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Opinion of the Scientific Panel on Biological Hazards

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Opinion of the Scientific Panel on Biological Hazards on the assessment of the likelihood of the infectivity in SRM derived from cattle at different age groups estimated by back calculation modelling [1]

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Summary

The European Food Safety Authority (EFSA) was invited to provide an opinion on the assessment of the likelihood of the infectivity in SRM derived from infected cattle at different age groups, estimated by a back calculation modelling as indicated in "approach 4" in the annex to the Opinion of 28 April 2005 of the Scientific Panel on Biological Hazards on the assessment of the age limit in cattle for the removal of certain Specified Risk Materials.

Following extensive and repeated scientific discussions it appeared that scientific consensus at the back calculation modelling could not be achieved. This possibility was already mentioned in the previous Opinion as one of the limitations of the approach. Therefore, the assessment of the likelihood of the infectivity in SRM derived from cattle at different age groups was based on data of experimental pathogenesis and dose/incubation period studies and on the descriptive epidemiology of BSE with respect to risk populations, to age at infection and age at detection by clinical and active surveillance.

Experimental studies of the distribution of BSE infectivity relative to the period post exposure in cattle have been conducted in the UK and more recently in Germany. Complete data from sequential kill pathogenesis studies and additional data from attack rate studies are now available to provide the basis for a revised calculation on incubation period ranges according to low (1g of fresh brain material obtained from clinical cases) and high (100g) infectivity doses. Moreover, initial data from the German pathogenesis study using the high dose have become available. These studies have used detection of the disease-associated prion protein (PrP^{TSE}) in tissue as a proxy but not perfect surrogate for infectivity.

In view of the the results, the panel considers its earlier opinion of 28 April 2005 still valid, which concluded that the likely detectable infectivity in the CNS appears at about ¾ of the incubation time. The situation has not changed either with regard to tissues comprised of, or containing, lymphoid tissue designated as SRM.

There are now completed pathogenesis data available from the experimental low-dose scenario that appears now more likely to resemble the field situation than an exposure in the field with a high dose of BSE infectivity. If PrP^{TSE}/infectivity is modelled as present in CNS at 75% of the incubation period, as in the previous opinion, it can be predicted that the infectivity would be sub-detectable or still absent in CNS in cattle aged 33 months.However, when interpreting the significance of the experimental data some points require to be considered, including the field occurrence of at least one BSE infected case in animals younger than 33 months in EU cohorts born after 2000, and the problem that failure to detect PrP^{TSE} does not guarantee absence of infectivity in a tissue.

The BSE epidemic is on decline in the different EU Member States, which is linked to a reduction in exposure. However, there is good reason to group member states for separate considerations or as individual cases. To date, the three youngest out of 22 BSE infected cases in cattle born after 2000 were aged 32, 36 and 39 months, respectively. Another case tested positive at an age reported as 25 months but there is uncertainty about its age. The number of cattle infected with BSE is likely to continue to reduce. It is now apparent that cases detected by active surveillance may be closer to clinical onset than previously estimated.

Key Words: BSE, Specified Risk Material (SRM)

1. Introduction

Since BSE was reported for the first time in 1986 in the UK, the EU has developed a comprehensive set of Community measures on TSEs in order to protect human health from BSE and to control and eventually eradicate TSEs in animals. That legislation has continuously been reviewed in the light of new scientific evidence, the evolution of the TSE epidemics and the practical implementation in the field. In the last few years, the Commission has generated 70 primary and implementing acts which set stringent measures at Community level.

The key piece of legislation to protect human and animal health from the risk of BSE and other TSE's was adopted on 22 May 2001. This Regulation (EC) No 999/2001 of the European Parliament and of the Council lays down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies, and is commonly known as the "TSE Regulation". This Regulation was applicable, within a very short time frame, as of 1 July 2001.

The different risk reducing measures listed hereafter are not exhaustive but summarize the most important key measures to combat and eradicate BSE:

– Removal and destruction of tissues which are potentially harbouring the BSE agent: the Specified Risk Material (SRM). Since 1 October 2000, these tissues must be removed from the food and feed chains to avoid the risk of recycling the TSE agent;

– A total EU wide ban (January 2001) on the use of processed animal protein in feeds for any animals farmed for the production of food, with some exceptions (use of fishmeal in non-ruminants).

– A comprehensive surveillance program including the testing of all risk animals over 24 months of age (fallen stock, emergency slaughtered animals and animals with clinical signs at ante-mortem inspection) and of all healthy slaughtered bovine animals above 30 months of age

– Eradication measures following the detection of a BSE case in a holding, including the killing and destruction of animals of birth and feed cohorts and offspring of female BSE cases.

– A series of measures to control TSE in sheep and goats including monitoring, killing and destruction of animals in infected flocks and breeding for resistance.

Since the implementation of the TSE Regulation in 2001, more than 50 million of adult bovine animals have been tested across the EU and around 7.000 cases have been detected. A constant decline (about 35 % per year) in the number of cases has been recorded: from 2.167 cases in 2001 to around 520 cases in 2005. Only 22 cases were diagnosed born after the total feed ban.

Under the terms of reference of the mandate, the European Food Safety Authority (EFSA) is invited to provide an opinion on the assessment of the likelihood of the infectivity in SRM derived from infected cattle at different age groups, estimated by back calculation modelling as indicated in “approach 4” in the annex to the Opinion

of 28 April 2005 of the Scientific Panel on Biological Hazards on the assessment of the age limit in cattle for the removal of certain Specified Risk Materials:

Estimation of the risk for each using a conventional epidemiological/risk assessment process. The process will include estimation of the risk for each age group using both observational and experimental data. The process can be either deterministic (point estimates) or stochastic (distribution) for the input/output.

Requirements:

- *Specific assumptions in terms of incubation period, infectivity, and population dynamic.*
- *Surveillance and experimental data to initiate selected inputs.*
- *Risk assessment modeling either in qualitative or quantitative types.*
- *Expertise in conducting risk assessment modeling with consideration as to input from experts.*

Advantage:

- *Precise risk estimates for each age group that can be quantitative with the potential to obtain distributions that can be used in the risk management decision process.*
- *Science-based approach to determine the relative magnitude of the risk excluding zero risk.*

Limitations:

- *Model assumptions are critical and require substantial planning as well as inputs from various experts and specialists. Consensus will be difficult if not impossible. Data acquisition for this model represents a serious technical problem.*
- *The risk assessment modeling is a dynamic process and once begun will be never-ending. The users therefore could get frustrated.*
- *Output from the model can be interpreted differently by some users. Some interpretations may be erroneous. Therefore, team involvement is important in the interpretation and writing of the final outcome.*
- *The effectiveness of the control measures introduced during the period of the data used for this calculation is not considered. Thus, there is a potential for biased estimations of the risk.*

The SRM or Specified Risk Materials mentioned in this report are according to the list given in Regulation (EC) 999/2001

Table: **List of tissues designated as Specified Risk Material (SRM) in bovine animals according to Regulation (EC) 999/2001**

Bovine tissues	**Age of bovine animals**
Tonsils	All ages
Intestines (from the duodenum to the rectum)	All ages
Mesentery	All ages
Skull (excluding the mandible)	Over 12 months
Brain	Over 12 months
Eyes	Over 12 months
Spinal cord	Over 12 months
Vertebral column*	Over 24 months

* Excluding the vertebrae of the tail, the spinous and transverse processes of the cervical, thoracic and lumbar vertebrae and the median sacral crest and wings of the sacrum, but including the dorsal root ganglia

Measuring infectivity: **Infectivity**

With respect to the data used in this report, the detection of misfolded host prion protein (PrP^{TSE}) as the infectious agent has proven to be a reliable indicator of infectivity, and PrP^{TSE} results are presented in parallel with bioassay data. The correlation between the PrP^{TSE} PrP amount and infectivity depends on the type of TSE agent. Analytical sensitivity of abnormal PrP^{TSE} PrP biochemical detection is still lower than most efficient bioassays hence failure to detect PrP^{TSE} does not guarantee absence of infectivity in a tissue. New methods of detection of abnormal prion protein (e.g. PMCA, Castilla *et al* 2005) will require further refinements of robustness and repeatability before they can be used to quantify prion protein. Bioassay in rodents can, in itself, be insensitive to infectivity compared to transmission studies of scrapie and BSE within the same species (e.g. sheep and cattle). Transgenic mice over-expressing ovine or bovine prion protein have been used to improve sensitivity and the efficiency of transmission from cattle or sheep tissues. However, these transmissions in terms of human or native species risk must be considered in the context of the general exposure risk. While absolute quantification of prions by biochemical methods is difficult, and the experiments needed to correlate their outputs to bioassay titres costly and time- consuming, measurements of abnormal PrP in two tissues of the same animal may be compared as a first approach to an assessment of the ratio of infectivity in each tissue, and their intrinsic relative risk following exposure to humans.

Referring to the WHO guidelines on tissue infectivity distribution in Transmissible Spongiform Encephalopathies (2006) (High-infectivity tissues, Lower-infectivity tissues, and Tissues with no detectable infectivity), it should be taken into account that categories of infectivity are not the same as categories of risk, which require consideration not only for the level of infectivity in a tissue, but also of the amount of

that tissue to which a person or animal is exposed, and the route by which infection is transmitted.

Current SRM risk management measures.

The SSC opinion of 9 December 1997 and the following revisions led to the management decision to set an age limit for the removal of SRM (excluding intestine and tonsils) at 12 months.

Following the conclusions of the EFSA opinion of 28 April 2005, the Commission proposed an increase of the age limit for the removal of the vertebral column of bovines to 24 months. The proposal received a favourable opinion from the Member States and the new age limit has been applicable from 1 January 2006.

Data from the on-going BSE monitoring programme

From 2001 to 2005, a total of 51,089,354 bovine animals were tested in the EU in the framework of the BSE monitoring programme. Of these 7,093 animals were positive. These included 1,117 out of 44,470,300 healthy slaughtered animals (25 per million) and 3,559 out of 6,434,001 risk animals (553 per million), while testing schemes differed between MS (Germany tested younger healthy stock than most MS, and the UK older healthy stock during much of its Over Thirty Months Scheme.

In the passive collection of data for the surveillance framework (animals reported as BSE suspects by the farmer or the veterinary practitioner and subject to laboratory examination) 15,122 bovine animals were tested and 2,354 were positive. In addition, of 169,931 animals tested in the framework of culling of animals with an epidemiological connection to a BSE case 63 turned out to be positive (371 per million).

Detailed information on the the BSE monitoring programmme, the age distribution of animals tested and on the age distribution of the positive cases for the period 2001-2005 (partially 2006) is appended (see tables in annex 1, 2 and 3.)

Evaluation of the overall situation by the Commission

According to Regulation (EC) 999/2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (the TSE Regulation), the age for the removal of the bovine vertebral column may be adjusted by amending this Regulation in the light of the statistical probability of the occurrence of BSE in the relevant age groups of the Community's bovine population, and based on the results of BSE monitoring programme as established by the Regulation.

The Communication from the Commission of 15 July 2005 on the future of TSE measures, the “TSE roadmap”, sets the strategic goal, with regard to SRM, to ensure and maintain the current level of consumer protection by continuing to assure the safe removal of SRM but modify list/age based on new and evolving scientific opinion. It is also stated that any amendment of the current list of specified risk material should be based on new evolving scientific knowledge while maintaining the existing high

level of consumer protection within the European Union. In addition, data from BSE active monitoring and surveillance should also be used to revise SRM policies.

A scientific assessment of the likelihood of the infectivity in SRM derived from infected cattle at different age groups will be a very useful tool in the framework of the discussions on the future of SRM measures envisaged by the "TSE roadmap".

2. Terms of Reference

The European Food Safety Authority (EFSA) is invited to provide an opinion on the assessment of the likelihood of the infectivity in SRM derived from infected cattle at different age groups, estimated by a back calculation modelling as indicated in "approach 4" in the annex to the Opinion of 28 April 2005 of the Scientific Panel on Biological Hazards on the assessment of the age limit in cattle for the removal of certain Specified Risk Materials.

3. Assessment

The assessment of the likelihood of the infectivity in SRM derived from cattle at different age groups estimated by a back calculation modelling should be based on data of ongoing experimental pathogenesis and dose/incubation period studies and on knowledge of the epidemiology of BSE with respect to risk populations, to age at infection and age at detection by clinical and active surveillance.

In accordance with the recommendations given in the 2005 opinion, a back calculation approach has been attempted for the assessment of the age-specific risk. During further work-up of the mandate, it became obvious that the modelling part would need detailed input of a specific working group. This independent WG would have to agree on the modelling approach to identify the data needs and any data gaps for the model. This is similar to the approach followed by the QRA sheep in the EFSA 2007 opinion. Ideally, this WG would be able to use a large body of specific surveillance data from all member states and model the future expected number of cases in the different cohorts.

The establishment of such an independent modelling-oriented WG has been attempted during the current mandate, but has failed so far due to the unavailability of key persons. As an alternative, the WG considered a less complex deterministic modelling approach to predict the number of BSE cases in age cohorts for the coming years. While this model was developed, its design and results did not meet unanimous agreement within the WG. Discussions within the WG indicated that the assumptions behind the predictive model, as developed, were not unanimously acceptable. Further, it was concluded that, without further elaboration, suitable modelling could not be undertaken at this time by the current WG.

These difficulties had been anticipated in the EFSA 2005 opinion, where it is stated as "limitations" that "*Model assumptions are critical and require substantial planning as well as inputs from various experts and specialists. Consensus will be difficult if not impossible. Data acquisition for this model represents a serious technical problem….. Output from the model can be interpreted differently by some users. Some interpretations may be erroneous.*"

Hence, the WG proposed to address the current mandate by focussing on the analysis of the BSE risk according to age based on data from experimental pathogenesis studies together with the descriptive epidemiology results available.

3.1. The relationship between PrP^{TSE} and infectivity

The relationship between disease-associated prion protein (PrP^{TSE})[2] and infectivity is clearly a fundamental issue which is still a subject of continuing scientific debate. According to the prion hypothesis, PrP^{TSE} is an infectious protein and the causative agent of TSEs (Prusiner, 1982). In TSEs, the accumulation of PrP^{TSE} in tissues of infected individuals is correlated with the presence of infectivity (McKinley *et al*., 1983, and inter alia, Race *et al*., 2001). While titration of infectivity through bioassay remains the only effective tool for quantifying the TSE agent,, the development of sensitive PrP measurement tools, combined with the use of recombinant PrP (as external standard), has allowed a robust quantification of PrP^{TSE} in various tissues (Moudjou *et al* 2001, Andréoletti *et al* 2002, Andréoletti *et al* 2004). In recent studies, the PrP^{TSE} quantities (after PK digestion) were compared to infectious titre as assessed in a transgenic (VRQ PrP protein) ovine mouse model (Andreoletti *et al*. 2004) and an apparent linear relationship was established over a limited range of PrP concentrations. In this experiment infectious titre could still be detected in the absence of a PrP^{TSE} positive signal (~10^2 LD_{50} per g) by TeSE Sheep and goat BIORAD tmPrP^{TSE} detection.

The biochemistry of PrP^{TSE} varies with the prion strain or type of disease. This can be illustrated by recent data obtained on atypical scrapie in sheep. Le Dur and colleagues titrated a "discordant" case of sheep TSE in tg338 mice over-expressing the VRQ allele of ovine prion protein (Le Dur *et al*., 2005). They found high levels of infectivity (> 108 LD50 per g) in brain with a very low content of protease-resistant prion protein. In a recent field trial, no PrPTSE signal could be detected below 1/500 dilution by any rapid tests in cerebral cortex of Nor98 atypical cases (EFSA, 2005a).

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[2] The complicated terminology in prion science can be summarised as follows:
prion: An acronym for "proteinaceous infectious particle." All known prions contain misfolded isomers of a normal cellular protein (PrP^{c}). Aggregates of the misfolded protein of sufficient quantity and size are associated with TSE infectivity and neurodegenerative diseases in both animals and humans. According to the methodology used for detection of the disease associated, misfolded protein, different terms have been used for its designation (see below). In mammals, prions are, at the present time, found primarily in nerve cells and lymphoreticular cells. The preponderance of evidence suggests that prions may be the infectious agent of TSEs. However, a minority of respected TSE experts believe that the protein-only theory has not been proven beyond question (Erdtmann & Sivitz, 2003).
PrP^{res}: Abnormally folded prion protein that is highly resistant to proteinase K digestion and is strongly associated with prion disease. It is sometimes used synonymously with PrP^{sc}.
PrP^{sc}: Term originally derived from scrapie associated PrP, but also more generally used in all TSEs. Abnormally folded prion protein that has a gradient of resistance to proteinase K digestion. It is associated with infectious potential and with prion disease even in circumstances where it may be sensitive to proteinase K digestion.
PrP^{d}: disease associated, abnormally folded prion protein. Sometimes this acronym is used when methods for detection of disease-associated PrP are employed that are not based on proteinase resistance nor infectivity assays, such as in immunohistochemistry.
PrP^{TSE}: TSE associated, abnormally folded prion protein. Sometimes "TSE" is replaced by the acronym of the respective disease, e.g. PrP^{CJD}, PrP^{GSS}, PrP^{BSE}, PrP^{sc}, PrP^{CWD} etc

Taken together these data appear to indicate that:

- The correlation between the PrP^{TSE} amount and infectivity depends on the type of TSE agent.
- Sensitivity of PrP^{TSE} detection is still lower than certain bioassays: failure to detect PrP^{TSE} does not guarantee absence of infectivity in a tissue.

3.2. Experimental studies

Experimental studies of the distribution of BSE infectivity relative to the period post exposure in cattle have been conducted by the VLA in the UK since 1991 (Wells *et al.* 1996, 1998, 2005; Terry *et al*. 2003; Grassi *et al*. 2001; EC 2002; WHO 2006) and more recently by the FLI in Germany (Buschmann and Groschup, 2005; Hoffmann *et al*., 2007). The UK studies have been summarized in the context of the current mandate in the previous EFSA Annex to the Opinion, Report of the Working Group on the assessment of the age limit in cattle for the removal of certain specified risk materials (SRM) (EFSA, 2005b).

With regard to tissues comprised of, or containing, lymphoid tissue designated as SRM, the situation has not changed despite some new information. Whereas PrP^{TSE} has not been detected in the ileum of natural cases of BSE in the UK using conventional mice (Terry *et al* 2003), infectivity, assayed in transgenic (TgbovXV) mice overexpressing the bovine PrP gene, has been detected in the distal ileum of a single case of BSE in Germany (Buschmann and Groschup, 2005). A small amount of infectivity has also been detected in a pool of nictitating membrane lymphoid tissue, from clinically suspect cases of BSE, by intracerebral inoculation assay in cattle (VLA, unpublished data). In contrast to this, albeit inconsistent, involvement of primary lymphoid tissue in BSE, various assays in RIII mice, cattle and TgbovXV mice of lymph nodes and spleens from BSE field cases (Fraser and Foster 1994; Buschmann and Groschup, 2005) or experimental exposure studies (Wells *et al* 1998, 2005) have proven negative. These data continue to support the view that in BSE, in contrast to sheep scrapie and some other TSEs, involvement of secondary lymphoid tissue in pathogenesis is restricted. Nevertheless, with regard to tonsil and intestine (primary lymphoid tissue sites), there is no scientific basis in experimental studies of the pathogenesis of BSE in cattle to change the age limit for their removal as SRM.

The assessment of the occurrence of initial infectivity in the Central Nervous System (CNS) relative to incubation period in cattle is, as previously stated (EFSA, 2005c), based on:

- VLA, UK studies of oral exposure of cattle to the BSE agent and sequential kills to examine the spread of infectivity and/or PrP^{TSE} in relation to time (pathogenesis studies),
- VLA, UK studies of incubation period range relative to dose (attack rate studies) in cattle infected with the BSE agent, and
- a statistical approach to estimate the time at which infectivity/ PrP^{TSE} might first be detectable in relation to incubation period.

Concerning this, the previous Opinion (EFSA, 2005c) indicated that data of particular interest and relevance relating to pathogenesis would come from cattle dosed orally

with 1g of BSE affected brainstem tissue, because, in the attack rate studies, the incubation period values of this group would fit more closely to the incubation period range estimated for naturally occurring cases of BSE. It also indicated that such data would not be available until 2006. These data from sequential kill pathogenesis studies and additional data from the attack rate studies are now available (see below 3.2.1.) to provide the basis for a revised calculation on incubation period ranges according to dose (Wells *et al*. 2007; Arnold *et al*. in prep.[3]).

Moreover, initial data from the German pathogenesis study have become available meanwhile and are summarised below (3.2.2)

3.2.1. Update of pathogenesis studies in UK

The additional data from the VLA, UK BSE oral exposure studies were obtained by the examination of CNS (midbrain, rostral medulla and medulla at the obex and cervical, thoracic and lumbar spinal cord) and certain peripheral nervous system ganglia (dorsal root ganglia (DRG), trigeminal ganglion, stellate ganglion and cranial cervical ganglion) for PrP^{TSE}, from cattle dosed orally with 100g or 1g of BSE infected brain. Transmission studies were conducted as two experiments: the first (Wells *et al* 1996; 1998) in which 30 cattle were dosed with 100g of brainstem with an infectivity titre, determined by end-point titration in RIII mice of $10^{3.5}$ mouse i.c.+ i.p. ID_{50}/g, and the second in which groups of 100 cattle were dosed with either 100g or 1g of brainstem with an infectivity titre in RIII mice of $10^{3.1}$ mouse i.c.+ i.p. ID_{50}/g and killed sequentially throughout the disease course. Techniques applied were based on those utilised in the routine diagnosis of BSE: immunohistochemical labelling (IHC), a Western blot incorporating a sodium phosphotungstic acid precipitation step (WBNaPTA) (applied only to CNS) and an ELISA test (BioRad TeSeE). The examination of certain peripheral ganglia was undertaken to give insights into the sequence and time of involvement of parts of the peripheral nervous system relative to the CNS, during the incubation period.

Experimental studies of attack rate and dose/incubation period response (Wells *et al* 2007) provide the data, for the estimation of the incubation periods according to dose, required for the calculations in a modelling study.

Estimating the relationship of the timing of detected PrP^{TSE} relative to clinical onset is facilitated by the development of a statistical model, which accounts for the differences in incubation period and probability of infection between the different dose groups (Arnold *et al*, in prep). This model relies firstly on the data from the two oral attack rate studies performed at VLA (Wells *et al* 2007), from which the dose-dependent attack rate and incubation period were estimated. Secondly, using these distributions, the probability density of the number of months before onset could be estimated for the subclinical animals and maximum likelihood methods employed to estimate the timing of detectable infectivity relative to clinical onset for animals in the pathogensis studies. Also, a likelihood ratio test was used to determine whether there was any significant difference between the timing of detection for the 1g and 100g dosed cattle.

[3] During the mandate the WG has been provided with an extended presentation of the work done by Arnold *et al.*

In the UK studies the earliest tissue to be detected positive was the obex by IHC at 30 months post exposure (Fig. 1). For the tissues taken from 100g dosed cattle, there were small differences in the proportion positive at each time point between the different CNS tissues tested by IHC. For the tissues tested from the 1g dosed cattle there were no differences between the results for IHC for the spinal cord tissues, or between the brainstem tissues.

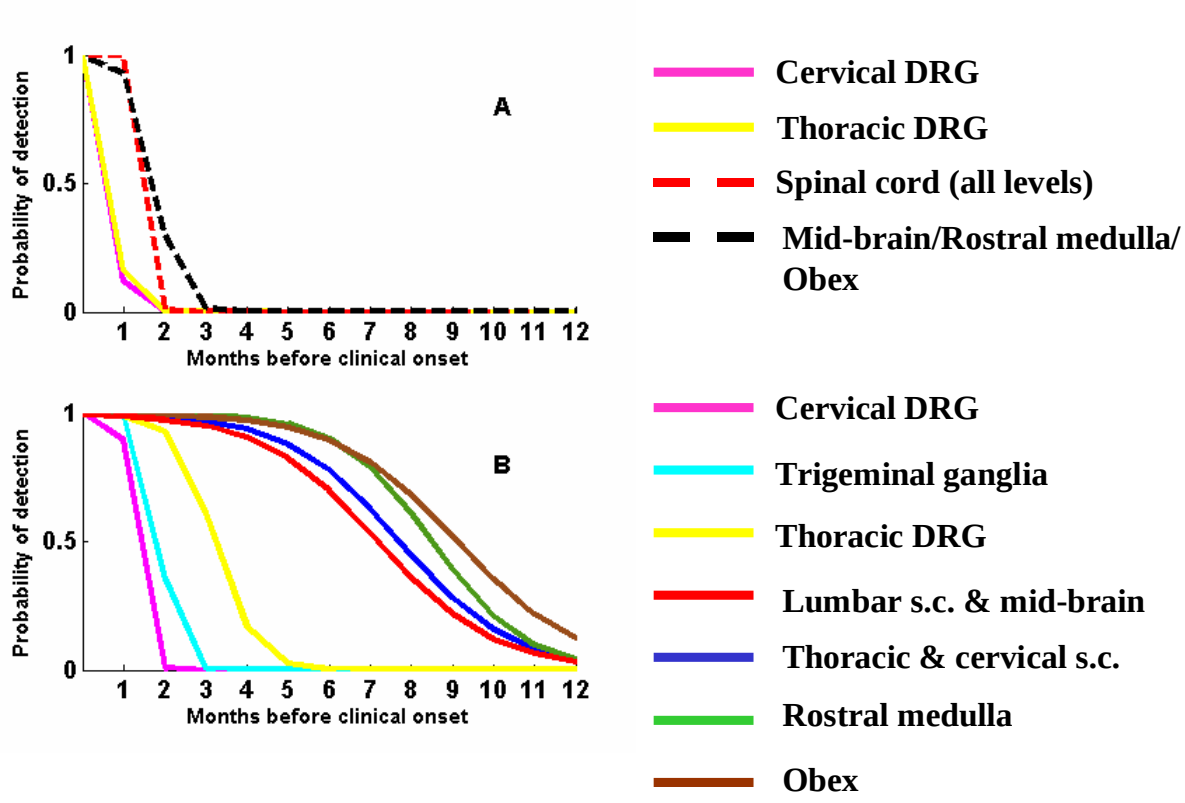

Figure 1: **The estimated probability of detected PrP^{TSE} relative to clinical onset for various CNS and peripheral nerve ganglia tissues applying IHC (with R145 antibody) for A) cattle dosed with 1g and B) cattle dosed with 100g, as predicted using an analytical modelling (Arnold *et al.* in prep).**

Initial detection of PrP^{TSE} during incubation was invariably in the brainstem and the earliest was at 30 and 44 months post exposure, for the 100g and 1g dose groups respectively. There was little difference in the timing of detection of PrP^{TSE} in different CNS tissues, but a significant difference in the estimated timing of detection between the 1g and 100g dosed cattle. It was estimated that the point at which 50 percent of the animals would be detected by immunohistochemistry applied to medulla was at 1.7 months (95% confidence interval of 0.2-4.0) and 9.6 months (95% confidence interval 4.6-15.7) before clinical onset for the 1g and 100g dosed cattle respectively, with a very low probability of detection in any of the tissues examined at more than 12 months before clinical onset. The timing of detected PrP^{TSE} with respect to the proportion of the incubation period completed was also significantly different for each of the dose groups ($P<0.01$). The model predicted that 50% of infected animals would have detectable PrP^{TSE} at 97% and 79% of the incubation period for the 1g and 100g groups respectively (Fig.2). However, the fit of the model to the data

(as measured by the log-likelihood) was poorer than the fit of the models giving the timing of detected PrP^{TSE} in terms of the number of months before clinical onset.

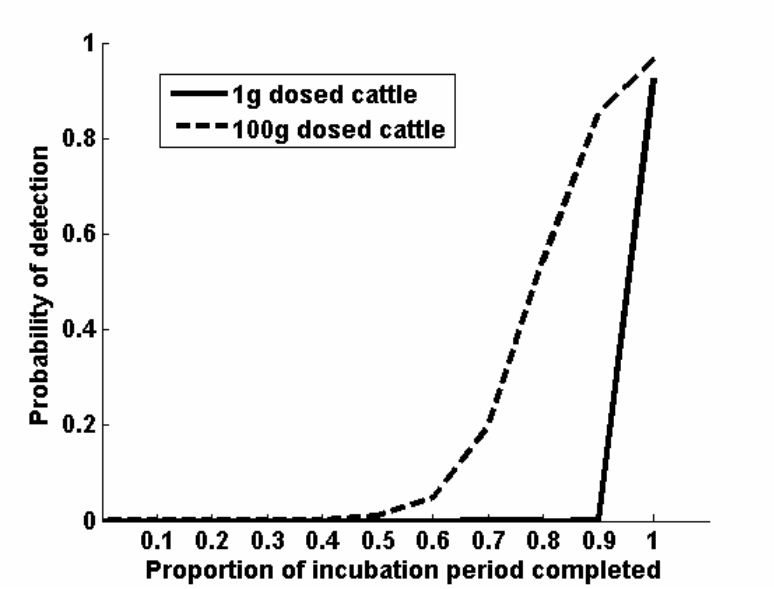

Figure 2: **The probability of detected PrP^{TSE} using IHC according to the proportion of the incubation period completed for brainstem material, as predicted using an analytical modelling (Arnold *et al*. in prep).**

PrP^{TSE} was detected inconsistently in the trigeminal ganglion and dorsal root ganglia, concurrent with, or after, CNS positivity and not at all in the sympathetic nervous system ganglia examined.

The study has shown that in addition to an expected dose response effect on the timing post exposure of detection of PrP^{TSE}, there is also a difference between doses in the timing of detected PrP^{TSE} relative to clinical onset.

The results give valuable information but caution is necessary in the interpretation of these results with respect to the field situation. Firstly, the estimates of the timing of detected PrP^{TSE} relative to clinical onset are dependent on estimates of the incubation period from the attack rate studies. The onset of definite clinical status in those studies was assessed against set criteria from routinely monitored animals. This is not the case for field animals, and therefore it is possible that the incubation periods indicated by observation in the field could differ from those in the attack rate study. Furthermore, the attack rate and incubation period distributions are themselves subject to uncertainty since they have been obtained from experimental studies with limited numbers of animals. Therefore, while the analysis shows that the differences in the estimated timing of detection are significantly different between the dose groups, even when the uncertainty in the incubation period and attack rate is allowed for, there are wide confidence intervals for the point at 50% detection.

Secondly, experimental factors may also have affected the incubation periods and probability of infection recorded in these studies, as indeed they do in laboratory rodent models, but, given the practicalities of such large scale transmission studies, not all of these factors can be controlled. Recent work (Juling *et al.*, 2006), suggesting that in certain cattle breeds, polymorphisms in the regulatory region of bovine *PRNP*

might be linked to BSE incidence/susceptibility, requires investigation/clarification in the animals in this and related experimental studies of BSE in cattle to evaluate of their possible effects on disease incidence and incubation period. Furthermore, the age of dosing could also impact on the incubation period and probability of infection; the results in this study relate to cattle orally dosed between 4-6 months of age, and infections in the field situation have potentially occurred over a wider range of ages, since the majority of infections in the field appear to result from exposures in the first six months of life (Wilesmith *et al*. 1988; Arnold and Wilesmith 2004).

Thirdly, the statistical model that estimated the relationship between the timing of detected PrP^{TSE} and the incubation period was a logistic regression model, with either the number of months before onset, or the proportion of the incubation period completed, as the independent variable. However, a more complex relationship between time points of onset and of detectable infectivity might be appropriate, but this cannot be determined with the data currently available.

3.2.2. Update of ongoing pathogenesis studies in Germany

In the German BSE pathogenesis study fifty six Simmental cross-breed calves were orally challenged at four months of age with a macerate of 225 BSE positive brainstems (100g in a 50% w/v mash containing 5% sucrose per animal). Another 18 animals received a non-infectious cattle brainstem homogenate to serve as negative controls. The infectivity in the BSE brain stem homogenate used for the cattle infection study was $10^{6.1}$ ID_{50} per gram of tissue as determined by endpoint titration in Tgbov XV mice (Buschmann *et al.*, 2005). The cattle were housed in a special TSE infection facility and were clinically assessed every two months. Every four months four or five randomly selected animals were euthanised and necropsied under TSE sterile conditions, and more than 150 tissue and bodily fluid samples were collected from each animal.

Samples are currently being analysed by IHC, PTA-Westernblot and transgenic mouse bioassay (TgbovXV) to a) reveal the time of the earliest detection of BSE prions in the CNS and b) to elucidate the route BSE prions take from the gastrointestinal tract to the CNS of bovines.

The initial results from the German BSE pathogenesis study demonstrate that BSE prions can reach the brain as soon as 24 months after a massive oral challenge. In the UK pathogenesis studies (Wells *et al*., 1998; Wells *et al* 2005; Arnold *et al*.,in preparation) which were of similar design (including a 100g dose), the first PrP^{TSE} deposition was observed in the brain stem 30 months post exposure. Moreover, CNS tissue pools of 3 animals sacrificed at 22 months and one animal sacrificed at 26 months post challenge did not contain infectivity when bioassayed in cattle (Wells *et al*., 2005, and VLA unpublished data). These results were in contrast to those of a recent study (Espinosa *et al*. 2007), utilising material derived from the second VLA, UK pathogenesis study (100g dose group), which reported the detection of infectivity (by assay in BoPrP-Tg110 mice) in the brainstem of cattle killed 27 months post exposure. Differences between the UK and German results with respect to PrP^{TSE} detection may be due to biological variation in the timing of the pathogenesis of BSE in cattle in individual animals and other differences in experimental design. An influence of the breed (Holstein-Friesian cattle used in the UK studies versus Simmental cross breed calves used here) as well as individual genetic differences in

the prion gene of cattle (Sander *et al.* 2004, Juling *et al.,* 2006) cannot be ruled out, although at present there is no evidence from epidemiological data that genetic factors impact on susceptibility or incubation period. Therefore a more detailed genetic analysis on the prion gene of the experimental animals will be carried out. Last but not least, it cannot be excluded that gut-associated inflammatory processes might be infection modulators and lead to shorter or prolonged incubation periods, although at present data on gut inflammation is lacking in BSE.

Two cows which were PrP^{TSE} positive in the brain stem after 24 (Cow A) and 28 (Cow B) month post challenge, respectively, were clearly at the threshold of the earliest detection of PrP^{TSE} by currently available IHC and PTA-WB methods. Light microscopic examination for PrP^{TSE} labelling was carried out on 5 sections in order to discover even minor traces of PrP^{TSE} labelling which may not be observed with routine diagnostic approaches using a single section. In accordance with previous experimental studies there was an accumulation of PrP^{TSE} in the Peyers` patches of the distal ileum and in the ENS of this animal. The most likely route for the BSE prion spread in cattle in the gastrointestinal tract is therefore a locally restricted uptake of BSE prions in the ileum and a subsequent replication in the local follicles followed or accompanied by a centripetal spread via the coeliac and mesenteric ganglion complex to the spinal cord and then to the brain.

The comprehensive sampling strategy used in the German pathogenesis study included the abdominal autonomic nervous system ganglia which were not available in the UK study. PrP^{TSE} deposition was also detected in the coeliac mesenteric ganglion complex (CMGC) and in the Ganglion mesentericum caudale (GMC) of this animal. Both, CMGC and GMC ganglia contain sympathetic and parasympathetic nerve fibres.

In agreement with the UK studies PrP^{TSE} was also detected in the dorsal motor nucleus of the vagus nerve (DMNV) as well as in the *Substantia intermedia centralis* and *lateralis* of the spinal cord of BSE infected cattle.

The results also suggest that the spread of infection to the CNS is principally by two routes. The first follows the efferent sympathetic fibres of the *Nervi splanchnici majores* and *minores* (which contain nerve fibres crossing over in the CMGC) to the thoracic and/or lumbal spinal cord (T6-L2). It must be emphasised that both parts of the spinal cord can innervate the same part of the intestine. The importance of this pathway is also supported by the immunolabelling of the sympathetic (in parts splanchnici-associated) pre-ganglionic neuronal cells in the *Substantia intermedia centralis* and *lateralis* of the spinal cord. All spinal cord segments were evenly affected, indicating an almost simultaneous prion invasion through the *Nn. splanchnici.* Moreover, a lack of involvement of the thoracic dorsal root ganglia which contain the afferent neurons (sensory innervation of the intestines) indicates also the spread of PrP^{TSE} along the efferent nerve fibres via the *Radix ventralis* directly to the pre-ganglionic neuronal cells.

The second possibility for the spread of BSE prions from the CMGC to the brain follows the parasympathetic nerve fibres of the vagus nerve, although it was not possible to demonstrate PrP^{TSE} accumulation in this nerve trunk. This result supports the hypothesis of McBride *et al.* (2001) that in peripheral nerve fibres PrP^{TSE} is in transit rather than actively replicated. However, both cows showed a clear DMNV associated PrP^{TSE} immunolabelling of singleton neurons at the level of the obex and

Cow B displayed minimal immunolabelling at this site without any involvement of other neuronal nuclei. This pattern is indicative for centripetal spread along the parasympathetical nerve fibers. Moreover in Cow B there was no PrP^{TSE} immunoreactivity detectable by IHC or PTA-immunoblot in the spinal cord. This result additionally indicates an early BSE prion transmission along the vagus nerve.

In conclusion, the results obtained in the German BSE pathogenesis study clearly suggest a neural rather than a lymphoreticular/haematogenous progression of BSE prions to the CNS. It must be assumed that there is a simultaneoussimultaneous spread in the early pathogenesis of BSE along the parasympathetic nerve fibres of the vagus nerve to the brain and via the sympathetic pathway of the splanchnic nerves to the spinal cord and subsequently to the brain.

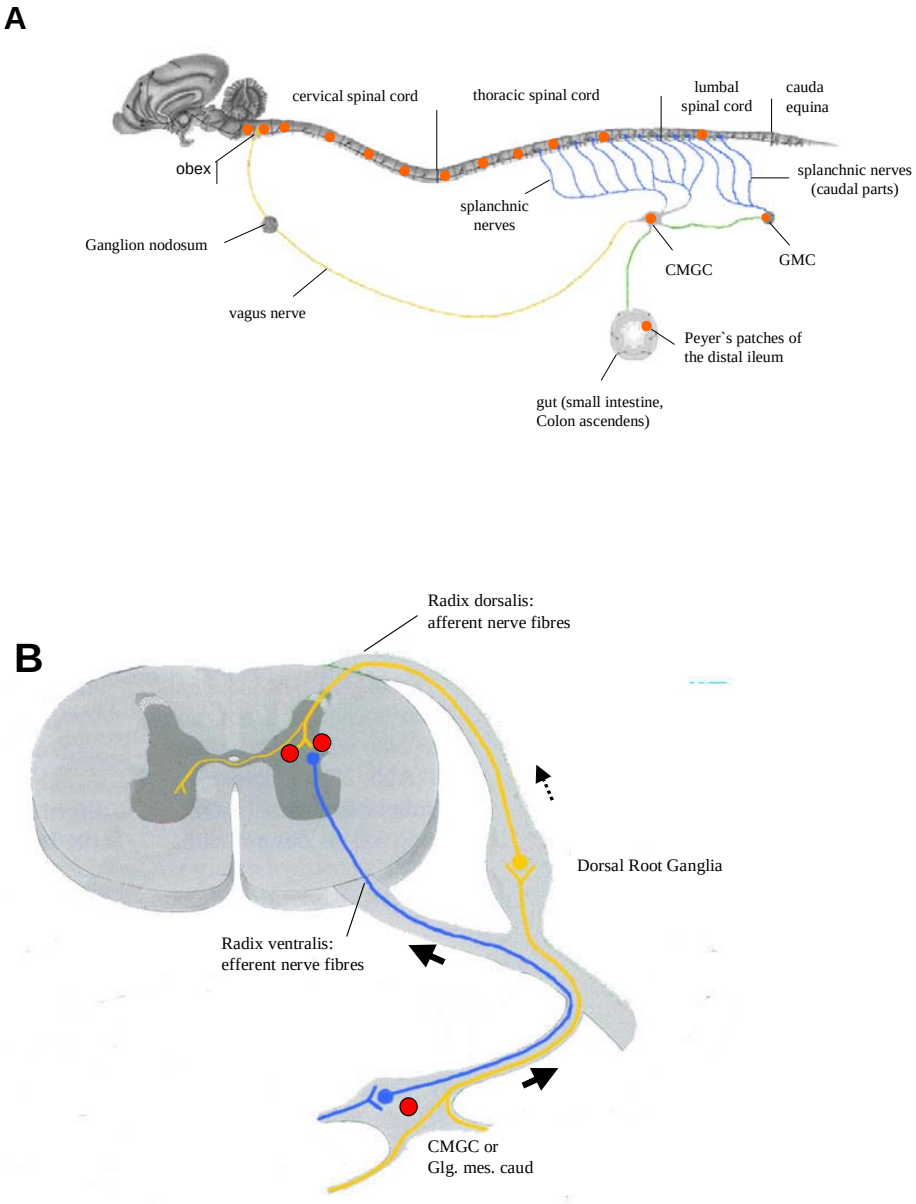

Figure legend (from Hoffmann *et al*. 2007):

Schematic overview. Regions with distinct PrP^{TSE} deposition are drawn in red.

(A) showing the most likely routes of BSE prions from the gut into the brain via the autonomic nervous system. Sympathetic nerve fibres are drawn in blue, parasympathetic nerve fibres of the vagus nerve in yellow, mixed autonomic fibres in green. It is important to know that as a result of the crossing over in the CMGC there is no localized projection zone for the splanchnic territory in the spinal cord.

B) BSE prion spread from the coeliac and mesenteric ganglion complex to the spinal cord. Efferent nerve fibres are drawn in blue, afferent nerve fibres in yellow. The thick arrows indicate the most probable infectivity routes into the spinal cord.

3.2.3. Conclusions on pathogenesis studies

Oral pathogenesis experiments in cattle have broadly mapped a transport pathway of infectious agent from gut via sympathetic and parasympathetic PNS pathways to the CNS. This routing is similar to that inferred from rodent studies. Infection of spinal cord and brainstem seems to occur almost at the same time and PrP^{Sc} is sometimes detected in the brainstem of individual animals without being found in their spinal cords; this supports the usefulness of targetting the medulla oblongata for early detection of PrP^{TSE} in the CNS.

The detection of infectivity/ PrP^{TSE} in the CNS at different stages of the disease is variable in both different studies and the effects of several factors such as exposure levels, age at exposure, cattle breed and PrP genetics remain to be quantified. Critically, detection of cases by active surveillance is predicted by modelling to be only 1-2 months prior to the onset of clinical disease if a realistic level of exposure (1g) is considered. Arnold *et al* (in prep) estimate that that 50% of infections for 1g dosed cattle were only detectable at less than 1.7 months before clinical onset (95% confidence interval of 0.2-4.0 for median detectability). In contrast at high dosage (single oral exposure to 100g affected brain tissue), 50% of animals were detected at less than 9.6 months (95% confidence interval 4.6-15.7 for median detectability) prior to clinical signs.

This new insight has implications for control policies as it implies that the vast majority of cases of BSE are not detected until very close to clinical onset at a time when infectivty is widespread, and that past estimates of BSE prevalence based on the observed numbers of cases in the healthy slaughter population may have been too low.

In respect to this mandate, there are two aspects of these studies which impact on decisions with respect to the age at which vertebral column should be removed to minimise possible human exposure to the BSE agent:

1. Earliest detection Earliest detection of infectivity/PrP^{TSE} at different stages of disease

   Experimental attack rate studies indicate the incubation period range for the 1g dose group is 45-73 months, (mean 60 months, SEM 2.7)(Wells *et al.* 2007). This overlaps considerably with the incubation period of a 100g dose (31-60 months, mean 44 SEM 1.2) but suggests that the incubation period in the majority of cases in the BSE epidemic will be longer and hence the timing at which infectivity reaches the CNS could be anticipated to be proportionately later (at an older age) than with a 100g dose. Analysis of the experimental pathogenesis data indicated that for both dose levels there was a low probability of detection of PrP^{TSE}/infectivity at more than 12 months before clinical onset.

2. Effect of dose on the incubation time and timing (age) at detection of PrP^{Sc} in the CNS

   Infectivity may be more widespread in an animal prior to the time of detection of PrP^{TSE} by routine testing in the CNS (medulla oblongata) if it is exposed to a low (1g) rather than high (100g) dose. Some caution is needed when using these data to decide at what age to exclude CNS-related SRM (including vertebral

column) from the food chain. It seems reasonable to assume that PrP^{TSE}/infectivity is present in CNS (DRG) at 79% of the incubation period. In the light of EU SRM and feed controls, it is more sensible to use incubation time data based on oral challenge using 1g rather than 100g exposure dose. The shortest incubation period for 1g dose is 45 months and the mean is 60 months. This would suggest that the infectivity in CNS-related SRM (including vertebral column) would be sub-detectable or absent in cattle aged 35 months. If PrP^{TSE}/infectivity is modelled as present in CNS at 75% of the incubation period, as in the previous opinion (EFSA, 2005c), it can be predicted that the infectivity would be sub-detectable or still absent in CNS in cattle aged 33 months.

Such inferences reflect only uptake of the BSE agent via the gut and other modes of prion uptake, e.g. via the oral mucosa and neural spread, cannot be completely excluded and theoretically might significantly shorten the incubation time (EFSA, 2005c). However, there continues to be no data to support even occasional cases of such an alternative pathogenesis, and preliminary results from the German BSE pathogenesis study would bear this out, since this has already indicated in more detail than hitherto, the dual sympathetic and parasympathetic autonomic nervous system routing of PrP from the intestine to the CNS.

In consideration of the significance of BSE pathogenesis studies, the WG concurs with SEAC (SEAC Annual Report 2006) that there is no threshold dose of BSE at which the probability of infection becomes negligible, that reliable detection of PrP^{Sc} in the CNS is only possible in the few months prior to, and during, the clinical stage of the infection, and that low levels of PrP^{Sc} in peripheral nervous system tissues could be detected at the same time, or after, PrP^{Sc} was detected in the CNS.

3.3. Back calculation modelling

Different attempts were made to model the future expected number of cases in the different cohorts using specific surveillance data from all member states.

The WG developed a deterministic modelling approach to predict the number of BSE cases in age cohorts for the coming years. However, discussions within the WG indicated that the assumptions behind the predictive model, as developed, were not unanimously acceptable.

In addition it became clear during the mandate that possible risk management actions based on this opinion would be restricted to the age limit for the vertebral column removal. The WG and the Panel considers that the pathogenesis data together with the descriptive epidemiology are suitable to answer this question.

3.4. Descriptive epidemiology: BSE-positives born after Re-inforced Feed Ban (BARB) of 1 January 2001 in European Union.

UK's re-inforced feed ban, which came into force on 1 August 1996, pre-dated the EU-wide implementation. Table 1 shows BARBs born after 1 January 2001 by member-state, birth month and year, age at slaughter and slaughter route.

From these data it is clear that the mean BSE survival is about 5 years, while a low proportion of BSE-infected cattle develop late-stage disease within 3 years of exposure.

EU SRM controls were introduced in October 2000 and EU feed controls were introduced in 2001. The exposure of cattle to contaminated feed has reduced significantly. This is shown by the decline in BSE cases in the EU.

To date, only 4 cattle born after 2000 have tested positive for BSE at < 40 months (UK, 36 and 39 months; Portugal, 32 months; Poland, 25 months) and there is some uncertainty about the age of the youngest case[4]. The number of cattle infected with BSE is likely to reduce even if it is now apparent that cases detected by active surveillance may be closer to clinical onset than previously estimated.

Cattle aged over 30 months will be born well after the introduction of the EU SRM and feed controls (born in or after Q4, 2004).

[4] The “25 months old” Polish case was first reported as "33 months old" in a July 2005 paper in the Veterinary Record (Polak & Zmudzinski, 2005). A 2005 FVO mission report referred to it as a "32 months old" case (DG/SANCO/7693/2005). It was finally officially classified as 25 months old in the Commission's Annual TSE Report 2005. This confusion casts some doubt on the real age of this Polish case.

Table 1: **BARBs by year, age and route of detection**

Year of detection (month)	Member State (adult cattle >24 months in millions)	Birth year (month)	Age in months at detection	Slaughter route	Comment
2004 (08)	Slovak (0.3m)	2001 (02)	42	Healthy slaughter	
2004 (09)	Slovak (0.3m)	2001 (01)	44	Healthy slaughter	
2005 (05)	UK (5.0m)	2001 (09)	44	BSE eradication	Ceteris paribus, member states with larger adult herds would be expected to signal first BSE positives in later birth-cohorts. However, alternative reason is higher residual BSE exposure
2005 (05)	UK (5.0m)	2002 (05)	36	BSE eradication	
2005 (11)	Czech (0.6 m)	2001 (02)	57	BSE eradication	
2005 (01)	UK (5.0m)	2001 (10)	39	Risk stock	
2005 (05)	Ireland (3m)	2001 (09)	44	Risk stock	
2005 (07)	Ireland (3m)	2001 (03)	52	Risk stock	
2005 (06)	Portugal (0.8m)	2002 (10)	32	Risk stock	
2005 (06)	Spain (3.5m)	2002 (01)	41	Risk stock	
2005 (12)	Netherlds (1.6m)	2001 (02)	58	Risk stock	
2005 (04)	Germany (6m)	2001 (05)	47	Risk stock	
2005 (03)	Germany (6m)	2001 (06)	51	Healthy slaughter	
2005 (11)	Luxemb. (0.1m)	2001 (11)	48	Healthy slaughter	
2005 (06)	Poland (3m)	2001 (06)	48	Healthy slaughter	
2005 (11)	Poland (3m)	2001 (01)	58	Healthy slaughter	
2005 (02)	Poland (3m)	2003 (01)	25	Healthy slaughter	
2006 (09)	Ireland (3m)	2001 (03)	66	Suspect	Note the absence so far in 2006 of 2002-born BARBs
2006 (01)	UK (5.0m)	2001 (01)	60	Risk stock	
2006 (04)	UK (5.0m)	2001 (03)	61	Risk stock	
2006 (01)	France (10m)	2001 (01)	60	Healthy slaughter	
2006 (01)	Poland (3m)	2001 (01)	60	Healthy slaughter	

To date the majority of BARBs have been 2001-born (18/22). Three have so far been born in 2002 (UK, Portugal and Spain, respectively BSE eradication, risk stock, and risk stock) and one healthy slaughter bovine in 2003 (Poland), which tested positive at only 25 months of age4.

Excepting the Czech Republic and Slovakia (where BARBs were discovered in 2004), there have been 14 BARBs in 2005 in eight MSs (with adult herds which total 23 millions of EU's 43 millions adult cattle). Twelve of the 15 BARBs in 2005 were healthy slaughter or risk stock, the remaining three being BSE eradications (two in

UK and one in Czech). Across Europe the BARB rate in 2005 has been 15/43,000,000 = 0.35 per 1 million adult cattle, and so reassuringly low.

Risk stock have been subject to BSE post-mortem testing since 2001, including the UK, and surveillance was robust from 2002. For UK cattle, this slaughter route is therefore a good beacon of how BSE exposure risk has decreased year-on-year after the introduction of a re-inforced feed ban. Table 2 illustrates the phenomenon for UK cattle born in 1995 or later and subject to BSE surveillance in 2002 or later.

Table 2: **UK's BSE exposure risk year-on-year since 1995 revealed by tracking birth cohort and BSE positivity in risk stock**

Birth cohort	Year of detection				Comment
	2002	2003	2004	2005	
1995	**137**	83	42	22	Diagonals to compare year-on-year exposure risk: NB a change from 1995 to '96 and further reduction from '96 to 1997 = all born after re-inforcement
1996	*22*	**16**	5	4	
1997	8	*11*	**2**	3	
1998	3	*7*	*6*	**3**	
1999	1	3	5	*5*	

By comparison, Table 3 (for other member states) shows that, although both UK and other member states' data evidence some change between 1995 and 1996 born cattle, the decrease in age-specific BSE positivity is sustained and re-inforced to a much greater extent for UK's 1997+ born cattle than in other member states.

Table 3: **Other member states' BSE exposure risk year-on-year since 1995 revealed by tracking birth cohort and BSE positivity in risk stock**

Birth cohort	Year of detection				Comment
	2002	2003	2004	2005	
1995	**174**	88	47	21	Pre-enlargement, comparison of Tables 2 & 3 for 1996+ borns shows impact of UK's re-inforcement
1996	*112*	**66**	35	20	
1997	41	*58*	**38**	14	
1998	17	27	*49*	**21**	
1999	1	8	24	*23*	

By 2005, BSE surveillance was comprehensive also in UK so that surveillance of both risk stock and healthy slaughter cattle can be used to monitor the impact of EU's re-inforced feed ban of 1 January 2001. Table 4 may still suffer some confounding due to enlargement (May 1, 2004) and the start date for UK's comprehensive surveillance. Nonwithstanding these reservations, there are grounds for cautious optimism.

According to previous SSC opinions, each BARB born after 1 January 2001 in any member state should be subject to an intensive case-control study according to a common protocol across member states, to help to understand possible residual infection routes.

Table 4: **EU member states' BSE exposure risk year-on-year since 2000 revealed by tracking birth cohort and BSE positivity in risk stock+healthy slaughter**

	Year of detection				
Birth cohort	2004	2005	2006	2007	Comment
2000	**23**	54	9 to date		Early evidence of exposure reduction in 2001 but beware confounding: start of UK's comprehensive testing + enlargement
2001	2	**9**	4 to date		
2002	0	2	**0** to date		
2003	0	1	0 to date		

4. Conclusions

Pathogenesis experiments

1. The situation has not changed despite some new information with regard to tissues comprised of, or containing, lymphoid tissue designated as SRM.
2. The medulla oblongata remains optimal for the initial detection of PrP^{TSE} in the CNS.
3. While now more complete data of experimental pathogenesis studies have become available, the panel considers the earlier opinion of 28 April 2005 still valid, which concluded that the likely detectable infectivity in the CNS appears at about ¾ of the incubation time.
4. The results of experimental pathogenesis studies must be interpreted with caution with respect to the field situation. With regard to dose, however, epidemiological data is consistent with a low field exposure scenario (equivalent or similar to 1g of fresh brain material from clinical BSE cases rather than 100g in these experimental studies).
5. The shortest incubation period in bovines experimentally infected by 1g is 45 months.
6. If PrP^{TSE}/infectivity is conservatively modelled as present in CNS at 75% of the incubation period, as in the previous opinion, it can be predicted that the infectivity would be sub-detectable or still absent in CNS in cattle aged 33 months.
7. When interpreting the significance of the experimental data the following points require to be considered:
    - At least one BSE infected case has been detected in animals aged 33 months or younger in EU cohorts born after 2000.
    - Pathogenesis studies show significantly different timing of PrP^{TSE} detection between dose groups, and wide confidence intervals for the time point at 50% detection (in particular in the high dose model).
    - Infectivity may be more widely distributed prior to the time of detection of PrP^{TSE} by routine testing in the CNS (medulla oblongata) after the animal was exposed to a low (1g) rather than high (100g) dose.
    - The cattle experiments of oral exposure to the BSE agent, in common with oral exposure studies in laboratory animals do not provide such consistent incubation times as those obtained with experimental TSE models in rodents using passaged agents, after parenteral exposures.
    - The sensitivity of PrP^{TSE} detection is still lower than certain bioassays: failure to detect PrP^{TSE} does not guarantee absence of infectivity in a tissue.

Epidemiology

1. There is a decline in BSE cases in the different EU member states which is linked to a reduction in exposure. To date, the three youngest out of 22 BSE infected cases in cattle born after 2000 were aged 32, 36 and 39 months, respectively. Another case tested positive at an age reported as 25 months but there is uncertainty about its age.
2. The number of cattle infected with BSE is likely to continue to decline.
3. It is now apparent that cases detected by active surveillance may be closer to clinical onset than previously estimated.

5. Recommendations

1. The probability of BSE agent presence in SRM should be periodically re-examined with regards to:
    - The risk management history outlined in OIE regulation and the Geographical BSE Risk (GBR) as outlined in the EFSA opinion.
    - The continuing accumulating data related to BSE prevalence and age of cases as detected through the active surveillance programme
    - The outcome of new data related to infectivity detection and measurement in tissues through bioassay or comparable assays.

2. There is good reason to consider each Member State separately or consider as groups with similar characteristics because of differences at the start of the various control measures and surveillance between EU member states, as well as differences in the country specific level of exposure.

6. Documentaton provided to EFSA

Letter from the European Commission, DG SANCO (D(2005)JOV/khk/421306) including the mandate and supporting documents

- Age distribution of bovine animals tested in the EU in the period 2001-2004 (Annex II)
- Age distribution of BSE positive cases detected in the EU in the period 2001-2004 (Annex III)

Annual Reports on the Monitoring and testing of ruminants for the presence of TSE for 2001, 2002, 2003 and 2004. Available from the following link:

http://europa.eu.int/comm/food/food/biosafety/bse/annual_reps_en.htm

Relevant SSC and EFSA opinions

- Opinion of the Scientific Steering Committee on Listing of specified risk materials: a scheme for assessing relative risks to man. Adopted at its meeting of 9 December 1997.
- Opinion of the Scientific Steering Committee on the Human Exposure Risk (HER) via food with respect to BSE. Adopted at its meeting of 10 December 1999.
- Opinion of the Scientific Steering Committee on Oral exposure of humans to the BSE agent: infective dose and species barrier. Adopted at its meeting of 13-14 April 2000.
- Opinion of the Scientific Steering Committee on TSE infectivity distribution in ruminant tissues (State of knowledge, December 2001). Adopted at its meeting of 10-11 January 2002.
- Update of opinion on TSE infectivity distribution in ruminant tissues, initially adopted by the Scientific Steering Committee at its meeting of 10-11 January 2002 and amended at its meeting of 7-8 November 2002
- Opinion of the Scientific Panel on Biological Hazards on the assessment of the age limit in cattle for the removal of certain Specified Risk Materials (SRM). Adopted at it meeting of 28 April 2005

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Annex I

BSE systematic surveillance 2001-2006

2001	Eradication Measures		Healthy slaughtered animals		Risk animals		Suspects subject to laboratory examination		Total	
	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed
Austria	28	0	202 809	1	8 752	0	2	0	**211 591**	**1**
Belgium	3 522	1	359 435	28	14 710	8	242	9	**377 909**	**46**
Denmark	4 286	0	250 414	3	22 192	2	73	1	**276 965**	**6**
Finland	31	0	9 882	0	17 960	1	3	0	**27 876**	**1**
France	11 117	3	2 382 225	83	133 889	100	469	91	**2 527 700**	**277**
Germany	13 849	4	2 565 341	36	276 933	78	214	7	**2 856 337**	**125**
Greece	95	0	15 360	1	1 655	0	3	0	**17 113**	**1**
Ireland	12 196	4	636 930	34	25 507	85	482	123	**675 115**	**246**
Italy	2 660	0	377 201	27	65 258	23	9	0	**445 128**	**50**
Luxemburg	2	0	19 475	0	1 395	0	14	0	**20 886**	**0**
Netherlands	2 558	0	454 649	11	44 337	6	97	3	**501 641**	**20**
Portugal	2 012	3	28 384	19	8 033	29	326	62	**38 755**	**113**
Spain	3 700	1	328 517	35	53 581	38	464	9	**386 262**	**83**
Sweden	0	0	4 433	0	23 643	0	25	0	**28 101**	**0**
United Kingdom	408	0	20 767	1	73 912	383	1 209	814	**96 296**	**1 198**
Total:	**56 464**	**16**	**7 655 822**	**279**	**771 757**	**753**	**3 632**	**1 119**	**8 487 675**	**2 167**

2002	Eradication Measures		Healthy slaughtered animals		Risk animals		Suspects subject to laboratory examination		Total	
	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed
Austria	0	0	215 075	0	13 564	0	4	0	**228 643**	**0**
Belgium	3 277	0	408 934	17	37 929	16	279	5	**450 419**	**38**
Bulgaria			1 619	0	51	0			**1 670**	**0**
Denmark	2 640	0	254 668	1	35 995	2	38	0	**293 341**	**3**
Finland	0	0	114 669	0	22 333	0	6	0	**137 008**	**0**
France	15 881	1	2 896 182	74	271 727	124	207	41	**3 183 997**	**240**
Germany	2 626	3	2 767 958	42	259 612	50	346	11	**3 030 542**	**106**
Greece	22	0	21 457	0	2 256	0	0	0	**23 735**	**0**
Ireland	18 659	4	610 002	33	78 372	186	511	108	**707 544**	**331**
Italy	4 034	0	623 913	21	103 539	15	99	0	**731 585**	**36**
Luxemburg	0	0	16 443	0	1 941	1	14	0	**18 398**	**1**
Netherlands	3 000	0	491 069	10	64 321	13	39	1	**558 429**	**24**
Portugal	1 163	1	66 721	38	14 193	24	150	23	**82 227**	**86**
Spain	5 473	7	454 132	36	86 380	74	68	17	**546 053**	**134**
Sweden	0	0	12 073	0	25 398	0	26	0	**37 497**	**0**
United Kingdom	945	0	171 591	14	221 053	636	872	475	**394 461**	**1 125**
Total:	**57 720**	**16**	**9 126 506**	**286**	**1 238 664**	**1 141**	**2 659**	**681**	**10 425 549**	**2 124**

2003	Eradication Measures		Healthy slaughtered animals		Risk animals		Suspects subject to laboratory examination		Total	
	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed
Austria	0	0	205 658	0	16 990	0	2	0	**222 650**	**0**
Belgium	1 126	0	356 184	10	34 988	5	167	0	**392 465**	**15**
Bulgaria			9 174	0	512	0			**9 686**	**0**
Cyprus	0	0	6 401	0	1 325	0	0	0	**7 726**	**0**
Czech Republic	706	0	133 046	3	76 431	1	1	0	**210 184**	**4**
Denmark	1 774	0	250 558	1	37 332	0	38	1	**289 702**	**2**
Estonia			19	0	3 964	0			**3 983**	**0**
Finland	0	0	108 198	0	23 202	0	5	0	**131 405**	**0**
France	1 669	2	2 920 157	37	283 695	87	442	12	**3 205 963**	**138**
Germany	1 125	1	2 337 605	23	249 489	20	854	10	**2 589 073**	**54**
Greece	0	0	24 533	0	1 999	0	1	0	**26 533**	**0**
Hungary	0	0	86 595	0	10 795	0	98	0	**97 488**	**0**
Ireland	11 986	1	600 586	31	87 437	112	330	41	**700 339**	**185**
Italy	2 148	0	658 770	15	124 050	15	63	1	**785 031**	**31**
Latvia	0	0	4 838	0	1 277	0	11	0	**6 126**	**0**
Lithuania	0	0	7 418	0	2 328	0	0	0	**9 746**	**0**
Luxemburg	2	0	14 598	0	3 110	0	4	0	**17 714**	**0**
Malta	0	0	1 089	0	110	0	0	0	**1 199**	**0**
Netherlands	954	0	439 403	11	65 943	6	25	2	**506 325**	**19**
Poland	37	0	428 452	4	26 873	0	51	1	**455 413**	**5**
Portugal	1 271	0	81 633	44	26 393	61	102	28	**109 399**	**133**
Slovakia	11	0	65 192	1	21 805	1	2	0	**87 010**	**2**
Slovenia	27	0	54 751	0	11 357	1	32	0	**66 167**	**1**
Spain	2 356	6	471 252	74	94 183	68	73	25	**567 864**	**173**
Sweden	0	0	9 856	0	24 708	0	16	0	**34 580**	**0**
United Kingdom	555	0	237 490	19	222 251	409	456	186	**460 752**	**614**
Total:	**25 747**	**10**	**9 513 456**	**273**	**1 452 547**	**786**	**2 773**	**307**	**10 994 523**	**1 376**

2004	Eradication Measures		Healthy slaughtered animals		Risk animals		Suspects subject to laboratory examination		Total	
	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed
Austria	0	0	188 520	0	17 136	0	2	0	**205 658**	**0**
Belgium	172	0	356 813	6	36 715	2	169	3	**393 869**	**11**
Bulgaria	0	0	7 789	0	560	0	0	0	**8 349**	**0**
Cyprus	0	0	5 888	0	1 463	0	0	0	**7 351**	**0**
Czech Republic	1 135	0	130 124	2	69 458	5	0	0	**200 717**	**7**
Denmark	86	0	246 156	0	37 974	1	18	0	**284 234**	**1**
Estonia	0	0	21 277	0	5 754	0	0	0	**27 031**	**0**
Finland	0	0	107 168	0	18 916	0	1	0	**126 085**	**0**
France	919	0	2 624 634	17	266 123	29	96	8	**2 891 772**	**54**
Germany	1 267	2	2 292 714	34	236 213	26	1 866	3	**2 532 060**	**65**
Greece	0	0	26 161	0	2 645	0	0	0	**28 806**	**0**
Hungary	0	0	81 284	0	14 735	0	62	0	**96 081**	**0**
Ireland	8 556	1	605 396	20	87 613	69	275	31	**701 840**	**121**
Italy	572	0	851 014	2	130 704	6	27	0	**982 317**	**8**
Latvia	1	0	28 017	0	1 557	0	1	0	**29 576**	**0**
Lithuania	0	0	47 506	0	2 997	0	0	0	**50 503**	**0**
Luxemburg	0	0	13 575	0	3 123	0	2	0	**16 700**	**0**
Malta	0	0	2 068	0	316	0	0	0	**2 384**	**0**
Netherlands	283	0	467 448	5	66 130	1	19	0	**533 880**	**6**
Poland	65	0	447 332	8	33 708	3	11	0	**481 116**	**11**
Portugal	1 217	2	78 783	21	34 932	55	85	13	**115 017**	**91**
Slovakia	127	0	63 553	5	19 258	2	1	0	**82 939**	**7**
Slovenia	5	0	35 767	0	9 873	2	21	0	**45 666**	**2**
Spain	1 477	0	478 037	36	98 536	76	75	26	**578 125**	**138**
Sweden	0	0	10 318	0	25 773	0	20	0	**36 111**	**0**
United Kingdom	569	0	341 916	10	256 719	243	336	90	**599 540**	**343**
Total:	**16 451**	**5**	**9 559 258**	**166**	**1 478 931**	**520**	**3 087**	**174**	**11 057 727**	**865**

2005	Eradication Measures		Healthy slaughtered animals		Risk animals		Suspects subject to laboratory examination		Total	
	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed
Austria	28	0	184 486	1	17 120	1	8	0	**201 642**	**2**
Belgium	15	0	324 129	1	43 001	0	136	1	**367 281**	**2**
Bulgaria	0	0	8 338	0	2 130	0	0	0	**10 468**	**0**
Cyprus	0	0	7 749	0	1 344	0	0	0	**9 093**	**0**
Czech Republic	1 142	1	109 180	1	60 501	6	0	0	**170 823**	**8**
Denmark	6	0	216 687	0	38 258	1	11	0	**254 962**	**1**
Estonia	0	0	23 959	0	7 150	0	0	0	**31 109**	**0**
Finland	0	0	99 534	0	17 512	0	0	0	**117 046**	**0**
France	208	0	2 341 151	12	252 178	17	57	2	**2 593 594**	**31**
Germany	1 007	0	1 839 337	16	230 786	16	2 127	0	**2 073 257**	**32**
Greece	9	0	27 650	0	4 024	0	1	0	**31 684**	**0**
Hungary	0	0	67 770	0	15 745	0	38	0	**83 553**	**0**
Ireland	4 329	0	678 657	11	92 612	49	242	9	**775 840**	**69**
Italy	527	0	592 177	7	98 263	1	26	0	**690 993**	**8**
Latvia	0	0	35 017	0	1 945	0	1	0	**36 963**	**0**
Lithuania	0	0	81 769	0	4 426	0	0	0	**86 195**	**0**
Luxemburg	15	0	11 687	1	3 044	0	2	0	**14 748**	**1**
Malta	0	0	2 431	0	412	0	0	0	**2 843**	**0**
Netherlands	38	0	451 507	2	65 651	1	7	0	**517 203**	**3**
Poland	212	1	472 428	16	43 295	3	41	0	**515 976**	**20**
Portugal	548	0	74 352	9	38 415	40	17	2	**113 332**	**51**
Slovakia	145	1	55 334	1	13 743	1	0	0	**69 222**	**3**
Slovenia	5	0	27 657	1	9 098	0	24	0	**36 784**	**1**
Spain	1 346	5	519 051	27	101 366	51	55	20	**621 818**	**103**
Sweden	0	0	10 095	0	25 174	0	8	0	**35 277**	**0**
United Kingdom	3 969	8	353 126	7	304 909	172	170	39	**662 174**	**226**
Total:	**13 549**	**16**	**8 615 258**	**113**	**1 492 102**	**359**	**2 971**	**73**	**10 123 880**	**561**

2006	Eradication Measures		Healthy slaughtered animals		Risk animals		Suspects subject to laboratory examination		Total	
	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed	Animals tested	BSE confirmed
Austria	31	0	165 906	2	15 770	1	5	0	**181 712**	**3**
Belgium	8	0	262 332	1	37 906	0	59	0	**300 305**	**1**
Cyprus			4 014	0	782	0			**4 796**	**0**
Czech Republic	271	0	108 809	2	65 387	1			**174 467**	**3**
Denmark	3	0	141 146	0	28 508	0	4	0	**169 661**	**0**
Estonia			21 073	0	6 619	0			**27 692**	**0**
Finland			90 533	0	14 495	0	1	0	**105 029**	**0**
France	60	0	1 494 290	2	174 992	2	25	0	**1 669 367**	**4**
Germany	293	0	1 538 695	7	215 591	7	1 638	0	**1 756 217**	**14**
Hungary			29 578	0	6 930	0	12	0	**36 520**	**0**
Ireland	2 056	0	682 919	6	96 482	24	179	5	**781 636**	**35**
Italy	93	0	405 893	4	68 804	2	8	0	**474 798**	**6**
Latvia			30 490	0	1 160	0			**31 650**	**0**
Lithuania			64 656	0	5 649	0			**70 305**	**0**
Luxemburg			10 574	0	2 836	0	1	0	**13 411**	**0**
Malta			2 100	0	242	0			**2 342**	**0**
Netherlands	29	0	268 830	1	40 798	0	9	1	**309 666**	**2**
Poland	1	0	440 234	6	45 851	3	157	1	**486 243**	**10**
Portugal	319	0	56 220	9	26 571	18	6	1	**83 116**	**28**
Slovakia			43 942	0	12 946	0			**56 888**	**0**
Slovenia			16 818	0	7 948	0	11	0	**24 777**	**0**
Spain	452	0	307 397	10	71 428	18	26	3	**379 303**	**31**
Sweden	4	0	34 084	0	15 352	1	6	0	**49 446**	**1**
United Kingdom	849	1	207 935	1	158 696	69	106	13	**367 586**	**84**
Total:	**4 469**	**1**	**6 428 468**	**51**	**1 121 743**	**146**	**2 253**	**24**	**7 556 933**	**222**

Annex II

Age distribution of tested bovines

Table 1: **Estimated age distribution of all bovine animals tested during 2001 in the EU BSE monitoring programme**

Age	UK	DE	ES	IRL	IT	FR	BE	NL	DA	AU	FI	PT	EL	LU	SV	Total
<24	0	**726.275**	**0**	**3.991**	**0**	**0**	**1.014**	**0**	0	0	0	0	0	0	0	731.280
24-30	14.653	**405.652**	**22.113**	**11.974**	**71.035**	**76.721**	**2.660**	**4.398**	2.778	14.084	1.960	2.370	2.505	1.400	660	634.963
31-36	799	**262.903**	**23.507**	**269.707**	**53.190**	**341.142**	**35.503**	**46.531**	31.613	23.306	2.152	1.975	821	3.351	2.550	1.099.051
37-42	6.099	**189.047**	**15.023**	**86.027**	**38.395**	**327.014**	**39.562**	**48.110**	31.978	16.185	2.701	6.045	923	2.612	2.600	809.262
43-48	6.777	**162.757**	**23.560**	**39.317**	**36.540**	**216.898**	**45.113**	**49.208**	34.912	14.374	2.589		1.017	1.879	2.350	640.318
49-54	5.424	**148.009**	**17.473**	**23.693**	**29.449**	**171.754**	**40.193**	**46.693**	32.329	12.725	3.092	4.973	1.187	1.661	2.692	538.811
55-60	12.088	**137.014**	**27.011**	**28.872**	**27.751**	**153.662**	**37.501**	**45.082**	28.881	13.153	2.690		1.185	1.321	2.360	521.075
61-66	13.922	**123.784**	**14.841**	**17.663**	**27.138**	**142.343**	**31.277**	**43.333**	25.124	12.518	2.788	4.746	1.283	1.295	2.675	462.339
67-72	11.464	**112.811**	**26.551**	**17.238**	**23.742**	**130.258**	**28.469**	**40.495**	21.312	13.107	2.186		1.185	1.113	1.914	434.249
73-78	4.109	**99.955**	**14.137**	**10.446**	**20.660**	**118.989**	**23.660**	**35.445**	16.748	11.647	1.940	4.157	1.168	1.017	1.736	363.734
79-84	4.637	**87.903**	**24.008**	**18.342**	**20.660**	**107.674**	**20.622**	**28.832**	13.059	11.815	1.492		892	839	1.090	343.961
85-90	4.654	**74.818**	**12.517**	**8.832**	**16.037**	**95.989**	**15.575**	**28.832**	10.022	10.277	1.191	3.253	866	834	443	282.504
91-96	3.997	**64.624**	**20.392**	**20.211**	**15.110**	**87.466**	**13.293**	**19.163**	7.633	10.668	884		610	620	1.553	267.871
>96	7.457	**260.785**	**145.129**	**118.802**	**84.039**	**557.790**	**43.467**	**65.519**	20.577	61.729	2.212	4.157	3.471	2.946	5.478	1.318.170
all	**96.081**	**2.856.337**	**386.262**	**675.115**	**463.746**	**2.527.700**	**377.909**	**501.641**	**276.965**	**225.588**	**27.876**	**31.675**	**17.113**	**20.886**	**28.101**	**8.512.995**

Table 2: **Estimated age distribution of all bovine animals tested during 2002 in the EU BSE monitoring programme**

Age	BE	FR	DE	DK	EL	ES	IRL	IT	LU	NL	AU	PT	SV	FI	UK	EU
<24	1.527	99	774.723	2.569	210	2.792	6.595	6.642	1	480	1.306	114	1.581	164	35	798.838
24-30	4.279	213.567	371.780	10.098	489	52.789	45.338	89.224	20	3.320	1	1.673	2.736	1.862	12.962	810.140
31-36	38.453	431.261	863.819	34.266	1.361	28.421	215.027	59.087	3.304	48.395	1	5.927	3.620	4.235	19.752	1.756.929
37-42	48.528	400.768	124.958	33.621	1.558	22.912	79.504	51.326	2.356	54.227	0	4.912	3.472	4.431	23.055	855.628
43-48	52.049	260.166	112.206	35.249	1.641	28.521	47.130	53.416	1.677	51.573	0	4.358	3.419	4.718	28.159	684.282
49-54	49.464	220.622	109.500	33.626	1.759	24.080	45.906	53.853	1.473	50.963	0	4.811	3.575	4.623	29.878	634.134
55-60	43.773	179.727	97.166	30.645	1.720	28.826	30.722	52.632	1.197	44.523	0	5.036	3.394	4.493	32.466	556.319
61-66	37.237	177.441	90.652	24.935	1.762	21.023	16.845	48.331	1.122	41.835	0	4.654	3.266	4.016	39.922	513.040
67-72	32.497	153.845	77.476	21.939	1.728	26.371	24.968	44.622	939	37.458	1	4.653	2.659	3.472	30.182	462.811
73-78	28.301	153.373	71.318	17.779	1.672	19.426	21.441	40.826	838	36.047	1	4.256	2.189	2.575	17.910	417.952
79-84	24.634	130.712	59.979	14.272	1.494	24.401	24.506	35.495	809	30.479	0	4.334	1.771	2.185	9.676	364.747
85-90	20.374	124.602	53.009	10.905	1.398	17.087	6.853	31.171	658	26.533	0	3.692	1.415	1.657	11.553	310.907
91-96	15.987	103.555	43.427	8.495	1.168	21.065	16.639	26.923	584	20.215	0	3.529	1.054	1.339	9.955	273.936
>96	53.317	680.143	180.529	23.332	5.775	128.161	127.047	153.353	3.395	64.822	0	30.275	3.344	3.518	67.615	1.524.626
<30	0	0	0	0	0	0	0	0	0	0	2.351	0	0	0	3.057	5.408
>24	0	0	0	0	0	0	0	0	0	0	13.564	0	0	0	0	13.564
>30	0	0	0	0	0	0	0	0	0	0	212.724	0	0	0	0	212.724
Total	**450.419**	**3.229.881**	**3.030.542**	**301.731**	**23.735**	**445.875**	**708.522**	**746.901**	**18.373**	**510.870**	**229.949**	**82.224**	**37.497**	**43.288**	**336.178**	**10.195.985**

Table 3: **Estimated age distribution of all bovine animals tested during 2003 in the EU BSE monitoring programme**

Age	AT	BE	DK	DE	EL	ES	FI	FR	IE	IT	LU	NL	PT	SV	UK	EU15
<24	388	1.031	2.731	518.446	272	2.618	875	0	170	1.796	3	1.510	0	1.355	151	531.348
24-35		36.641	38.230	586.987	2.025	64.906	19.897	610.409	230.523	145.396	3.395	59.286	7.547	5.816	27.608	1.838.667
36-47		85.998	65.145	330.496	3.523	61.844	28.702	672.233	95.173	113.681	3.981	94.832	13.151	6.105	63.943	1.638.805
48-59		80.751	62.843	294.191	3.795	69.148	27.742	405.384	45.658	114.382	2.654	94.281	14.754	6.405	89.778	1.311.766
60-71		61.440	47.978	246.948	3.569	64.152	21.294	331.935	40.755	102.045	9.935	78.437	13.427	5.665	87.219	1.114.801
72-83		44.794	31.345	193.096	3.430	56.694	14.240	282.090	38.637	81.873	1.586	63.134	12.779	3.939	74.299	901.936
84-95		32.340	20.314	144.474	2.859	50.107	8.464	232.189	18.018	64.203	1.271	48.364	10.926	2.215	32.076	667.819
96-107		0	11.452	0	2.245	35.578	0	0	0	46.858	0	27.994	8.613	0	23.372	156.113
108-119		0	5.908	0	1.599	28.090	0	0	0	33.806	0	15.093	6.714	0	17.413	108.624
120-131		0	3.246	0	1.074	21.444	0	0	0	23.771	0	9.259	4.593	0	15.938	79.325
132-143		0	1.698	0	792	16.698	0	0	0	17.511	0	5.011	3.456	0	8.988	54.154
144-155		0	905	0	537	15.248	0	0	0	13.128	0	2.565	2.776	0	7.814	42.974
156 & >		0	1.130	0	813	44.728	0	0	0	27.605	0	2.467	11.236	0	13.121	101.102
96 & >		49.469	0	274.435	0	29.424	10.227	671.723	232.142		2.842	6.486	0	3.075	0	1.279.823
< 30	1.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.012
> 30	204.649	0	0	0	0	0	0	0	0	0	0	0	0	0	0	204.649
> 24	16.601	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.601
Total	**222.650**	**392.465**	**292.925**	**2.589.073**	**26.533**	**560.681**	**131.441**	**3.205.963**	**701.076**	**786.057**	**25.667**	**508.720**	**109.972**	**34.575**	**461.720**	**10.049.518**

Table 4**: Estimated age distribution of all bovine animals tested during 2003 in the EU BSE monitoring programme**

Age	CY	CZ	LT	LV	SI	SK
< 24	0	101	25	50	175	99
24-35	173	32.532	560	382	19.585	11.158
36-47	661	40.218	937	579	7.220	14.647
48-59	1.452	36.825	821	606	6.020	13.282
60-71	2.247	29.710	865	682	5.998	12.295
72-83	2.586	22.233	752	700	5.569	9.869
84-95	519	16.866	1.008	686	5.234	7.933
96-107	57	11.913	1.148	534	4.641	5.978
108-119	17	8.065	900	487	3.450	3.973
120-131	9	5.306	910	408	2.718	3.005
132-143	0	2.780	621	351	1.921	1.826
144-155	4	1.783	562	303	1.459	1.364
156 & >	0	2.124	636	358	2.178	1.597
96 & >	0	0	0	0	0	0
Total	**7.726**	**210.456**	**9.746**	**6.126**	**66.167**	**87.025**

Table 5: **Estimated age distribution of all bovine animals tested during 2004 in the EU BSE monitoring programme**

Age	BE	DK	DE	ES	FR	IE	IT	LU	NL	AT	PT	FI	SV	UK	EU 15
< 24	1.151	1.419	370.828	2.685	0	0	1.708	1	225	15	2	793	1.129	133	380.669
24-35	34.268	37.511	532.916	63.273	445.314	231.404	145.560	3.276	49.766	23.285	8.366	18.844	5.826	39.715	1.644.119
36-47	78.263	65.454	370.509	61.788	601.164	91.101	110.997	5.878	100.199	29.382	12.463	28.094	6.066	71.685	1.637.681
48-59	77.961	60.102	323.170	68.693	395.926	45.244	112.927	3.336	95.863	25.315	14.680	26.885	6.251	99.485	1.360.402
60-71	61.457	45.788	268.111	65.073	324.754	40.909	99.676	2.055	87.085	24.009	13.724	20.803	5.407	99.966	1.162.543
72-83	46.886	30.660	210.810	57.970	268.102	40.850	80.367	1.129	69.366	22.353	12.235	13.970	4.032	95.485	956.577
84-95	31.541	18.798	155.873	49.281	220.217	39.687	61.841	588	51.520	19.511	10.841	8.269	2.493	79.663	751.366
96-107	21.231	11.954	0	35.917	171.169	0	47.070	1	33.968	17.222	8.947	0	0	33.012	381.118
108-119	13.365	6.364	0	28.173	130.572	0	33.677	0	20.402	13.412	7.182	0	0	20.137	273.583
120-131	7.513	3.447	0	26.076	97.075	0	24.949	0	10.510	9.574	5.620	0	0	17.254	202.152
132-143	4.160	1.948	0	18.327	71.775	0	18.356	0	5.747	6.800	4.218	0	0	9.941	141.330
144-155	2.329	1.122	0	14.900	52.925	0	13.739	0	2.998	4.416	3.057	0	0	8.963	104.477
156 & >	2.856	1.314	0	52.179	112.779	0	32.271	0	2.767	6.746	13.686	0	0	13.614	238.249
96 & >	0	0	297.931	33.381	0	211.172	0	367	7.116	0	0	9.991	3.008	0	562.966
> 24	0	0	0	0	0	0	0	0	0	3.678	0	0	0	0	3.678
Unknown	10.888	517	2.719	0	0	549	1.620	0	0	0	0	0	1.915	12.543	30.751
Total	393.869	286.398	2.532.867	577.716	2.891.772	700.916	784.758	16.631	537.532	205.718	115.021	127.649	36.127	601.596	9.831.661

Table 6: **Estimated age distribution of all bovine animals tested during 2004 in the EU BSE monitoring programme**

Age	CZ	EE	CY	LV	LT	HU	MT	SI
< 24	98	19	36	61	29	46	2	161
24-35	31.518	2.493	1.070	2.102	5.426	9.950	225	7.178
36-47	38.407	3.767	1.189	3.287	5.080	17.576	341	6.090
48-59	36.183	3.751	1.137	2.910	4.402	17.426	988	5.243
60-71	28.303	3.436	1.056	2.888	4.496	14.414	468	4.950
72-83	21.051	3.349	761	3.207	4.760	11.418	128	4.774
84-95	15.316	2.862	927	2.855	4.436	8.088	35	3.987
96-107	10.997	2.407	664	2.587	5.151	5.961	2	3.681
108-119	7.495	1.793	327	2.461	4.937	4.155	0	3.096
120-131	5.179	1.149	115	2.102	4.823	2.699	0	2.204
132-143	2.850	754	45	1.636	2.154	1.762	0	1.518
144-155	1.518	566	8	1.445	2.144	1.020	0	1.067
156 & >	1.958	682	16	2.035	2.665	1.760	0	1.684
96 & >	0	0	0	0	0	0	0	0
Unknown	0	3	0	0	0	0	98	59
Total	**200.873**	**27.031**	**7.351**	**29.576**	**50.503**	**96.275**	**2.287**	**45.692**

Annex III

Age distribution of BSE positives cases detected in the period 2001-2004 in the EU BSE monitoring programme

Table 7: **Age distribution of BSE positives cases detected in 2001 in the EU BSE monitoring programme (EU-15)**

Age (months)	BE	DK	DE	EL	ES	FR	IE	IT	NL	AT	PT	FI	UK	Total
<24	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
24-35	0	0	2	0	0	0	0	0	0	0	0	0	0	**2**
36-47	0	1	1	0	4	1	0	0	0	0	0	0	2	**9**
48-59	3	1	38	1	15	6	1	13	3	0	10	0	11	**102**
60-71	22	3	64	0	24	75	60	22	11	1	23	0	127	**432**
72-83	14	0	14	0	16	125	98	9	0	0	27	1	375	**679**
84-95	4	1	3	0	14	57	46	3	2	0	33	0	277	**440**
96-107	2	0	1	0	4	13	17	2	3	0	12	0	196	**250**
108-119	1	0	1	0	0	0	8	0	0	0	2	0	84	**96**
120-131	0	0	0	0	0	0	6	0	0	0	0	0	46	**52**
132-143	0	0	1	0	2	0	1	0	0	0	2	0	20	**26**
144-155	0	0	0	0	1	0	2	0	0	0	1	0	24	**28**
156 & >	0	0	0	0	2	0	3	1	1	0	0	0	42	**49**
Total	**46**	**6**	**125**	**1**	**82**	**277**	**242**	**50**	**20**	**1**	**110**	**1**	**1.204**	**2.165**

Table 8: **Age distribution of BSE positives cases detected in 2002 in the EU BSE monitoring programme (EU-15)**

Age (months)	BE	DK	DE	ES	FR	IE	IT	LU	NL	PT	UK	Total
<24	0	0	0	0	0	0	0	0	0	0	0	0
24-35	0	0	0	0	0	0	0	0	0	1	1	2
36-47	0	1	0	1	0	2	0	0	0	1	0	5
48-59	2	0	13	17	7	1	3	0	3	5	8	59
60-71	13	1	22	30	27	13	8	0	9	13	16	152
72-83	10	1	48	35	81	98	15	1	6	17	138	450
84-95	9	0	15	23	83	110	7	0	3	24	325	599
96-107	1	0	4	11	28	45	2	0	1	18	231	341
108-119	1	0	2	6	5	32	0	0	1	4	113	164
120-131	2	0	0	1	4	11	0	0	1	1	118	138
132-143	0	0	0	0	3	4	1	0	0	0	45	53
144-155	0	0	1	1	1	9	0	0	0	0	48	60
156 & >	0	0	1	2	1	9	0	0	0	2	80	95
Total	**38**	**3**	**106**	**127**	**240**	**334**	**36**	**1**	**24**	**86**	**1123**	**2118**

Table 9: **Age distribution of BSE positives cases detected in 2003 in the EU BSE monitoring programme (EU-15)**

Age (months)	BE	DK	DE	ES	FR	IE	IT	NL	PT	UK	Total
<24	0	0	0	0	0	0	0	0	0	0	**0**
24-35	0	0	0	0	0	0	0	0	0	0	**0**
36-47	0	0	2	1	0	0	0	0	0	1	**4**
48-59	1	0	13	10	1	0	0	2	3	10	**40**
60-71	3	1	9	50	10	2	3	2	25	21	**126**
72-83	4	0	16	41	16	2	10	10	30	18	**147**
84-95	3	1	12	32	29	67	8	3	19	69	**243**
96-107	2	0	1	15	29	55	3	0	13	148	**266**
108-119	1	0	0	8	18	27	3	0	22	117	**196**
120-131	0	0	0	5	1	14	1	0	14	90	**125**
132-143	0	0	0	1	3	7	1	0	3	53	**68**
144-155	1	0	0	0	1	3	0	1	1	31	**38**
156 & >	0	0	1	4	3	6	2	0	2	55	**73**
Unknown	0	0	0	0	0	0	0	0	1	0	**1**
Total	**15**	**2**	**54**	**167**	**111**	**183**	**31**	**18**	**133**	**613**	**1327**

Table 10: **Age distribution of BSE positives cases detected in 2004 in the EU BSE monitoring programme (EU-15)**

Age (months)	BE	DK	DE	ES	FR	IE	IT	NL	PT	UK	Total
<24	0	0	0	0	0	0	0	0	0	0	**0**
24-35	0	0	0	0	0	0	0	0	0	0	**0**
36-47	0	0	2	2	0	0	0	0	1	0	**5**
48-59	0	0	18	9	0	1	1	0	0	7	**36**
60-71	1	0	13	30	6	3	0	0	11	6	**70**
72-83	3	0	9	45	4	3	1	2	16	6	**89**
84-95	5	0	13	23	10	9	3	2	17	9	**91**
96-107	1	0	5	12	6	31	3	1	9	37	**105**
108-119	0	0	3	7	11	31	0	0	11	67	**130**
120-131	1	0	1	3	10	17	0	0	14	70	**116**
132-143	0	0	0	4	1	16	0	0	8	43	**72**
144-155	0	0	0	1	2	6	0	1	1	29	**40**
156 & >	0	1	1	1	1	9	0	0	3	53	**69**
Unknown	0	0	0	0	0	0	0	0	1	8	**9**
Total	**11**	**1**	**65**	**137**	**51**	**126**	**8**	**6**	**92**	**335**	**832**

Table 11: **Age distribution of BSE positives cases detected in the period 2001-2004 in the EU BSE monitoring programme (EU-10)**

Age (months)	CZ	PL	SK	SI	Total
< 24	0	0	0	0	**0**
24-35	0	0	0	0	**0**
36-47	2	0	2	1	**5**
48-59	3	1	3	1	**8**
60-71	3	4	2	0	**9**
72-83	5	5	9	3	**22**
84-95	0	4	1	0	**5**
96-107	2	3	2	0	**7**
120-131	0	1	0	0	**1**
132-143	0	1	1	0	**2**
144-155	0	1	0	0	**1**
156 & >	0	0	0	0	**0**
Total	**15**	**20**	**20**	**5**	**60**