglia and trigeminal ganglia constitute the major hazards for direct human consumption.

*From the SSC's opinion and report* of 11 January 2002 on TSE Infectivity distribution in ruminant tissues *the following can be deduced:*

> Available data are incomplete and much of the information emanates from a single study of the distribution of infectivity after experimental oral exposure. Values of infectivity for the few tissues containing infectivity in experimentally exposed cattle, estimated from incubation period assay in mice, suggests that in most of the infected tissues infectivity is close to the limit of detection of the assay, even in central nervous system. Preliminary 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. In the experimental study of the pathogenesis of BSE in cattle after oral exposure to a relatively high dose of untreated BSE infective material, 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, this study does not provide interpretable data on the relationship between the earliest detectable infectivity in CNS (or any other tissue) and incubation period, because the incubation period range of all animals in the study cannot be determined (because of the sequential kill design of the study). In naturally occurring BSE, the age (or stage of incubation) at which CNS material may contain infectivity is unknown and it is not possible from available results of experimental studies to predict when a case of BSE will show infectivity in the CNS. 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). From experimental studies of scrapie in rodents, after peripheral routes of exposure, and from data on naturally occurring sheep scrapie (Opinion on SRM of Small Ruminants Adopted 13-14 April 2000) infectivity in CNS occurs approximately 50% through the incubation period. It is not known if such a constant relationship might be applicable to BSE of cattle, but based therefore 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.

In its opinion of 12 January 2001 on the safety with regard to BSE of certain bovine tissues and certain animal derived products, the SSC considered that in general, as a reasonable worst case assumption, the dorsal root ganglia and the spinal cord are considered to pose a higher risk as from the second half of the

incubation period. The SSC concluded that meat on the vertebrae of animals above 12 months of age should not be consumed whenever it cannot be demonstrated that the animal is unlikely to be incubating BSE. The SSC also stated that the results of monitoring with rapid tests should add information in this respect.

Following the SSC opinion of 12 January 2001 (EC, 2001), bovine vertebral column was classified as SRM in animals over 12 months. Derogation was foreseen in certain countries and under certain conditions. Furthermore, a review of the age limit for removal of vertebral column was foreseen, in the light of the statistical probability of the occurrence of BSE in relevant age groups of the Community's bovine population. This review should be based on the results of BSE monitoring.

In the monitoring carried out between January and December 2001, some 8.5 million rapid BSE tests were carried out on bovine animals in the Community. Target groups for testing were healthy stock over 30 months (animals over 24 months in certain Member States) and risk animals and suspect cases.

2. In October 2001, Commission Services received the results of an *Assessment of risk from possible BSE infectivity in dorsal root ganglia* (DRG), carried out for the Food Safety Authority of Ireland (DNV, 2001). Although this risk assessment is applied to the specific conditions of Ireland, it provides also a methodological and scientific update of the DNV's risk assessment of 1997 (DNV, 1997) The latter risk assessment and its outcome has been widely quoted and exploited in the SSC's opinion of 14 April 2000 on *The UK decision to lift the ban on the consumption of meat on the bone* (E.C., 2000a) and in the SSC opinion of 15 September 2000 on *Export from the uk of bone-in veal* (E.C., 2000b).

3. In the light of (a) the results of the BSE monitoring carried out so far and in particular the age distribution of positive BSE cases and (b) the recent assessment of the possible risk posed by bovine dorsal root ganglia (DNV, 2001), the Scientific Steering Committee is asked:
   - to assess a recent quantitative assessment of risk from possible BSE infectivity in dorsal root ganglia, produced for the Food Safety Authority in Ireland. The assessment is attached.
   - to give a quantitative assessment of the BSE risk [for human consumers] posed by bovine vertebral column including dorsal root ganglia.
   - to address the question of whether evidence can be found to justify an increased age limit for treating vertebral column as SRM in bovine animals? If yes, to which extent and under which conditions? If no, what would be the conditions for increasing the age limit?

4. A report was prepared under the joint rapporteurship of Dr.G.Wells (bovine BSE pathogenesis aspects) and Dr.S.Bird (data analysis). The report was discussed, finalised and adopted by the TSE/BSE *ad hoc* Group at its meeting of 2.05.02.

## II. QUANTITATIVE ASSESSMENT OF THE BSE RISK FOR CONSUMERS POSED BY BOVINE VERTEBRAL COLUMN INCLUDING DORSAL ROOT GANGLIA.

The only available scientific analyses on the quantification of the BSE risk for consumers resulting from exposure to the bovine vertebral column and dorsal root ganglia, are the quantitative assessments carried out by DNV for the UK (in 1997) and Ireland (2001). These reports estimate, for *consumers of these countries*, the risk of consuming infected dorsal root ganglia and the corresponding levels of infectivity expressed as human oral $ID_{50}$. The DNV (1997) report also estimates the risk resulting from a vertebral column contaminated with residual spinal cord material.

Depending upon the scenario and assumptions made[3], the results of these assessments are as follows:

UK, in 1997 (all consumed meat from animals below 30 months) (DNV, 1997):

- The median value of the total infectivity in DRG to which the whole UK population would have been exposed in 1997 (= the societal risk) ranges from 0.004 to 0.25 human oral $ID_{50}$ units (with 0.05 human oral $ID_{50}$ units for the most likely scenario). The corresponding 95% percentiles are $2x10^{-5}$ to 63 human oral $ID_{50}$ units.
- The median value of the average individual risk ranges from $7x10^{-11}$ to $5x10^{-9}$ human oral $ID_{50}$ units consumed in 1997 by each individual. The corresponding 95% percentiles are $4x10^{-13}$ to $1x10^{-6}$ human oral $ID_{50}$ units.

Ireland, in 2000 (DNV, 2000): (before the obligation to remove the vertebral column from animals above 12 months and before the generalised rapid testing of animals, but taking into account that approx. 89% of the Irish meat production is exported):

- The median value of the total infectivity in DRG to which the whole Irish population would have been exposed in 2000 (= the societal risk) ranges from 0.008 to 0.6 human oral $ID_{50}$ units. The corresponding 95% percentiles are $5x10^{-5}$ to 110 human oral $ID_{50}$ units.
- The median value of the average individual risk ranges from $3x10^{-9}$ to $2x10^{-7}$ human oral $ID_{50}$ units consumed in 2000 by each individual. The corresponding 95% percentiles are $2x10^{-11}$ to $4x10^{-5}$ human oral $ID_{50}$ units.

The above risk estimates cannot be generalised for other countries, because consumption patterns[4] and BSE incidence are different. Preparing similar assessments for other countries, or for the EU's continental part as a whole, would require the preliminary collection of the corresponding information, part of

---

[3] The scenarios and assumptions cover, for example: the ratio boneless meat / meat-on-the bone; % of dorsal root ganglia removed with the bones, % of DRG eaten with the bone-in meat, etc.

[4] Quantities consumed by individuals, parts of the carcass used for the production of meat-on-the-bone, frequency of consumption of meat-on-the-bone and other carcass parts to which dorsal root ganglia may be attached, age distribution of the animals slaughtered, ...

which is not likely to be readily available but would need to be collected by surveys.

It can, however, be reasonably assumed that the *current* risk (in 2002) in the EU Member States is unlikely to be significantly higher than risks in the UK in 1997 and in Ireland in 2000. At the time of these assessments, the BSE incidence in these 2 countries was higher than in any other EU country (with the exception of Portugal as compared to Ireland) and no improved surveillance using rapid BSE tests were in place.

Nevertheless, the estimated risks are not zero. However, as they result mainly [exclusively] from the infectivity present in animals in the last 12 months of incubation, it can be concluded that dorsal root ganglia and spinal cord residues on vertebral column bones do not pose a risk if they are sourced from animals that are sufficiently early into the incubation period for the risk that infectivity is present in those tissues being negligible.

## III. INTERPRETATION OF THE BOVINE BSE PATHOGENESIS STUDIES WITH RESPECT TO THE TIME AFTER EXPOSURE AT WHICH INFECTIVITY CAN BE DETECTED IN THE CENTRAL NERVOUS SYSTEM AND SPINAL AND CRANIAL GANGLIA.

a. The SSC opinion of 11 January 2002 provides the state of knowledge in December 2001 on TSE Infectivity distribution in ruminant tissues (E.C., 2002.) It summarises the completed results of the bioassay of tissues from cattle experimentally infected with BSE agent and killed sequentially (VLA Pathogenesis study) by inoculation of mice. It also provides interim results of the bioassay of tissues from cattle in the Pathogenesis study by inoculation of cattle.

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

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

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

   Tissues were sampled aseptically for infectivity assays in mice. After each sequential kill, inocula were prepared from 44 tissues, representing principally the lymphoreticular system (LRS), the peripheral nervous system (PNS) and the central nervous system (CNS), alimentary tract, striated muscles and major viscera. All inocula were prepared as ten per cent suspensions in saline, with the inclusion of antibiotics for certain tissues. Single tissue inoculum pools were made from the exposed cattle at each time point.

Inocula were similarly prepared from control animals, but from single tissues of each animal. Test and control inocula were injected by intracerebral (20µl) and intraperitoneal (100µl) routes into inbred mice for standard qualitative assay of infectivity.

Qualitative assays by the i.c. and i.p. inoculation of mice (RIII and/or C57BL) of a large range of tissues from the UK VLA Pathogenesis study of BSE have been completed (Wells *et al.*, 1996, 1998, 1999 and unpublished data). No titration of infectivity in positive tissues has been carried out but an approximation of infectivity titre has been obtained from mean incubation period and data on titrations of BSE affected brain in the same mouse strains. For all tissues in which infectivity has not been detected it can be stated that they contain less than $10^{1.4}$ mouse (i.c./i.p.) $\log_{10}$ $LD_{50}$/g.

A study (VLA/CSG SE1821) of infectivity of a pool of brains from BSE affected cattle by simultaneous titration in cattle and mice, was also conducted to provide a measure of the underestimation of the titre of infectivity in tissues across the species barrier in mice (described in detail in E.C 2002). This established that the underestimation is a factor of 500 fold (G.A.H.Wells and S.A.C.Hawkins, unpublished data). Expressed as relative titres, $10^0$ mouse (i.c./i.p.) $LD_{50}$/g is equivalent to $10^{2.7}$ cattle (i.c.) $LD_{50}$/g, or the limit of detection of the mouse bioassay (at approximately $10^{1.4}$ mouse [i.c./i.p.] $LD_{50}$/g ) is equivalent to $10^{4.1}$ cattle [i.c.] $LD_{50}$/g. From this study also an approximate dose-incubation curve for infectivity of brain from BSE affected cattle was constructed. Following these results additional assays of selected tissues from the original pathogenesis study were conducted by the intracerebral inoculation of cattle. As yet this assay study has confirmed infectivity only in certain tissues which were already found to be positive by the mouse bioassay.

Utilising available dose-incubation response data from titrations of BSE affected brain material in mice and in cattle (see Sections II.4 and II.5 and **Tables 4-6** of EC, 2002) Results, relevant to spinal cord, from the above experimental studies, together with available equivalent information for natural clinical cases of BSE (Foster and Fraser 1994) are summarised in **Table 1**. The Table provides an iterim classification of the levels of infectivity detected in a small number of animals and apparent differences between tissues from experimental studies and natural cases cannot be considered significant. They may relate to stage of clinical disease and other factors.