

## SCIENTIFIC OPINION

### Scientific Opinion on BSE Risk in Bovine Intestines<sup>1</sup>

EFSA Panel on Biological Hazards (BIOHAZ)<sup>2,3</sup>

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#### ABSTRACT

According to Regulation (EC) No 999/2001 certain ruminant tissues, designated as Specified Risk Material (SRM), must be removed from the food and feed chain to protect the health of consumers against the risk of BSE. The intestines, from the duodenum to the rectum, of bovine animals of all ages are currently included in the list of SRM. The “TSE roadmap” of the European Commission (EC) foresees amendments of the current SRM list based on newly evolving scientific knowledge while ensuring and maintaining a high level of consumer protection. EFSA was requested by the EC to evaluate the current risk of BSE linked with the use of bovine casings for the production of sausages. EFSA’s assessment was based on new but limited experimental scientific data demonstrating that in addition to ileum, also jejunum may harbour infectivity when a large BSE inoculum dose was used to experimentally infect cattle, and a recent report that attempted to quantify the BSE infectious load in bovine sausage casings if produced in the EU from intestines excluding ileum. The calculated results considered in that report show that the individual human exposure from bovine casings, excluding ileum, produced in the EU (based on the calculated BSE prevalence in 2007) were “very low”. When the total human exposure in the EU per year is considered by the Panel, the obtained figures cannot be considered negligible, even when ileum is excluded. Several input assumptions of the report bore considerable uncertainties, in particular with regard to the amount of tissue calculated for production of bovine casings and the amount of infectivity potentially present in bovine intestine. EFSA concludes that the previous assessment of the BSE related risk of bovine intestines after processing into natural sausage casings remains valid.

#### KEY WORDS

BSE, cattle, intestines, casings, sausage, SRM

#### SUMMARY

Following a request from the European Commission (EC), the Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific opinion on the BSE related risk of bovine intestines used

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1 On request from the European Commission, Question no EFSA-Q-2009-00226, adopted on 10 September 2009.

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for casings. Regulation (EC) No 999/2001 of the European Parliament and of the Council stipulates that certain tissues from bovine, ovine and caprine animals must be considered as Specified Risk Material (SRM) and must be removed from the food and feed chain to protect the health of consumers against the risk of bovine transmissible spongiform encephalopathies (BSE). The intestines, from the duodenum to the rectum, of bovine animals of all ages are currently included in the list of SRM. The “TSE roadmap” prepared by the EC details the short, middle and long term actions on TSE measures such as SRM removal and sets the objectives to ensure and maintain the existing high level of consumer protection. It allows for amendments of the current SRM list based on new evolving scientific knowledge while ensuring and maintaining a high level of consumer protection.

Specifically, the mandate asked the BIOHAZ panel to evaluate the scientific validity of a report prepared by Det Norske Veritas Ltd" (DNV) for the Swiss Cervelas task force. This report provides an assessment of the current potential human exposure to BSE infectivity that could result from eating sausages made with EU bovine casings. The BIOHAZ panel was further requested to evaluate the conclusions of the DNV report and, if it was considered necessary based on the report and any other new relevant scientific information, to provide a re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings.

The BIOHAZ panel evaluated the risk assessment as described in the DNV report, and took into account the relevant previous EFSA opinions as well as new scientific data on the same subject.

New but limited experimental scientific data demonstrate that in addition to ileum, also jejunum may harbour infectivity when a large BSE inoculum dose was used to experimentally infect cattle. With regard to the DNV Report, the BIOHAZ Panel considers its approach (concept and methodology) scientifically sound, whereas the interpretation of the results as obtained is not shared by the Panel. Its assumptions were based on limited scientific data obtained from a single morphometric study (which was already found to be inadequate in the previous EFSA Opinion on bovine casings) and on limited and earlier data on the presence of PrP<sup>sc</sup>/infectivity in bovine gut after experimental oral BSE inoculation. There is uncertainty about the relative BSE risk of neural and lymphoid tissues in casings compared to CNS that might have significant impact on the calculated results of the DNV Report. The Panel notes that the DNV Report considers the individual human BSE exposure risk from bovine casings, excluding ileum, to be “very low”. However, when the upper confidence limits are taken into account, along with the uncertainties in key parameter assumptions, the calculated total human exposure per year in the EU from bovine casings, even when ileum is excluded (based on the calculated BSE prevalence in 2007) is 11.000 cattle oral ID<sub>50</sub> units per year (when all casings would have been sourced in the UK) and about 1.000 cattle oral ID<sub>50</sub> units per year (when all casings would have been sourced in the Netherlands) and therefore cannot be considered negligible. Thus the conclusion in the DNV report that sausage casings sourced from intestines of cattle in EU Member States would lead to a negligible risk for human consumption cannot be considered valid. Moreover, when considering other new relevant scientific information it is concluded that the previous EFSA assessment of the BSE related risk of bovine intestines after processing into natural sausage casings remains valid. The Panel recommends that future considerations on the risk in bovine casings should take into account the BSE prevalence in cattle at that time.

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## **BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION**

According to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies, certain tissues from bovine, ovine and caprine animals must be considered as specified risk material (SRM) and must be removed from the food and feed chain to protect the health of consumers against the risk of BSE. The intestines, from the duodenum to the rectum, of bovine animals of all ages are currently included in the list of SRM.

As regards SRM, the Communication from the Commission of 15 July 2005 on the future of TSE measures (the “TSE roadmap”) sets the objective to ensure and maintain the existing high level of consumer protection within the European Union by continuing to assure the safe removal of SRM. The TSE roadmap also states that any amendment of the current list of SRM should be based on new evolving scientific knowledge.

On 17 December 2008, the Federal Veterinary Office of Switzerland submitted to DG SANCO a report elaborated for the Swiss Cervelas task force by "Det Norske Veritas Ltd" (DNV) on the TSE risk assessment for use of bovine casings. This report provides an assessment of the current potential human exposure to BSE infectivity that could result from eating sausages made with EU bovine casings. It concludes that this exposure is very low and that changes to the SRM removal rules could be considered without any significant increase in the risk to consumers.

## **TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION**

In view of the above, and following consultation with the EU Commission Services, the terms of reference were left as follows:

EFSA and its Panel on Biological Hazards are requested:

- to evaluate the scientific validity of the report
- to evaluate the conclusions of the report and, if it was considered necessary based on the report and any other new relevant scientific information, to provide a re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings.

## **ACKNOWLEDGEMENTS**

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## ASSESSMENT

### 1. Introduction

The objective of this assessment is to evaluate the scientific validity of the DNV report taking into account any other scientific data or reports on the BSE related risk in bovine intestines, and to advise whether an update of the previous risk assessment is needed. By means of a letter from the European Natural Sausage Casings Association (ENSCA), EFSA was informed of a scientific study commissioned by ENSCA and carried out by the Veterinary Laboratory Agencies (VLA) and has considered this study and its conclusions in the assessment. Additional scientific publications on the subject and preliminary new data from the German BSE pathogenesis study have also been analysed.

While there are some conclusions in the DNV report that relate to other issues, this assessment is restricted to the parts of the DNV report directly related to the risk of bovine intestines.

### 2. Current legislation

According to Regulation (EC) No 999/2001 (EU, 2001) laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (the TSE Regulation), certain tissues from bovine, ovine and caprine animals must be considered as Specified Risk Material (SRM) and must be removed from the food and feed chain to protect the health of consumers against the risk of BSE. The intestines, from the duodenum to rectum, from bovine animals of all ages are included in the list of SRM.

### 3. TSE roadmap

The Communication from the Commission of 15 July 2005 on the future of TSE measures, the “TSE roadmap” (EC, 2005), sets the strategic goal, regarding to SRM, to ensure and maintain the current level of consumer protection by continuing to ensure the safe removal of SRM but to modify the SRM list based on new and evolving scientific advice while maintaining the existing high level of consumer protection within the European Union.

### 4. Background information

#### 4.1. Background related to the usage of bovine intestines for casings

It is well known that size and proportion of different parts of the intestinal tract of slaughtered cattle at abattoirs can vary considerably. In the context of this Opinion, it is therefore important to know whether bovines from extensive farming systems (common in some beef producing countries overseas) differ from those from intensive farming systems (common in EU) with respect to the amount/distribution of BSE-relevant tissues along the intestines. Some studies indicated that high concentrate rations increase both the absorption surface of the rumen papillae and the height of villi in the small intestine of intensively fed cattle as compared to extensively fed animals (Zitnan *et al.*, 2003) and that morphology of small intestinal mucosa and intestinal weight change with types of cattle having distinct metabolic characteristics (Zitnan *et al.*, 2008). However, no published information is available on whether such, or other, anatomical variations have any relevance for the BSE-related concerns with bovine sausage casings.

Literature data does not indicate different usages of intestines from different age categories of slaughtered cattle for production of natural sausage casings. It is assumed that the harvested intestines come mainly from cattle destined for beef production. Beef production systems in the European Union differ in regard to the age at which animals are slaughtered. Analysis of data for the 1997-1999

period indicated that large proportions of the offspring of the 11.7 million beef suckler cows and of the 21.7 million dairy cows in the EU were destined for the beef fattening units (SCAHAW, 2001). With respect to the slaughter age of these beef animals, younger concentrate-fed beef animals are slaughtered at ages ranging from 12 to 18 months, and older grass-fed beef animals are slaughtered at ages ranging from 2 to 2.5 years (SCAHAW, 2001). In addition, older cows excluded from dairy production are also slaughtered and enter the beef chain.

The bovine intestinal tract can be divided into several parts; each is designated by a meat industry- and/or sausage casings industry-related technical term, which does not necessarily correspond to the standard anatomical term for that intestinal part. The intestinal “set” used by the sausage casings industry (Praendl *et al.*, 1998; Rust, 2004; INSCA, 2009) comprises:

- For industrial purposes, the small intestine (jejunum and ileum; technically called “runner” or “beef round”) starts at the duodenum-jejunum junction and ends at the ileum-caecal junction. It is 25-30 m long in total (of which the ileum is <1-1.5 m) and 3-6 cm in diameter. “Beef rounds” casings derive their name from their characteristic “ring” or “round” shape and are used for various types of sausages, e.g. Ring Bologna, Ring Liver Sausage, Mettwurst, Polish Sausage, Blood Sausage, Kishka and Holsteiner. In the documents, no explicit distinction is made between ileum and jejunum. Caecum (technically called “beef bung cap”) is 1-1.5 m long and 7.5-20 cm in diameter. “Beef bung caps” are used for various large-diameter sausages, e.g. Mortadella.
- The large intestine (colon; technically called “beef middle”) is 6-12 m long and 3-6 cm in diameter. “Beef middles” are often used for Leona Style Sausage, Dry and Semi-dry Cervelats, Dry and Cooked Salami, and Veal Sausage.
- The end part of large intestine (colon descendens and rectum; technically called “fat end”) is around 1 m long. It is used for similar purposes as “Beef bung caps”. In addition, oesophagus and bladder of cattle are also used for the preparation of some sausages casings.

Regarding some non-EU countries e.g. Canada, (Meat Hygiene Manual of Procedures, Chapter 4 Annex N., Canadian Food Inspection Agency) the distal ileum of all slaughtered cattle, regardless of their age, is designated as SRM. This has to be done by one of the following options: a) removal and disposal of the entire cattle small intestine; or b) separation of the distal ileum from the jejunum and disposal of the distal ileum as SRM. In the latter case, it is in practice done by removal of the ileo-caecal junction together with at least a 200 cm-long section of the attached and uncoiled small intestine proximal to the ileo-caecal junction. After the removal of the distal ileum section, the remaining sections of the small intestines from cattle of all ages can be harvested for edible meat products, provided at post-mortem meat inspection no pathologies were found in the intestines and corresponding carcasses were judged as fit for human consumption.

#### 4.2. Background on the terminology of pathological PrP

In scientific studies, the presence of TSE agents or prions can be best assessed in infectivity bioassays. As these are time consuming and expensive, the presence of the prion protein (PrP) in its disease-associated misfolded form can be assessed by various diagnostic methods as a surrogate marker. However, the terminology used to describe such pathology associated PrP is not uniform, hence for reasons of clarity, explanations are given in table 1. In this document, the term PrP<sup>sc</sup> is used throughout as designation for the disease-associated PrP with potential infectivity.



**Table 1:** The terminology used to describe pathology associated PrP.

PrP <sup>d</sup>	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 immunohistochemistry (IHC). It is sometimes used synonymously with PrP <sup>sc</sup> .
PrP <sup>res</sup>	Abnormally folded prion protein that is highly resistant to proteinase K digestion and is strongly associated with prion disease. This acronym is used when methods for detection of disease-associated PrP are employed that are based on proteinase resistance, such as immunoblotting or ELISA. It is sometimes used synonymously with PrP <sup>sc</sup> .
PrP <sup>sc</sup>	Term originally derived from scrapie associated PrP, but also more generally used in all TSEs. Disease associated, 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 <sup>TSE</sup>	TSE associated, abnormally folded protein. It is used synonymously with PrP <sup>sc</sup> or PrP <sup>d</sup> . Sometimes “TSE” is replaced by the acronym of the respective disease, e.g. PrP <sup>CJD</sup> , PrP <sup>GSS</sup> , PrP <sup>BSE</sup> , PrP <sup>sc</sup> , PrP <sup>CWD</sup> , etc.

## 5. Previous EFSA Risk assessment on BSE risk in Bovine Intestines

A previous opinion on the subject, based on a similar request for an evaluation of a scientific study (EFSA, 2007) has also been consulted. The EFSA Scientific Panel on Biological Hazards (BIOHAZ) analysed the design of a morphometric study by Wijnker *et al.* (2008) in detail and considered the study inadequate for the purpose of demonstrating the safety of bovine casings of cattle originating from BSE risk countries.

In the EFSA opinion of 2007 (EFSA, 2007) on the Wijnker *et al.* quantitative histological studies and the re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings, there was a clear statement on the significance of negative findings:

*“Future studies need to address problems of sampling; for example, the lower frequency of lymphoid follicles in parts of the intestine other than the distal ileum suggests that possible future studies of the pathogenesis of BSE need to harvest a large number of samples to improve confidence in a negative result. Improved knowledge of the distribution of infectivity within the component tissues of the distal ileum also impacts on any future sampling strategy. The numbers of animals from which the required tissues are available from experimental studies invariably provides a challenge for statistical analyses of the results because of the practical and funding limitation on such large animal experiments.”*

In answering to the ToR in the EFSA 2007 opinion it was considered that the design of the study evaluated for the opinion was considered inappropriate for the purpose of demonstrating the safety of bovine casings of cattle originating from BSE risk countries. It was concluded that for a re-assessment of the BSE risk of bovine casings there were at the time insufficient data.

In addition to this conclusion, a number of recommendations on the topics that should be addressed in future studies on the subject were made. *“If a relevant re-assessment of the BSE risk of bovine casings would be desired, appropriately designed morphometric, experimental PrP<sup>sc</sup> and infectivity*

studies would be necessary. Moreover, it would be necessary to study under real-life, commercial conditions the potential for cross-contamination of the processed intestines.” The studies required to be carried out were summarized as follows:

**“An appropriately designed morphometric study would require:**

- *To assess the amount of lymphoid tissue and neural tissue in a specific anatomical region. The 3D volume of the whole region is estimated by weighing it and transform it to a volume using the volume/weight ratio of intestinal tissue. This estimation can also be performed by the use of the principle of Cavalieri, systematic uniformly random sampling (SURS) and point counting of ~10 slices of each sample (Gundersen et al., 1987). If possible, the same slices of fresh tissue can be used to estimate the area fraction of lymphoid tissue using point counting. Sections (paraffin or frozen) taken from the same sites and stained with a specific antibody against lymphoid tissue should be used to estimate the average height of the lymphoid tissue. The area fraction and average height of the lymphoid tissue combined provides the volume fraction of lymphoid tissue. If this design is not practical, the paraffin or frozen sections would be used to estimate a volume fraction of lymphoid tissue. Similar sections stained with specific antibodies against neural tissue would be used to estimate the volume fraction of the neural tissue. The total volume of lymphoid tissue and neural tissue would be calculated by multiplying the total volume of the anatomical region with the relevant volume fractions. Sections should be checked for global and differential tissue deformation and if present, the estimates corrected.*

**An appropriately designed experimental PrP<sup>sc</sup> and infectivity study would require:**

- *To determine the distribution of prion protein and/or infectivity in relation to specific intestinal regions (jejunum and duodenum relative to ileum) and the anatomical substructure of the intestinal wall. Without large scale prospective BSE infection studies in cattle this can probably only be achieved by utilizing archived material from previous studies of the pathogenesis of BSE after oral exposure. Infectivity studies (titrations or incubation period assays, following a single titration of ileal tissue), utilising a sensitive mouse assay and biochemical estimation of PrP<sup>sc</sup> using appropriate ELISA tests or Western blot methods should be used to provide quantification of infectivity/PrP<sup>sc</sup> according to intestinal region. Specific tissue localisation of infectivity is impractical because of the difficulty of dissection of components from the fresh intestine, but localization and frequency estimates of PrP<sup>sc</sup> can be achieved in paraffin sections of the intestine at multiple levels immunolabelled with antibodies to the protein.*
- *PrP<sup>sc</sup> and, ideally, infectivity studies should be performed on bovine casings after real life production conditions*

**An appropriately designed cross-contamination study would require:**

- *To consider the issue whether the casings production process can enable cross-contamination of the final product with the BSE agent as well as the related food safety implications (including legislative aspects), should such a cross-contamination occur.”*

It is against this background that the current report has been considered.

## 6. Overview of current scientific knowledge on BSE risk in Bovine Intestines.

The previous EFSA Opinion on BSE risk from bovine intestine summarised the scientific knowledge that was available until early 2007. Since then, additional publications have become available on a natural BSE case in Japan (Kimura and Haritani, 2008) and two experimental studies that examined



presence of PrP<sup>Sc</sup> and/or infectivity in the intestines of cattle challenged orally with 100g (Espinosa *et al.*, 2007; Hoffmann *et al.*, 2007). Moreover, a new study performed by the VLA in the UK on PrP<sup>Sc</sup> in BSE-infected cattle (Stack, 2009) and preliminary results from the German BSE pathogenesis study have recently be made available to EFSA and were also taken into account.

### 6.1. New experimental studies on intestines of BSE infected cattle

Espinosa *et al.* (2007) examined pooled tissues from 13 cattle inoculated at ages between 4 and 6 months and culled at ages between 24 and 39 months. Infectivity in Tgbov mice but not PrP<sup>Sc</sup> by ELISA/WB was found in Peyer's patches dissected from distal ileum at all ages. Hoffmann *et al.* (2007) demonstrated PrP<sup>Sc</sup> by IHC in Peyer's patches of distal ileum in one of two preclinical animals sacrificed at 24 and 28 months post inoculation (mpi). Most recently, Arnold *et al.* (2009) estimated the titre of infectivity in the distal ileum from the incubation time found by bioassay in wild type mice. Over time, the infectivity in the distal ileum showed an initial increase up to 14-18 months post exposure, followed by a decrease, which was likely to be highly variable between animals. However, these estimates were based on mouse titration of brain material, while the incubation period to dose relationship may differ between brain and intestines (Robinson *et al.*, 1990).

### 6.2. Infectivity of intestines in cattle with natural BSE infection

Data on presence of PrP<sup>Sc</sup> or infectivity in intestines of natural BSE cases are sparse. The immunohistochemistry (IHC) and Western blot examinations of three BSE infected cattle detected in Japan in the course of active surveillance (but showing locomotor deficits) found PrP<sup>Sc</sup> in distal ileum of two (by IHC confined to the myenteric plexus) (Iwata *et al.*, 2006). No PrP<sup>Sc</sup> was detected in Peyer's patches of distal ileum, or in samples of other regions of small and large intestine, or in a range of other lymphoid tissues. Labelling of myenteric plexus was also detected in 9/29 confirmed field cases of BSE examined in the UK (Terry *et al.*, 2003). Infectivity by wild-type mouse assay or the presence of PrP<sup>Sc</sup> has not been found in the distal ileum, or other levels of intestine in a total of some six natural BSE cases studied (Fraser and Foster, 1994; Buschmann and Groschup 2005; Iwata *et al.*, 2006). In one of these cases in Germany, however, infectivity was detected in the distal ileum by bioassay in TgBov XV mice (Buschmann and Groschup, 2005). More recently, another BSE case (94 months of age) in Japan showed definite or equivocal immunoreactivity in nerve cells of the myenteric plexus in ileum, caecum and colon, and in Schwann cells of the myenteric plexus in duodenum, jejunum, ileum, caecum and colon (Kimura and Haritani, 2008).

### 6.3. Study commissioned by ENSCA

This ENSCA commissioned study investigated the presence of BSE PrP<sup>Sc</sup> in small intestines of cattle that had been orally challenged at 4-6 months of age with 100g or 1 g doses of BSE affected brain tissue. These animals were culled and examined 18-30 months post inoculation (p.i.). Three methods to identify PrP<sup>Sc</sup> were applied: a commercial ELISA test, Western immunoblotting, and IHC. Results confirmed previous observations that PrP<sup>Sc</sup> was mainly confined to lymphoid tissue of the ileum, whereas the duodenum was negative and no part of the enteric nervous system tested positive. The lymphoid tissue of the jejunum of one high-dosed animal tested positive. As expected, the low-dosed animals had a much lower frequency of positive ileum samples (1/18 vs. 15/18 in the high-dose group) and some longer incubation times (24 months in the one animal with positive ileum), whereas the high-dose group included animals positive at all ages examined.

As the ENSCA commissioned study was performed retrospectively on archival tissue, sampling was limited by availability, and the study authors themselves concede that "*it is possible tissue sampling was not optimal*" for duodenum and jejunum of low-dosed animals. The 1g-dosed group included 6 animals sampled at 18 months p.i., 6 at 24 months, and 6 at 30 months. The 100g-dosed group included 6 animals sampled for ileum at 18 months p.i., 6 at 24 months, and 6 at 30 months;

duodenum and jejunum, however, were sampled only in 2 animals each at 18, 24 and 30 months p.i., respectively. From each level of the intestine, three sections were examined by IHC per case. While at least two of the three sections of the ileum per case contained lymphoid follicles, in 36% of the duodenum cases, and in 39% of the jejunum cases lymphoid follicles were absent in any of the examined sections. The frequency of positive follicles per section ranged between 1% and 14% in ileum of the high-dose group, and 0,7% in the one positive ileum of the low-dose group, and was 6,7% and 11,1% in the two positive jejunum sections of one high-dosed animal.

#### Conclusions on the ENSCA commissioned study:

- This study confirms that detectable PrP<sup>sc</sup> is mainly confined to lymphoid tissue of the ileum in cattle orally challenged with 100g of BSE brain and culled at 18, 24 and 30 months post-inoculation (p.i.)
- One out of 18 animals challenged orally with 1g of BSE brain was positive in ileum.
- One out of 18 animals challenged orally with 100g of BSE brain was positive in jejunum.
- The duodenum was always negative.
- However, the sampling in particular of duodenum and jejunum was limited and contained lymphoid tissue only in a part of sections examined.
- In contrast to previous reports on natural BSE cases in older animals, the enteric nervous system was always negative.
- In consideration of the previous EFSA opinion on bovine intestine that gives detailed advice for future studies, in particular concerning the lower frequency of lymphoid follicles in parts of the intestine other than the distal ileum, the present ENSCA commissioned study meets some but not all recommendations; in particular the mostly negative results obtained for jejunum and duodenum should not be over-interpreted when tissue sampling was limited.

#### 6.4. New preliminary data on bovine intestine from the German BSE Pathogenesis study

In the German BSE pathogenesis study performed at the Friedrich-Loeffler-Institute (FLI), 56 Simmental cross-breed calves aged about four months were challenged orally with 100g brainstem-homogenate pooled out of clinically BSE diseased cattle. The infectivity load in the homogenate was about  $10^{6.1}$  ID<sub>50</sub> (grams of tissue)<sup>-1</sup> as determined by end-point titration in Tgbov XV mice (Buschmann & Groschup, 2005, Hoffmann *et al.*, 2007). Furthermore, as controls, 18 calves were inoculated orally with a BSE-negative brainstem homogenate. Four to five animals were selected randomly and euthanised every four months. More than 150 tissue and body fluid samples were sampled at subsequent necropsies from each animal under TSE-sterile conditions.

After oral exposure with the TSE agent, previous studies had demonstrated consistently early prion accumulation in the gut associated lymphatic tissue, about six months post infection (mpi) in cattle (Terry *et al.*, 2003), and at two mpi in scrapie infected sheep (van Keulen *et al.*, 2002) and in 21 days old lambs (Andreoletti *et al.*, 2002). In contrast to scrapie, the accumulation of PrP<sup>d</sup> in the distal ileum of BSE-infected cattle was confined to an only minor proportion of follicles respectively neurons/glial cells of the enteric nervous system (Terry *et al.*, 2003).

Normally when performing IHC, a three micrometer section per paraffin block is used, reflecting a very small proportion of the tissue sample. Therefore a serial section procedure was newly established at the FLI to increase the total amounts of tissue structures examined per sample and consequently increasing the probability of detecting PrP<sup>sc</sup> accumulation. Thereby, five sections per paraffin block

with a plane distance of about 25-30  $\mu\text{m}$  were examined. Hence, a tissue depth of about 150-200  $\mu\text{m}$  per block was screened for positive immunosignals. Additionally two different PrP<sup>sc</sup>-specific monoclonal antibodies, highly sensitive for the detection of bovine PrP<sup>sc</sup> were used.

According to this method, representative samples of the small intestine, in particular Peyer's patches of the distal ileum but also the ileo-caecal junction from most of the infected animals of the German BSE Pathogenesis study were examined by IHC. From 4 mpi until 44 mpi in most animals (38/43), PrP<sup>sc</sup> was detectable, initially in the follicles of the Peyer's patches and at later stages of the incubation period in the enteric nervous system too.

### Conclusions on the German pathogenesis study

- With improved sampling, nearly all animals dosed with 100 g of BSE brain tissue showed PrP<sup>sc</sup> in distal ileum between 4 and 44 mpi, first in lymphoid tissue and later in enteric nervous system.

## 7. Review of the DNV report

### 7.1. Summary of the report

DNV makes an attempt to quantify the amount of BSE infectious load in bovine sausage casings. This is then extrapolated to the risk carried in an individual sausage, a normal persons risk per year and the overall exposure within the EU in a year. The key points of the DNV Report are as follows:

- The DNV Report assumes that the ileum is not used for the production of casings and is removed and discarded.
- The DNV Report is based on the assumption that potential infectivity in bovine intestine used for sausage casing production would be 2 logs less than in the ileum. Based on experimental data, the infectivity in the distal ileum was considered to be at a titre equivalent to that in the CNS at the late stage of infection. Thus infectivity in non-ileal parts of the intestines used for casings production was assumed to be 100 fold less than in the CNS.
- The DNV Report uses a value of 0.43g/m (obtained from Wijnker *et al.*) of casing to quantify the amount of lymphoid and neural tissue that might harbour infectivity in a sausage casing,
- The results of the DNV Report calculate that an exposure per person per year from bovine casings produced in the Netherlands "would be very low" even when a high consumption pattern like in Germany is assumed (upper range  $7 \times 10^{-6}$  cattle oral (CO) ID<sub>50</sub> units). For casings sourced in the UK, the exposure would be about one log higher.
- When the calculated total amount of cumulative human exposure per year in the EU is considered, the following scenario emerges: 11.000 CO ID<sub>50</sub> units per year when all casings would have been sourced in the UK, and about 1.000 CO ID<sub>50</sub> units when all casings would have been sourced in the Netherlands, a country with an about average prevalence of BSE in the EU<sup>4</sup>.

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4 How can the output of the DNV calculations be interpreted in terms of potential human infections? If we follow, as in the previously adopted EFSA Opinions on Tallow and MBM (EFSA 2005 a and b) the cautionary advice of the original QRA WG and assume the species barrier is 1 as a worst case scenario, then there would be up to 5500 infected person in the EU per year in the first scenario, and up to about 500 in the second. This would have to assume a linear dose-response curve of infectivity at very low doses. If the species barrier was given a more realistic value obtained from the analysis carried out on the exposure of the British population to the BSE agent (EFSA, 2006) of around 1000 - 4000, this would mean that there might be up to around 1 to 5 infected person in the EU per year in the first scenario, and less than 1 in the second.

## 7.2. The methodology applied in the DNV Report

### *Risk model structure*

The risk assessment method is based on a linear calculation that multiplies a few essential parameters. In describing the method, for ease of illustration, the report focuses on calculating the total European exposure (per year) for which the approach is based on the following parameters:

- a. The total weight of bovine sausage casings used to produce sausages in Europe each year.
- b. The fraction of the total casing weight that is comprised of lymphoid and neural tissue.
- c. The prevalence of BSE infected cattle in the slaughter population.
- d. The number of CO ID<sub>50</sub> units in a gram of neural and lymphoid tissue.

For a basic estimate of the total exposure in Europe the product of the individual parameters a, b, c and d above was used. Hence due to the form of the model, the final risk values will always scale in manner directly proportional to each parameter value. It is therefore straightforward to consider the impact of any uncertainties in parameter estimates. In practice, DNV further assumes a statistical distribution for some of these parameters, and hence obtains a range of estimates for the total exposure risk.

### *Parameter values and assumptions*

The parameter values used in the DNV model are as follows:

- a. The total weight of bovine sausage casings used each year is assumed to be 68.000 tonnes, which was quoted as the total amount imported into the EU in 2005.
- b. The fraction of the total casings weight that is lymphoid and neural tissues is assumed to be 0.7%. (fixed point estimate value from the DNV model) This is based on histological studies by Wijnker *et al.* (2008).
- c. The prevalence of BSE infected cattle in the slaughter population is based on two scenarios. First, the prevalence in 2007 in the UK, and secondly the prevalence in 2007 in the Netherlands. In both cases this prevalence is stratified by age and time to expected clinical onset. The values used were given in Tables 3 & 4 of the DNV Report:

### **Tables 3 and 4 of the DNV Report:**

**Table 3: Prevalence of BSE Infection in UK Slaughter Cattle – 2007**

Age at slaughter	All infected cattle	Within 12 months of onset
OTM but born after 1 August 1996	$2.7 \times 10^{-5}$	$4.4 \times 10^{-6}$
Under 30 months	$4.7 \times 10^{-5}$	$4.8 \times 10^{-6}$

**Table 4: Prevalence of BSE Infection in Slaughter Cattle in Netherlands – 2007**

Age at slaughter	Numbers of infected cattle slaughtered		Number slaughtered	Prevalence
	All infected cattle	Within 12 months of onset		
Over 30 months	1.95	0.91	472,000	$4.1 \times 10^{-6}$
Under 30 months	0.5	0.02	143,000	$3.5 \times 10^{-6}$

- d. The number of CO ID<sub>50</sub> units per gram of lymphoid and neural tissue is assumed to be log normal with a median of 0.05 and the 99<sup>th</sup> percentile is 1. This is extrapolated directly from the distribution of CO ID<sub>50</sub> found in experiments using brain homogenate from clinically diseased cattle (Wells *et al.*, 2007) and assuming that the neural and lymphoid tissue in bovine casings has a 100 fold less infectious titre than CNS of clinical BSE cases. The ileum is not considered a potential source of infectivity as it is assumed to be always removed.

*Uncertainty and variability in chosen parameter values*

The assumptions for the values or ranges of each parameter are considered here as follows:

- Sausage casing production: for the EU the exposure may be lower since some sausages will be exported and not all of the casings will be consumed. The worst case scenario in the EU would be that all the casings are consumed and include the ileum. The DNV report bases its calculations on a fixed value for this parameter, assuming all are consumed within Europe.
- The distribution applied for the proportion of sausage casings (by weight) that is comprised of lymphoid and neural tissue is taken directly from Wijnker *et al.* 2008. This parameter was commented on in the previous EFSA report on the safety of bovine sausage casings (EFSA, 2007), where it was concluded that the value would be dependent on the specific process applied for the production of the casing, and would not necessarily be valid for all the different industrial methods in operation throughout Europe. Therefore the value used for this parameter cannot be assumed to be a worst case assumption. It is however, the only published number for this parameter. The total exposure risk scales with this parameter in a linear fashion; hence if any higher or lower value is assumed the total risk will increase or decrease proportionally.
- The European BSE prevalence values applied here are reasonable. However, the applied estimates for 2007 can be considered as a worst case assumption due to the declining prevalence of BSE in Europe.
- The titration of CO ID<sub>50</sub> in brain homogenate of clinical BSE cases is well established. Hence the key assumption here is the relative risk of neural and lymphoid tissues in casings as compared to CNS. Estimates based on mouse titration of brain material carry uncertainty because the incubation period to dose relationship may differ between brain and intestines (Robinson *et al.*, 1990). While there is experimental evidence that the infectivity in the jejunum would be lower than in the ileum, there is uncertainty about the validity of the assumption of a two log difference. In the very limited cases where infectivity has been detected in the jejunum, the experiments have been performed on cattle after infection with a large BSE inoculum (100g) that is unlikely to be representative of the field situation.



### *Population approach versus individual risk*

The model makes the assumption that the infectious load is spread homogeneously throughout the European population. This is a worst case scenario with regard to the number of potentially exposed persons.

### **7.3. Conclusions on the DNV report**

- While the approach (concept and methodology) of the DNV Report was scientifically sound, the interpretation of the results as obtained is not shared by the Panel.
- The general method used in the analysis is sound and logical. The model is straightforward and its strengths lie in using several worst case assumptions, especially where there is little certainty on the actual value of the parameters.
- There are concerns about the level of uncertainty of the parameters and values used to feed the model and about the scientific interpretation of the results obtained.
- The calculated figures emerge to be low, essentially, because the model describes the situation where there is a very low prevalence of BSE combined with a relatively very low infectivity per gram likely to be present in bovine sausage casings. The range of estimates do allow for several worst case scenario assumptions as long as ileum is not included.
- The report does not add much additional information as compared to previous studies. Moreover, it does not specifically consider the recommendations for future studies made by the previous EFSA Opinion (2007) on bovine casings; in particular, the potential for cross-contamination.
- The DNV Report states that PrP<sup>sc</sup> or infectivity has not been found in parts of the bovine gut other than ileum after experimental oral BSE infection. However, the new data from the ENSCA commissioned study demonstrate that the jejunum may be positive, although after infection with a large BSE inoculum (100g) that might not be representative of the field situation.
- The morphometric data used in the DNV calculations are based on a study that was found inadequate by the previous EFSA Opinion (2007) on bovine casings.
- The input assumptions bear considerable uncertainties, in particular with regard to the amount of tissue calculated for production of bovine casings and the amount of infectivity potentially present in bovine intestine.
- The greatest uncertainty lies in the relative risk of neural and lymphoid tissues in casings compared to CNS.
- The main drawback in the DNV Report is that it does not consider cross-contamination. If there are errors in the estimate of infectivity in the casings, the overall exposure will scale proportionally. A second drawback is that the results of the analysis are not precise in describing the actual risk of bovine sausage casings, due to the use of worst case assumptions in the calculation (e.g. prevalence), combined with parameters for which there is little information (true infectivity levels).
- The calculated results considered in the DNV report show that the individual human exposure from bovine casings, excluding ileum, produced in the EU (based on the calculated BSE prevalence in 2007) were “very low”. When the total human exposure in the EU per year is considered by the Panel, the obtained figures cannot be considered negligible, even when ileum is excluded.



## 8. Potential cross-contamination with the BSE agent

In the DNV Report, the issue of potential cross-contamination with the BSE agent occurring during the production cycle has not been addressed. In the previous BIOHAZ Opinion (EFSA, 2007), this issue was considered as relevant:

*“According to the EU Food Hygiene legislation, removal of Specified Risk Material (SRMs) during slaughter and dressing of cattle must be conducted in such a way as to prevent cross-contamination of non-SRM materials including edible parts; should this occur, cross-contaminated parts must be treated as SRM. The issue as to whether the casings production process can give rise to cross-contamination of the final product with the BSE agent should be considered as well as the related food safety implications (including legislative aspects), should such a cross-contamination occur. These considerations would not be necessary only on condition that certain pre-conditions are reliably and verifiably met, including:*

- *any animal sub-clinically carrying the BSE agent is excluded among all those from which the intestines are harvested/processed on the day; or*
- *any remains of ileum attached to the jejunum, after their separation step, are excluded from all stages of casing production, or accidental (e.g. by mistake) inclusion of ileum in the batch being processed is excluded; and the presence of the BSE agent in jejunum is definitely excluded even in BSE-infected cattle.”*

That is, the opinion recommended that an appropriately designed cross-contamination study would require: *“To consider the issue whether the casings production process can enable cross-contamination of the final product with the BSE agent as well as the related food safety implications (including legislative aspects), should such a cross-contamination occur”*. The BIOHAZ Panel still regards these considerations as relevant.

Natural bovine casings used for sausages are generally considered as edible so can be consumed together with the sausage. This is particularly the case with small intestine casings (“beef rounds”) (Rust, 2004). Many consumers enjoy eating the casing with the sausage as they enjoy the particular bite-related sensation in the mouth; so-called “the snap” (INSCA, 2009). On the other hand, it is likely that some consumers prefer to peel off the natural beef casings before eating the sausage, and more so in case of larger-diameter bovine natural casings from large intestines (“beef middles”) from a palatability standpoint (Rust, 2004). No published systematic and reliable data on proportions of consumers eating or discarding bovine natural casings are available presently.

The DNV Report mentions: *“it is assumed that all the casings are consumed with the sausage. In reality, a significant proportion of the casings in most types of sausage would be removed and discarded before consumption. This would be expected to reduce exposures by at least a factor of 10 (e.g. only 10% of casings consumed)”*. More precise data related to, and source of information for, the stated assumption on proportion of consumers removing the casings are not provided.

In addition, should the BSE agent be present in the sausage casings, the cross-contamination of the sausage content from the bovine casings (due to contact and/or diffusion) cannot be excluded; in such a case, even removal of the casing before sausage consumption would not remove the risk for the consumer completely.

Furthermore, bovine natural casings are used for a variety of different sausages (indicated above), some of which can be mixed/cooked together with some other ingredients and consumed as a combined dish – depending on the culture and habits of the consumers. In such a case, should the BSE agent be present in the sausage casings, the cross-contamination of the meal from the sausage casings during food preparation cannot be excluded, unless the casing is completely removed before

mixing/cooking the dish. Information on how often sausages with natural bovine casings are used for combined dishes is lacking.

## CONCLUSIONS

1. New but limited experimental scientific data demonstrate that in addition to ileum, also jejunum may harbour infectivity when a large BSE inoculum dose was used to experimentally infect cattle.
2. While the approach (concept and methodology) of the DNV Report was scientifically sound, the interpretation of the results as obtained is not shared by the Panel.
3. The input assumptions in the DNV report bear considerable uncertainties, in particular with regard to the amount of tissue calculated for production of bovine casings, and the amount of infectivity potentially present in bovine intestine.
4. The DNV Report did not address the recommendations for future studies made by the previous EFSA Opinion (2007) on bovine casings, in particular the potential for cross-contamination.
5. The calculated results considered in the DNV report show that the individual human exposure from bovine casings, excluding ileum, produced in the EU was “very low”. When the total human exposure in the EU per year (based on the calculated BSE prevalence in 2007) is considered by the Panel, the obtained figures cannot be considered negligible, even when ileum is excluded. As the human exposure is correlated with the cattle BSE prevalence, the exposure can be expected to decrease in line with the decrease in cattle BSE prevalence in EU member states.

## ANSWERS TO THE TERMS OF REFERENCE PROVIDED BY THE EUROPEAN COMMISSION

- *EFSA was requested to evaluate the scientific validity of the report.*

While the approach (concept and methodology) of the DNV Report was scientifically sound, the interpretation of results as obtained is not shared by the Panel. Its assumptions were based on limited scientific data obtained from a single morphometric study (which was already found to be inadequate in the previous EFSA Opinion on bovine casings), and on limited and earlier data on the presence of PrP<sup>sc</sup>/infectivity in bovine gut after experimental oral BSE inoculation. There is uncertainty about the relative BSE risk of neural and lymphoid tissues in casings compared to CNS that might have significant impact on the calculated results of the DNV Report.

- *EFSA was requested to evaluate the conclusions of the report and, if it was considered necessary based on the report and any other new relevant scientific information, to provide a re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings.*

The Panel notes that the DNV Report considers the individual human BSE exposure risk from bovine casings, excluding ileum, to be “very low”. However, when the upper confidence limits of the selected scenarios are taken into account, along with the uncertainties in key parameter assumptions, the calculated total human exposure per year in the EU (based on the calculated BSE prevalence in 2007) from bovine casings, even when ileum is excluded, cannot be considered negligible. Thus the conclusion in the DNV report that sausage casings sourced from intestines of cattle in EU Member States would lead to a negligible risk for human consumption cannot be considered valid.

Moreover, when considering other new relevant scientific information, it is concluded that the previous EFSA assessment of the BSE related risk of bovine intestines after processing into natural sausage casings remains valid.

## RECOMMENDATIONS

Future consideration on the risk in bovine casings should take into account the BSE prevalence in cattle at that time.

## DOCUMENTATION PROVIDED TO EFSA

1. DNV, 2008. Report by "Det Norske Veritas Ltd" (DNV) on TSE risk assessment for use of bovine casings prepared on behalf of the Swiss Cervelas task force (17 December 2008) as part of the mandate submitted by the EC.
2. ENSCA (2009). European Natural Sausage Casings Association (ENSCA) Letter of 17th April 2009 including the VLA Customer Report VLA contract FT1394. "Immuno-histochemical and immuno-biochemical PrP<sup>d</sup> analysis of BSE infected small intestinal tissues used for sausage casings".
3. ENSCA (2009). European Natural Sausage Casings Association (ENSCA) Letter of 15<sup>th</sup> of July 2009 with reference to "Assessment of BSE risk in bovine intestines (EFSA-Q-2009-00226) – request permission on the use of data in report VLA FT1394.
4. Stack, M.J., 2009. Immuno-histochemical and immuno-biochemical PrP<sup>d</sup> analysis of BSE infected small intestinal tissues used for sausage casings. VLA Report No. FT1394. Veterinary Laboratories Agency - Weybridge, Addlestone, UK.
5. Wijnker, J.J., Tersteeg, M.H.G., Vernooij, J.C.M., Koolmees, P.A. (2008). Quantitative histological analysis of bovine small intestines before and after processing into natural sausage casings. *Journal of food protection*, Vol. 71, p.1199-1204.
6. Wijnker J. (2009). Aspects of Quality assurance in processing natural sausage casing. PhD Thesis University of Utrecht, the Netherlands. ISBN 978-90-393-4932-8.

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- EFSA, 2007. Opinion of the Scientific Panel on Biological Hazards on a request from the European Commission on quantitative histological studies and the re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings, *The EFSA Journal* (2007), 464, 1-14
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