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SCIENTIFIC OPINION

Scientific Opinion on BSE risk in bovine intestines and mesentery[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

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ABSTRACT

Bovine intestines and mesenteries in the European Union (EU) have to be removed from the food and feed chain. The opinion provides a quantitative assessment of the Bovine Spongiform Encephalopathy (BSE) infectious load that might enter the food and feed chain yearly if bovine intestine and mesentery from animals born and raised in the EU would be re-allowed for consumption. Data on the evolution of the BSE infectious titre; and of the weight of histological structures accumulating BSE infectivity, were collected. The Cattle TSE Monitoring Model (C-TSEMM) was used to estimate the number of BSE infected cattle entering undetected in the food and feed chain yearly. A model named TSEi was developed to estimates the BSE infectious load in tissues from infected animals at different ages and the total yearly infectious load that could enter the food and feed chain in the EU27. In BSE infected cattle, the infectivity associated with intestine and mesentery reaches its maximum in animals younger than 18 months and then progressively declines to a minimum value in animals older than 60 months. Due to the decline of the BSE prevalence in the EU, between 2007 and 2012, the yearly amount of BSE infectivity associated with intestine and mesentery (sent to destruction) from animals entering the food and feed chain was reduced by a factor of 10. However, over this period, the maximum level of exposure to the BSE agent for individuals that would have consumed these tissues remained stable. Finally, the TSEi model indicated that the removal of the last four metres of the small intestine and of the caecum from the food and feed chain would result in a major reduction of the BSE exposure risk associated with intestine and mesentery in cattle.

KEY WORDS

Bovine Spongiform Encephalopathy (BSE), cattle, intestine, mesentery, Specified Risk Material (SRM), Quantitative Risk Assessment (QRA)

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* Three minor editoral changes were made: The number of atypical cases in 2005 in Table 11 was changed from 16 to 4. The suggested citation was amended and the title of the reference to EFSA Journal 2011;9(1):1945 was modified. The changes have no impact on the opinion.

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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 risk in Bovine intestine and mesentery.

Currently bovine intestines and mesenteries in the European Union (EU) are classified as Specified Risk Material (SRM) according to Regulation (EC) 999/2001[4] (Reg. 999/2001) as amended - the TSE Regulation – and have to be removed from the food and feed chain. SRM are considered as the animal tissues potentially containing the highest level of Transmissible Spongiform Encephalopathies (TSEs) infectivity. Thus, the removal of SRM is the most important public health protection measure against TSEs in the EU.

In this context, the BIOHAZ Panel was requested to assess quantitatively the Bovine Spongiform Encephalopathy (BSE) infectious load that might enter the food and feed chain yearly if bovine intestines (fresh or after processing into casings) and mesentery (including mesenteric fat) from animals born and raised in the EU would be re-allowed for consumption.

In order to perform the assessment the main data collected were related to: i) the evolution of the BSE agent distribution and growth of infectious titre in the tissues of infected animals; and ii) the evolution of the weight of histological structures susceptible to accumulate BSE infectivity in intestine and mesentery. The Cattle TSE Monitoring Model (C-TSEMM)[5] was used to estimate the number of BSE infected animals (per age category) that might be present in the EU Member States (MS) and enter into the food and feed chain.

A new model (named TSEi) was developed to estimate the infectivity associated with the intestine and the mesentery in BSE infected cattle born in the EU and that enter undetected the food and feed chain. The model was also developed for future estimation of the TSE infectivity associated with tissues other than intestine and mesentery and can be applied to species other than bovine. In the absence of data related to L- and H- type Atypical BSE agent distributions in bovine tissues, the model cannot at this stage be applied to provide a quantitative risk assessment related to these diseases.

TSEi relies on a combination of experimental data and assumptions that might have an impact on its final accuracy. Four parameters are strongly affecting the model's results: i) infectivity titre of the ileum; ii) the age at slaughter of the animals; iii) ileocaecal plate weight in small intestines; and iv) conversion of the infectivity titre as measured by bioassay in conventional mice and in cattle.

In a BSE infected bovine, the relative distribution of the infectivity in the different portions of the intestines and in the mesenteric tissues varies with the age of the animal, reflecting the stage of incubation of the disease. In BSE infected cattle: i) up to 36 months of age the infectivity is mainly associated (on average more than 90%) with the last 4 metres of small intestine and the caecum; ii) over 36 and under 60 months of age, there is a substantial inter-individual variability in the relative contribution of intestinal and mesenteric structures to the total infectivity; iii) from 60 months of age the infectivity is mainly associated (on average more than 90%) with the mesenteric nerves and the celiac and mesenteric ganglion complex.

The total infectivity associated with those tissues also varies with the age of the infected animal. On average, it peaks at about 15 Bovine oral Infectious Dose 50% ($BoID_{50}$)[6] in animals younger than 18

[4] Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 147, 31.5.2001, p. 1-40.

[5] Adkin A, Simmons R and Arnold M 2012. Model for evaluation of different options for the monitoring of Transmissible Spongiform Encephalopathies in cattle in the European Union (C-TSEMM). EFSA Supporting Publications, 2012:EN-349, 55 pp.

[6] Bovine oral Infectious Dose 50% ($BoID_{50}$): The oral dose which infects 50% of bovine animals in an experimental test.

months before progressively declining to 8-9 $BoID_{50}$ (in animals between 24 and 48 months of age) and dropping to 0.7 $BoID_{50}$ in animals older than 60 months.

The TSEi model also allows a comparison between the level of BSE infectivity associated with the intestine and the mesentery collected from infected and undetected cattle in different years. On the basis of the model it was concluded that between 2007 and 2012, the yearly BSE infectivity in EU27[7] that was associated with intestine and mesentery (eliminated as SRM) from infected but undetected animals entering the food and feed chain was reduced by a factor of 10 (i.e. from about 23,000 to about 2,000 $BoID_{50}$).

It is important to note that, in a scenario where intestine and mesentery would not be considered anymore as SRMs, the intestine collected from one single infected bovine would be consumed by a limited number of people without any dilution effect. Thus, the infectivity titre per metre in the ileo-caecal plate (the most infectious part of the intestine) may be used to estimate the potential maximum level of exposure for an individual consumer. According to the TSEi model during the period 2007 – 2012 the potential maximum level of exposure to the BSE agent for an individual consumer would have remained stable (on average about 1.5-1.6 $BoID_{50}$/m).

TSEi was also used to simulate a re-emergence of the BSE in cattle in the EU27. The simulation assumed: i) that the current BSE surveillance system would be maintained and ii) a 10% increase of the prevalence of the disease by yearly birth cohort. After consultation with the risk manager, the detection of either 1 or 3 BSE cases yearly in EU27 cattle aged between 48 to 72 months were considered as the thresholds for identifying a BSE re-emergence. Under such a re-emergence scenario it was estimated that, on average, 16 and 36 years would be needed to identify respectively one or three BSE cases. As the re-emergence of the disease would be associated with the infection of young animals, the mean contribution of the ileocaecal plate and the caecum to the total amount of infectivity associated with intestine and mesentery would be prominent (about 99%). Moreover, in an infected animal that would enter the food and feed chain, the average level of infectivity per metre of ileocaecal plate (used to estimate the potential maximum level of exposure for an individual consumer) would be about 4.7 $BoID_{50}$/m. In comparison with 2012, this value would represent about a 3 fold increase.

The BIOHAZ Panel finally concluded that whatever the scenario, the removal of the last 4 metres of the small intestine and of the caecum from the food and feed chain would result on average in more than a 90% reduction of the total infectivity associated with intestine and mesentery in BSE infected cattle up to 36 months of age.

Since the TSEi model relies on a number of assumptions, the BIOHAZ Panel recommended updating it if more specific data would become available. If data related to L- and H- type BSE becomes available the model should be used to provide quantitative estimates of the exposure to these TSE agents. Moreover, TSEi would be useful to provide quantitative estimates of the infectivity in tissues i) other than intestine and mesentery of BSE infected cattle; and ii) of TSE infected small ruminants.

The BIOHAZ Panel further recommended to the risk manager to take into consideration a potential re-emergence of BSE in case a modification of the SRM measures would be envisaged.

[7] EU27: all the European Union Member States excluding Croatia

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Based on the results and recommendations of different scientific opinions adopted by the Scientific Steering Committee between December 1997 and June 2001, the intestines from the duodenum to the rectum and the mesentery of bovine animals are currently included in the list of Specified Risk Material (SRM), as laid down in Regulation (EC) No 999/2001, because they contain cells and tissues that may harbour BSE infectivity. Consequently, these tissues must be removed from the food and feed chain to protect public and animal health against the risk of BSE.

Before their ban in March 2001, bovine intestines could be used for human consumption either fresh (i.e. unprocessed) in certain food specialities or, most frequently, after processing into casings.

Before its ban in April 2002, the mesenteric fat associated with the mesentery was also used for human consumption. It has to be noted that the mesentery fat, although not posing a risk regarding BSE, is currently considered as SRM and shall be disposed of as such because it is very closely associated with the mesentery and cannot be safely separated from it.

The Communication of the Commission to the European Parliament and to the Council of 16 July 2010 on the possible future changes to EU measures on TSEs ("the TSE Roadmap 2") highlights that amendments to the TSE rules are and will continue to be made following a stepwise approach supported by a solid scientific basis. Any amendment of the current list of SRM should therefore be based on new evolving scientific knowledge, while maintaining the existing high level of consumer protection within the EU. Since it is impossible, however, to consider the complete elimination of risk as a realistic objective for any risk management decision, the scientific advice should aim for a quantitative or a semi-quantitative approach, taking into account the favourable epidemiological situation regarding BSE in the EU.

On 8 March 2007[8], EFSA adopted an opinion on quantitative histological studies and the re-assessment of the BSE-related risk of bovine intestines after processing into natural casings. The purpose of this opinion was to evaluate a report produced by the Institute for Risk Assessment Sciences (IRAS) and the Department of Farm Animal Health of Utrecht University on quantitative histological studies of bovine small intestine before and after processing into natural sausage casings. The report concluded that commercially processed casings do not pose a measurable risk, in terms of BSE, for consumers. After a detailed analysis of the report, EFSA considered the IRAS study inadequate for the purpose of demonstrating the safety of bovine casings of cattle originating from BSE risk countries and made a number of recommendations on the topics that should be addressed in future studies on the subject. Moreover, EFSA did not consider valid, from a scientific point of view, the conclusions of the report and did not consider it necessary, based on the information received and on currently available scientific information, to re-assess the BSE-related risk of bovine intestines after processing into natural sausage casings.

On 10 September 2009[9], EFSA adopted a new scientific opinion on BSE risk in bovine intestines. The purpose of this opinion was to evaluate the scientific validity and the conclusions of a report prepared by "Det Norske Veritas Ltd" (DNV) for the Swiss cervelas task force. This report, based on BSE prevalence data from 2007, was an attempt to quantify the amount of BSE infectious load in bovine sausage casings where ileum has been excluded and to extrapolate to the overall human exposure within the EU. The report concluded that the risk for humans was very low, but EFSA did not share this conclusion because, on the one hand, the assumptions made regarding the data input in the DNV report contained considerable uncertainties and, on the other hand, did not address the recommendations for these kind of studies made in the previous EFSA opinion of 8 March 2007 on

[8] 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

[9] EFSA Panel on Biological Hazards (BIOHAZ), 2009. Scientific Opinion on BSE Risk in Bovine Intestines on request from the European Commission. EFSA Journal 2009;7(9):1317, 19 pp. doi:10.2903/j.efsa.2009.1317

bovine casings (in particular the potential for cross-contamination). Therefore, EFSA concluded that the risk was not negligible and that its previous assessment of 8 March 2007 remained valid.

On 10 March 2011[10], EFSA adopted a reviewed opinion of the BSE-related risk in bovine intestines taking into account the new scientific information that became available after the previous 2009 opinion, including the favourable evolution, over the last few years, of the EU epidemiological situation of Classical BSE and the progresses made in ongoing Classical BSE pathogenesis studies, as well as new recent studies. In this opinion, EFSA concluded that new scientific data, which add some new elements, concur and confirm the presence of limited amounts of PrP^{Sc} and/or infectivity in parts of the intestine other than the ileum (i.e. jejunum and colon) of Classical BSE-infected cattle under experimental inoculation (jejunum) and natural exposure (distal jejunum and colon). Most of these results were obtained after the examination of a limited number of cases/samples, especially for the natural cases, and were reported in individual studies. In addition, the new scientific data further confirm the presence of consistent amounts of PrP^{Sc} and infectivity in the ileum of Classical BSE-infected cattle under experimental inoculation and natural exposure. Moreover, due to limitations in the data currently available, EFSA concluded that an accurate quantification of the amount of infectivity in the intestinal parts other than the ileum of Classical BSE-infected cattle at different stages of the incubation period cannot be provided. However, EFSA acknowledged that due to the continuous decline of BSE, the current BSE exposure risk from bovine intestines has declined correspondingly.

The BSE exposure risk from bovine mesentery and/or mesenteric fat has never been quantitatively assessed.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above, EFSA is requested to assess quantitatively the BSE risk posed by bovine intestines and mesentery.

This assessment should be based on an update of quantitative models already developed if relevant, or on a new methodology to be developed so as to deliver quantitative estimates of the BSE risk arising from the consumption of bovine intestines, fresh or after processing into casings, and mesentery (including mesenteric fat), and should take into account any new scientific data available on infectivity of bovine tissues and the current data prevalence as regards BSE in EU.

Clarification to the Terms of Reference

After discussion with the requestor it was agreed to modify the terms of reference as reported here below:

In view of the above, EFSA is requested to assess quantitatively the BSE infectious load that might enter the food and feed chain yearly if bovine intestines and mesentery from animals born and raised in the EU would be re-allowed for consumption.

This assessment should be based on an update of quantitative models already developed if relevant, or on a new methodology to be developed so as to deliver quantitative estimates of the BSE infectious load arising from bovine intestines, fresh or after processing into casings, and mesentery (including mesenteric fat), and should take into account any new scientific data available on infectivity of bovine tissues and the current data prevalence as regards BSE in EU.

[10] EFSA Panel on Biological Hazards (BIOHAZ), 2011. Scientific Opinion on a review of the BSE-related risk of bovine intestines. EFSA Journal 2011;9(3):2104, 21 pp. doi:10.2903/j.efsa.2011.2104

ASSESSMENT

1. Introduction

Specified Risk Material (SRM) – such as brain, spinal cord and intestine of animals of certain species/ages – are defined in Regulation (EC) No 999/2001[11] as amended and are considered as the animal tissues potentially containing the highest level of Transmissible Spongiform Encephalopathy (TSE) infectivity and that have to be removed from the food and feed chain. The removal of SRM is the most important public health protection measure against TSEs in the European Union (EU).

According to the current EU legislation, the intestines from the duodenum to the rectum and the mesentery of bovine animals of all ages originating from EU Member States (MS) are included in the list of SRM. The list of tissues currently designated as SRM in bovine animals originating from EU MS is reported in Table 1.

Table 1: List of tissues currently designated as SRM in bovine animals originating from EU MS according to Reg. (EC) 999/2001 as amended

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 30 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.

[11] Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 147, 31.5.2001, p. 1-40.

2. Approach to the mandate

Data related to:

- the evolution of BSE agent distribution and growth of infectious titre in the tissues of the infected animals; and
- the evolution of the weight of histological structures susceptible to accumulate BSE infectivity in the intestine and mesentery

were collected.

The mandate from the European Commission requested that the BSE infectious load in bovine intestines be assessed quantitatively. Being part of the intestine, the infectivity in the rectum should ideally be considered in the assessment. Although from a practical point of view, bovine rectum is usually excluded from the food and feed chain, there is no explicit ban of this part of the intestine in the European Food Hygiene Regulations. However, no specific information is currently available on the presence of infectivity and/or abnormal PrP deposition and on the weight of lymphoid tissue (which is considered responsible for the major part if the infectivity in intestine) in the rectum. For these reasons, an assessment of the BSE infectivity level in bovine rectum is not provided in this opinion.

In parallel, the Cattle TSE Monitoring Model (C-TSEMM) (Adkin et al., 2012) was used to estimate the number of BSE infected animals (per age category) that might be present in the EU Member States (MSs) and that enter the food and feed chain.

In order to quantitatively assess the BSE risk in bovine intestine and mesentery, these and other parameters were used to feed a model on "TSE infectivity level in animal tissues" (TSEi) that was specifically developed for EFSA by a contractor selected through an open call for tender (CFT/EFSA/BIOHAZ/2012/02) (Adkin et al., 2014).

It is important to highlight that the BSE infectious load potentially present in bovine intestines cannot be considered as homogeneously distributed along the food and feed chain. In fact, it would most probably be concentrated in relatively high amounts of infectious doses. This is due to the fact that:

i) the consumer will be eating a single portion (serving portion) of intestine coming from a single infected animal; and

ii) a heterogeneous distribution of the BSE infectious load along the intestine of BSE infected animals is expected.

As a result, most of the total BSE infectious load will be concentrated in a few portions of bovine intestine. In addition, the patterns of consumption will have a major impact on the individual exposure to the BSE agent.

Under these circumstances, an overall estimation of the mean exposure of the population is not a good representation of the risk for the individual consumer.

All the parameters and the related data and assumptions used to model the BSE infectivity in bovine intestine and mesentery are reported in Appendix A.

3. Preamble on Classical BSE and Atypical BSE (H- and L- type)

Beside Classical BSE (C-BSE), the systematic testing of cattle over 30 months of age implemented at EU level in 2001 allowed the identification of two atypical neuro-pathological and molecular BSE phenotypes, termed H-type and L-type Atypical BSE (H-BSE and L-BSE).

According to the data available in scientific literature or obtained through the EU active surveillance system, Atypical BSE cases have been reported in several European countries (Jacobs et al., 2007; Stack et al., 2009; EFSA, 2012), Japan (Yamakawa et al., 2003; Masujin et al., 2008), USA (Richt et al., 2007) and Canada (Dudas et al., 2010).

The pathogenesis of C-BSE was extensively investigated since the early 90's (in particular using oral challenge in cattle). These studies provide us with a large amount of information on the distribution and infectivity level of the C-BSE agent in infected cattle.

Unfortunately, there is no data available on the pathogenesis and the tissue infectivity distribution of H-BSE in ruminants.

In one L-BSE asymptomatic natural case, infectivity (detected by bioassay in Tgbov mice) was detected in skeletal muscles but not in kidney, spleen and lymph node (Suardi et al., 2012). However, these data remain too limited to draw any definitive conclusions.

In a recently published work, Torres and colleagues (Torres et al., 2011) showed that C-BSE can emerge spontaneously from a H-BSE type isolate (in the absence of any interspecies passage), which could indicate that H-BSE might be a source of the C-BSE agent.

In addition, studies carried out in transgenic mice that express the human PrP (Beringue et al., 2008; Kong et al., 2008; Padilla et al., 2011) and in cynomolgus monkeys (*Macaca fascicularis*) (Comoy et al., 2008) all concurred to indicate that L-BSE may be more virulent to humans than C-BSE.

Conclusion:

- The lack of specific data related to L-BSE and H-BSE agent tissue distribution in infected cattle, precludes the development of a quantitative risk assessment that would consider specifically these diseases.
- Such specific risk assessment will only become possible when adequate data on L- and H-BSE pathogenesis and infectivity level in peripheral tissues are collected.
- Considering the uncertainties related to L- and H-BSE infectivity distribution/levels in tissues and their potential capacity to propagate in cattle and other species, the consequences of dietary exposure to tissue derived from L- and H-BSE infected animals should not be neglected.

4. BSE agent detection and infectious titre measurements

In prion diseases the infectivity measurement traditionally relies on bioassay transmission in laboratory animals. In these experiments, groups of animals are challenged either by the intra-cerebral (i.c.) route only or both by the intra-peritoneal (i.p.) and the intra-cerebral route and the resulting disease attack rate, incubation period and the percentage of inoculated animals that develop clinical signs are recorded to estimate the infectious titre (Cf infra).

4.1. Classical BSE infectivity measurement models

4.1.1. RIII mice model

Up to the early 2000s, most of the bioassays for the detection/measurements of BSE infectivity relied on mouse inoculation bioassay with conventional RIII mice (Fraser and Foster, 1993; Wells et al., 1994a; Bradley, 1996).

A major drawback of the use of the RIII model is that, in order to propagate, the BSE agent has to cross the species barrier between cattle and mice. Therefore, negative results of experimental challenges employing tissues from BSE diseased cattle in conventional RIII mice cannot reliably exclude low levels of infectivity therein.

4.1.2. Transgenic mice lines expressing the bovine PrP

The development of transgenic mouse models for the PrP gene allowed the species barrier phenomenon that limits TSE agent primary passage in conventional rodents models to be reduced/suppressed. In particular, several mouse lines expressing the bovine PrP^C were developed (Scott et al., 1997; Castilla et al., 2003; Buschmann and Groschup, 2005) and these provided an increased sensitivity for BSE infectivity detection. A comparison of the BSE infectivity detection in RIII mice and in bovinized mice (Tgbov XV) was carried out by Buschmann and Groschup (2005) and indicated that Tgbov XV mice are approximately $10^{4.4}$ fold more sensitive to BSE infection than conventional RIII mice.

4.1.3. Cattle model

A comparison similar to the above-mentioned one was carried out between bovine animals and RIII mice (Hawkins et al., 2000). Groups of calves were intra-cerebrally (i.c.) challenged with a dilution series (1/10 diluted) of the same brain stems pooled from BSE affected cattle as the one used by Buschmann and Groschup (2005) (VLA brain pool: SE1809:BBP). A parallel titration in RIII mice was conducted according to standard mouse endpoint titration protocols. Mice were inoculated by the i.c. and i.p. routes simultaneously to maximise the efficiency of the assay.

In RIII mice, a titre of $10^{3.3}$ mouse (i.c. + i.p.) ID_{50}/g of tissue was obtained. The titration in bovines was terminated at 86 months post inoculation based on survival data and the estimation that the end point of the titration had been exceeded; the infectivity titre in bovines was estimated to be $10^{6.0}$ cattle (i.c.)ID_{50}/g of tissue.

More recently (Wells et al., 2007), an end point titration of the same inoculum was performed by the oral route using decreasing amount of brain homogenate (ranging from 100g to 1 mg). The study demonstrated that BSE transmission in cattle can be produced by oral exposure to as little as 1 mg brain homogenate ($\leq 10^{0.4}$ RIII mouse i.c./i.p. ID_{50} units). It has also indicated that one bovine oral ID_{50} ($BoID_{50}$) was, under the experimental conditions that were applied, approximately equivalent to $10^{2.8}$ RIII mouse i.c./i.p. ID_{50}.

4.1.4. Conclusions on the relative sensitivity of BSE detection bioassay models

Together these data support the view that 1 ID_{50} i.c./i.p. in RIII mice is equivalent to:

- $10^{4.4}$ ID_{50} i.c./i.p. in Tgbov XV mice

- $10^{2.7}$ ID_{50} i.c. in cattle
- $10^{-2.8}$ ID_{50} oral in cattle

However, when considering these figures it is important to remember that they rely on a limited number of experiments and measurements and that bioassay titrations have an intrinsic limited accuracy.

4.2. Background information on bioassay results and infectious titre estimates

When trying to estimate the infectious titre of a TSE agent in a sample using bioassay transmission results several approaches can be used.

4.2.1. End point titration approach

The most straightforward and accurate approach to estimate the infectious titre in the field of prion diseases is the Spearman-Karber method. In this approach, groups of animals are challenged with successive dilutions of an inoculum. The model provides a maximum likelihood infectious titre (LD_{50} or ID_{50}) and a confidence interval (Markus et al., 1995).

However to provide an accurate estimate of the infectious titre the Spearman-Karber method requires that at least one of the tested dilutions displays a 100% attack or infection rate. This requirement makes this approach improper for estimating titres in samples containing low infectivity.

4.2.2. Incubation period/attack rate derived models

Although regarded as less accurate than the end-point titration approach, dose-response relationships have been used as a method for infectivity estimation (Prusiner et al., 1982; Heikenwalder et al., 2007; Lacroux et al., 2008) when endpoint titration data are not available.

For a given TSE agent (strain), the approach relies on:

- the end point titration of a reference inoculum;
- the computation of a dose-response curve reporting for a given incubation period the associated infectious titre of the inoculum; and
- the use of this dose-response curve to estimate the infectious titre of other inocula on the basis of their inoculation in the reference animal model.

More recently Arnold et al. (2009) developed a method able to provide a more accurate estimation of the infectious titre than using attack rate alone and providing a natural way to deal with experimental animals that have died before the end of the experiment without having to decide debatable inclusion/exclusion criteria. The titre of infectivity is estimated using both the probability of survival (attack rate at each dilution) and the individual mouse incubation periods at each dilution using a Bayesian approach instead of the maximum likelihood approach.

The method considers not only the incubation period observed in mice but also the possibility that an infected mouse could die from causes unrelated to BSE (e.g. intercurrent disease or be sacrificed at the experimental end point) during the experiment.

4.2.3. Poisson distribution derived models

The use of the Poisson distribution to calculate the resulting titres and standard deviations in TSE agent inocula was initially used in the prion field to estimate the infectious titre in blood fractions prepared from infected mice (Brown et al., 1998). The complete methodology is described in an appendix to the paper of Brown et al. (1999). The approach relies on the principle of the random

distribution of physical particles in low concentration solutions. It is based on the attack rate/transmission rate observed following inoculation of a known volume of inoculum to a group of animals and it does not require any reference (dose-response) to provide a titre estimate.

The method has been extensively employed for measurements of infectivity in blood, blood components and urine of TSE infected rodents (under the denomination "Limiting Dilution Titration"). This statistical approach can be applied to inocula which do not fulfil the criteria of the Spearman-Karber method application (at least one dilution infecting/killing 100% of the animals).

This approach also provides an estimate of the maximum infectious titre (upper boundary IC 95%) that can be found in an inoculum when no transmission is observed in a bioassay experiment (Brown et al., 1999; Andreoletti et al., 2012).

The Poisson calculation returns a value for the Infectious Dose, ID that can be converted into ID_{50} (Fisher, 1936; Gregori et al., 2006).

4.2.4. Conclusions

Bovine intestine and mesentery harbour what can be considered as a low amount of infectivity. In this context, incubation period/attack rate and Poisson distribution derived models appear to be the most suitable approaches for estimating the infectious titres in these organs.

5. Anatomical data and evolution of bovine tissue length and weight

In assessing the BSE risk posed by bovine intestines and mesentery, several tissues have to be considered. For a better understanding, the descriptions of the most relevant anatomical structures involved in the spread of the BSE agent will be presented in this section.

5.1. Length and weight of intestine and lymphoid tissue in the intestine

The data in this section mainly refer to the thesis written by Olof Carlens in 1928 (Carlens, 1928). These data were further confirmed by information received from the European Natural Sausage Casings Association (ENSCA)[12]. Additional references are mentioned in the text. The tables are modified from Carlens (1928). These data might not be exactly representative of the diversity of the tissues weights and lengths that may be encountered in the different breeds and production systems in the EU today. However, they constitute the only dataset currently available.

The intestine is divided in to the small and large intestine. The former is subdivided into duodenum, jejunum and ileum. The latter consists of caecum, colon and rectum. The length of the whole intestine as well as the length of the different parts varies between breeds and individual animals. Overall the length of the cattle intestine is 20 fold that of the body length (Nickel et al., 1987).

The lymphoid tissue of the gut is mainly represented by the Peyer`s patches, which are localized in the submucosa of the intestine. They are defined as specialized lymphoid tissues containing four compartments (follicle, corona, interfollicular area, dome). These components may be aggregated to give Peyer's patches of various sizes. In cattle, several patches can be found in the jejunum, one in the ileum (ileocaecal plate) which extends into the caecum (ileocaecal-junction) and jejunum, one in the ascending colon and several patches in the rectum. Additionally nodules (that are isolated lymphoid follicules) are seen throughout the intestine (Pabst, 1987; Liebler-Tenorio and Pabst, 2006).

The neural parts of the intestine are represented by the enteric nervous system (ENS), which is composed of an extensive network of numerous nerves and ganglia, which are longitudinally connected by nerve fibers throughout all parts of the small and large intestine. Three ganglionated plexuses can be found in the gut wall, the myenteric plexus (between the longitudinal and circular layer of the tunica muscularis) as well as the inner (adjacent to the mucosa) and outer submucosal plexi. The ENS innervates not only the different parts of the gut, but also among others the gut-associated lymphoid tissues (Furness, 2012).

5.1.1. Small intestine

The duodenum originates at the pylorus, it consists of three parts: i) Pars cranialis, ii) Pars descendens and iii) Pars ascendens and it has a length of 0.9-1.2 metre (Nickel et al., 1987). Peyer`s patches (PP) are not visible in the duodenum (Liebler-Tenorio and Pabst, 2006) and the first PP can be found (in adult cattle) at 2.5-3.5 metres from the pylorus region.

The jejunum is the longest part of the intestine and has, including the ileum, an overall length varying between 16 to 56 metres. It includes numerous PP, which depending on age can vary from about 20 to 50.

According to different anatomy books, the ileum is defined by the insertion of the so-called *Plica ileocaecalis*. However, a concrete length is not reported in anatomy books. Data received from ENSCA indicate a length varying between 0.7 and 0.9 metres depending on the age of the animal[13]. A second approach to define the length of the ileum is the length of the ileocaecal plate which is (in particular in young animals) a long continuous lymphoid structure reaching far beyond the insertion of the *Plica ileocaecalis* into the jejunal parts (Liebler, 1985). However, the length of this ileocaecal plate

[12] Information received from the European Natural Sausage Casings Association (ENSCA) on 14 June and 24 August 2012.
[13] Information received from the European Natural Sausage Casings Association (ENSCA) on 14 June 2012.

depends on the age of the animal and has a range from about 0.3 to 4 metres. In animals older than two years, it is hardly visible.

For the purpose of this document it was agreed to define ileum as the part of small intestine contained within the *Plica ileocaecalis* (see Figure 1) since this is the operational identification of ileum used by industry for casing production.

The overall weight of the small intestine depends on the age of the animal and ranges from about 1000 to 7000 grams. Data on the length and weight of small intestine are shown in Table 2 and Table 4.

Table 2: Overall data concerning length of small intestine, number of PP and length of ileocaecal plate. Modified from Carlens (1928).

Age	N° samples (intestines)	Length (m)	N° of PP	Length (m) ileocaecal plate
1 – 5 days	10	16.0 – 19.5	24 – 49	1.87 – 2.86
15 – 30 days	8	19.0 – 25.0	29 – 43	1.98 – 3.05
6 – 8 weeks	12	26.0 – 32.0	26 – 45	2.26 – 3.65
3 – 6 months	10	28.0 – 35.0	32 – 46	2.24 – 3.85
6 – 12 months	12	32.0 – 36.0	28 – 42	2.07 – 3.90
12 – 18 months	8	34.0 – 39.0	35 – 48	2.35 – 4.06
1.5 – 2 years	10	38.0 – 44.0	26 – 45	0.35 – 2.56
2 – 5 years	12	40.0 – 52.0	28 – 39	-
5 – 10 years	10	45.0 – 56.0	22 – 43	-
10 – 14 years	8	42.0 – 55.0	14 – 36	-

5.1.1.1. Peyer's patches of the small intestine

The total amount of lymphatic tissue in the small intestine differs clearly among individual animals, in particular between individuals within the age group 3-24 months. This could be due to differences in the development of the ileocaecal plate (in particular in the age group 12-24 months) and differences in the feeding (in particular in the age group 3-12 months). Data on the length and size of PPs in the small intestine are shown in Table 3.

Carlens (1928) reported a ratio between the length of the lymphoid tissue over the total length of the small intestine ranging from 9 up to 23% depending on the age of the animals. Overall, the jejunal Peyer`s patch contributes one third and the ileocaecal plate two thirds of the gut associated lymphoid tissue of the small intestine (Liebler-Tenorio and Pabst, 2006). The cranial part of the small intestine contains higher numbers of PPs which are smaller in size and can be found more frequently. In contrast the caudal part contains PPs which are bigger in size and in particular in younger animals distinctly raised above the mucosal surface. The size of the PPs is variable and has a range from 1-45 cm in length (except for the ileocaecal plate that can reach up to 4 metres, see Table 2 above and section 5.1.1.2 below) and 0.2-3.0 cm width. The height of PPs decreases with the age of the animals. Cattle older than 10 years mostly reveal PPs that are sunken in, showing follicle structures the size of pinheads.

The weight of the jejunal Peyer`s patches depends on the age of the animals and ranges from about 30 to 190 grams (Table 4).

Table 3: Length and size of Peyer`s patches in the small intestine. Modified from Carlens (1928).

Age	Total length PP (cm)	Total length small intestine (m)	Ratio total length PP / small intestine	Total surface area PP (cm^2)	Total surface area small intestine (cm^2)	Ratio total surface area PP / small intestine
1 – 5 days	383	17.9	1 : 4.7	607.0	8,900	1 : 14.7
15 – 30 days	517	22.6	1 : 4.4	791.0	12,100	1 : 15.3
6 – 8 weeks	678	28.8	1 : 4.2	970.0	14,800	1 : 15.3
3 – 6 months	728	31.6	1 : 4.3	1080.0	18,410	1 : 17.1
6 – 12 months	760	34.0	1 : 4.5	1260.0	21,930	1 : 17.4
12 – 18 months	770	37.0	1 : 4.8	1290.0	26,050	1 : 20.0
1.5 – 2 years	718	40.8	1 : 5.7	970.0	32,680	1 : 34.0
2 – 5 years	576	47.0	1 : 8.1	639.0	35,840	1 : 56.0
5 – 10 years	600	49.5	1 : 8.2	629.0	40,160	1 : 64.0
10 – 14 years	470	50.0	1 : 10.6	611.0	41,510	1 : 68.0

5.1.1.2. Ileocaecal plate

The length of the ileocaecal plate depends on the age of the animal (Table 2). The biggest size, with a length up to about 4 metres, can be found in cattle between 12-18 months. However, it has to be borne in mind that the individual variation among animals is high. An involution of the ileocaecal plate can be seen in cattle older than 2 years. The structure is replaced by solitary follicles which are constantly visible even in very old animals.

The total ileocaecal plate measurements by Carlens (1928) included only the anatomical structures of the jejunum and ileum (the measurement in the caecum was not included). It can be assumed that the proportion of the ileocaecal plate located in the ileum can be estimated by dividing the length of ileocaecal plate in the ileum by the total length of the ileocaecal plate (in ileum and jejunum). Moreover, it can be assumed that the proportion of ileocaecal plate in ileum and jejunum increases and decreases at the same rate by age. In this way, the proportion of ileocaecal plate in each of these tissues at slaughter can be estimated using the data from those animals slaughtered under 6 months of age. Since at this age the ileocaecal plate covers the whole length of the ileum, it can be assumed that the length in the ileocaecal plate in the ileum is equal to the length of the ileum.
The weight of the ileocaecal plate varies between about 100 to 400 grams, depending on the age of the animal (Table 4).

Table 4: Weight of small intestine and its related PPs. Modified from Carlens (1928).

Age	Weight (g) ileocaecal plate	Weight of jejunal PP`s	Total weight (g) of PPs	Weight (g) small intestine	Ratio total weight PPs / small intestine	Ratio total weight ileocaecal plate / PPs
1 – 5 days	103.0	34.0	137.0	1184	1 : 8.6	1 : 1.33
15 – 30 days	185.0	58.0	243.0	2000	1 : 8.2	1 : 1.32
6 – 8 weeks	334.0	72.0	406.0	2770	1 : 6.8	1 : 1.22
3 – 6 months	369.0	105.0	474.0	3420	1 : 7.2	1 : 1.28
6 – 12 months	386.0	134.0	520.0	4000	1 : 7.7	1 : 1.35
12 – 18 months	350.0	196.0	546.0	4810	1 : 8.8	1 : 1.56
1.5 – 2 years	210.0	189.0	399.0	5600	1 : 14.0	1 : 90
2 – 5 years	-	160.0	160.0	6130	1 : 38.0	-
5 – 10 years	-	112.0	112.0	6500	1 : 58.0	-
10 – 14 years	-	67.0	67.0	6740	1 : 100	-

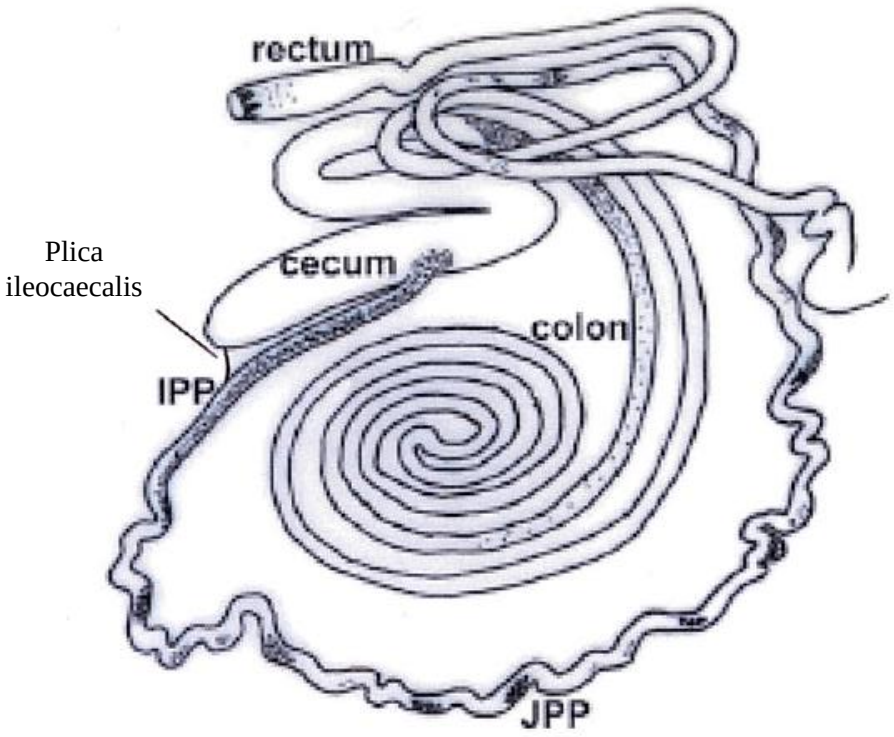

Figure 1: Distribution of Peyer`s patches in the intestine of cattle (modified from Liebler-Tenorio and Pabst, 2006. Available at http://www.vetres.org/). The insertion of the Plica ileocaecal defines the anatomical proximal end of the ileum. In younger animals the ileocaecal plate is a long and continuous structure reaching far beyond that point into the jejunum. JPP = jejunal Peyer`s patches; IPP = ileal Peyer`s patches (ileocaecal plate)

5.1.1.3. Involution of the lymphoid tissue of the small intestine

The involution of lymphoid tissue, in particular the decrease of the ileocaecal plate, starts at about one year of age. Subsequently the total length of PPs decreases as compared to the total length of the small intestine. Due to the total absence of the ileocaecal plate in older animals (2-10 years), the decrease in the total length of PPs is even more obvious (Table 3).

Animals between 10 and 14 years of age reveal a clear reduction of the PPs. However, it cannot necessarily be expected that the PPs are smaller because they are hardly visible and it is difficult to distinguish them from the normal mucosa. After staining of the native gut with Hämatoxylin (Hellmann), they are clearly distinguishable and the number and length do not necessarily show differences to younger animals. However, the surface area is clearly smaller as compared to younger cattle (Table 3).

Considering the total weight of all PPs, a rapid increase can be seen in the first six months followed by a slower increase to a peak at 12-18 months of age and a subsequent rapid decrease (Figure 2). However, these data include the ileocaecal plate which shows a completely different development as compared to the jejunal PPs:

- the peak of the ileocaecal plate is at about one year of age
- the peak of the remaining PP`s (jejunal PP`s) is at about 1.5-2 years of age

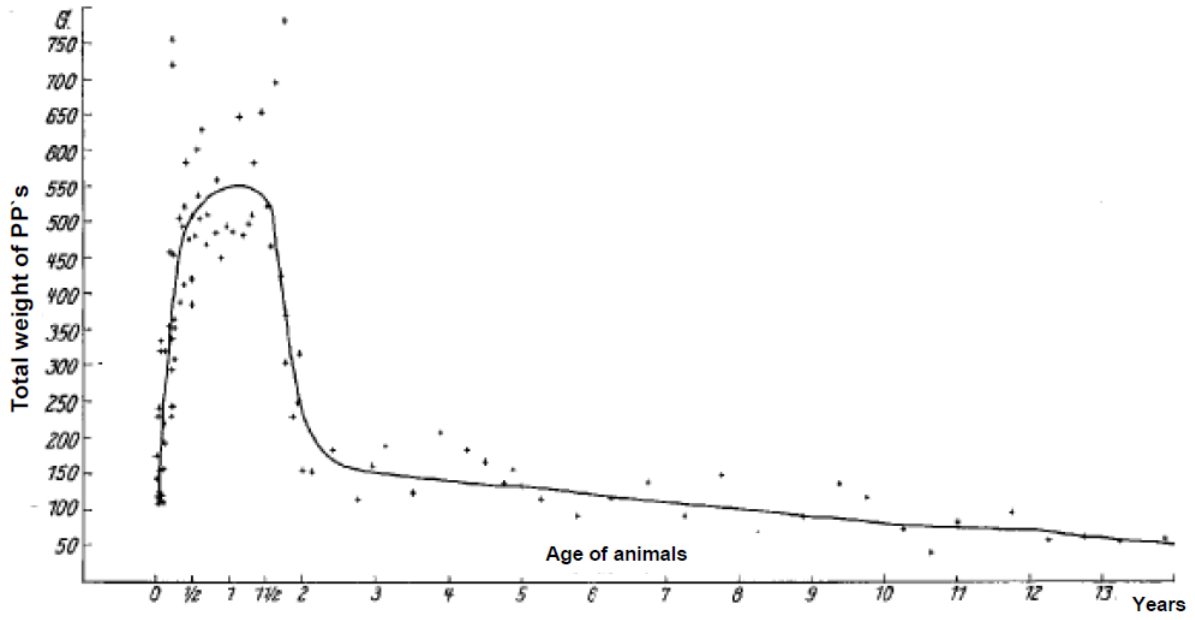

Figure 2: Age involution of the lymphatic tissue of all PP`s. The points indicate single animals. Modified from Carlens (1928).

Therefore, the amount of lymphatic tissue in the small intestine reveal two peaks: the first at about 12-18 months of age depending on the ileocaecal plate and the second at about 18-24 month of age depending on the remaining PPs.

The rapid decrease seen in the total amount of lymphatic tissue in the intestine is mostly due to the rapid decrease of the ileocaecal plate, the lymphatic tissue of the jejunal PPs decreases much slower and more constantly than that of the ileocaecal plate (Figure 3).

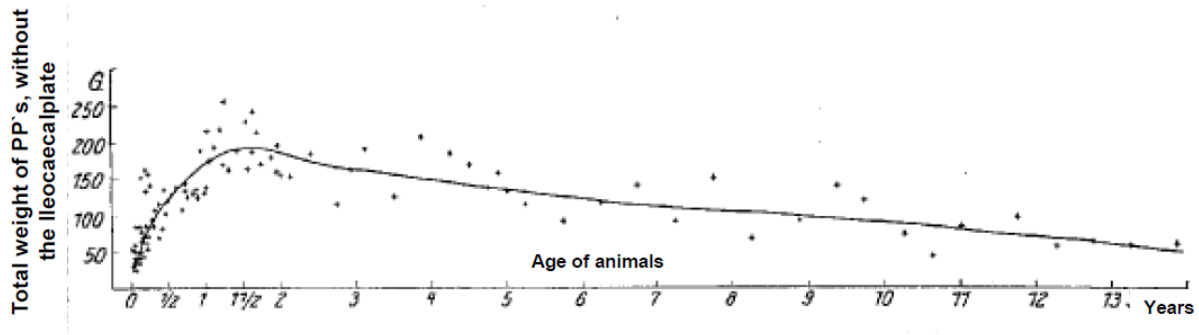

Figure 3: Age involution of the lymphatic tissue of all (jejunal) Peyer`s patches without ileocaecal plate. Points indicate single animals. Modified from Carlens (1928).

In summary the age dependent involution of the intestinal lymphoid tissue starts at about 18 to 24 months of age and the lymphatic tissue is developed at its best in guts weighting between 4000 and 5000g.

5.1.2. Large intestine

According to anatomical books, the length of the caecum is about 0.5-0.75m and the diameter about 12 cm (Sisson and Grossman, 1953; Nickel et al., 1987). The ostium ileale, the link between small (ileum) and large intestine (caecum), is surrounded by the so called papilla ilealis which represents a projection of the ileocaecal plate into the lumen of the caecum. Additional lymphoid structures randomly distributed within the caecal mucosa can be found in an area of 60-80 cm around the ostium

ileale. These "patches" are consisting of solitary follicles, only (Figure 4). In the remaining parts of the caecum neither solitary follicles nor PP`s can be found. In the absence of specific data, we assume that the weight of these follicles in the caecum can be approximated by the weight per surface area (g per cm^2) of ileocaecal plate that is estimated to be present in the ileum multiplied by the surface area of follicles measured in the caecum.

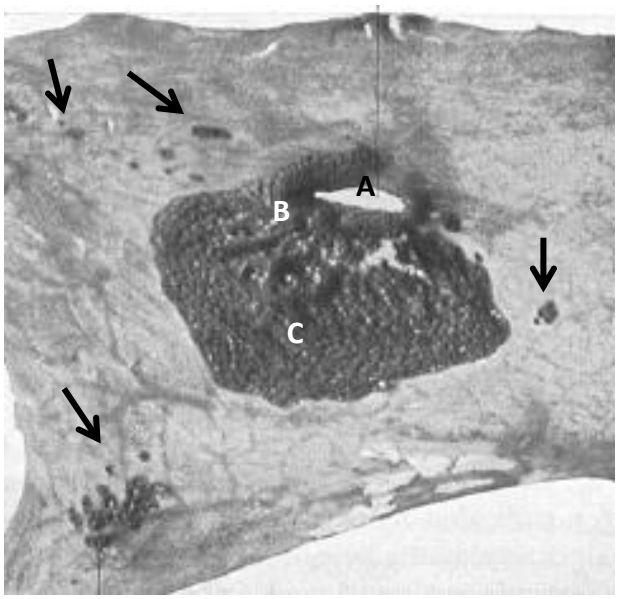

Figure 4: Lymphoid tissue of the caecum of a six week old calf. (A) Ostium ileale; (B) Papilla ilealis; (C) follicle plate of the caecum (projection of the ileocaecal plate); Arrows: randomly distributed accumulation of solitary follicles within an area of about 60-80 cm around the Ostium ileale. Modified from Carlens (1928).

According to anatomical books, the colon has a mean length of about 10 metres (Sisson and Grossman, 1953) with a range of 6 to 13 metres including the rectum (Nickel et al., 1987). The diameter is at first the same as that of the caecum, but diminishes to about 5cm (Sisson and Grossman 1953). All this information is in line with the estimation received from ENSCA[14].

In the proximal colon (proximal loop of the ascending colon), a big accumulation of lymph follicles can always be seen (Figure 5). The patch is localized, depending on the age of the animal, between 15-20 cm (newborn calf) and 70-130 cm (adult cattle) caudal from the *Ostium ileale* and extends circularly with a length of about 8 to 30 cm (Liebler-Tenorio and Pabst, 2006) or 25 - 50 cm (Carlens, 1928). Density and size of these accumulations increases up to an age of 1.5 years. At the end of the patch, there is a continuous change to isolated lymphoid follicles which can be found over the next two metres of large intestine (Liebler-Tenorio and Pabst, 2006).

[14] Information received by the European Natural Sausage Casings Association (ENSCA) on 14 June 2012.

Figure 5: Lymphoid tissue of the colon of a six week old calf. Modified from Carlens (1928).

5.1.3. Elements retained to model the evolution of the weight of the intestinal tissues of interest

A reasonable amount of information is available concerning the length of intestines, the weight and extension of lymphoid tissue in the small intestine at different ages.

However, little information is available as regards to the weight of caecum and on the weight and extension of lymphoid tissue in the large intestine at different ages.

In order to develop a quantitative model, the Panel had to make different assumptions with regard to the quantity and evolution of lymphoid tissues of different parts of the intestine, in particular:

- colon has the same quantity per metre of lymphoid tissue as the proximal jejunum[15];
- the proportion of ileocaecal plate at all ages in jejunum can be estimated using the data from those animals slaughtered under 6 months of age;
- the weight of lymphoid tissue in the caecum is derived from the ileocaecal plate and additional lymphoid follicles present in the caecum.

These assumptions can be considered as plausible and could be revised in the future if new data would become available.

5.2. Weight of lymphoid and nervous tissue in mesentery

The following data mainly refer to Nickel et al. (1987). Additional references are mentioned in the text. Figure 6 and Figure 7 illustrate the anatomical structures described in the text.

The *Mesenterium dorsale commune* extends from the pylorus region of the abomasum to the peritoneal parts of the pelvic cavity and is subdivided due to the different parts of the gut (*Mesoduodenum, Mesojejunum, Mesoileum, Mesocaecum, Mesocolon, Mesorectum).* The dorsal insertion point is the *A. mesenterica cranialis (=Radix mesenterii)* and the *A. mesenterica caudalis*. The mesenterium contains a network of nerves, blood and lymph vessels as well as lymph nodes to support the gut. Cattle in good body condition normally reveal distinct fat deposits in all parts, in particular along blood and lymphatic vessels.

[15] Proximal jejunum: it is intended as the part of jejunum not containing the ileocaecal plate. See section 5.1.1.2

5.2.1. Nervous tissue of the mesentery

The most important nervous tissues within the mesenterium consist of the *Plexus coeliacus* with the *Ganglion coeliacum* and the *Plexus mesentericus cranialis* with the *Ganglion mesentericum craniale*. These structures are localized ventral to the *Aorta abdominalis* on both sides of the origin of the *A. coeliaca* and the *A. mesenterica cranialis*. Both ganglia are connected with each other and with the one on the opposite side by strong nerve fibres, the so-called *Rami interganglionares*. The nerves regularly contain small ganglia too (Krediet (1910). In total, these plexi build up the so-called Celiac and mesenteric ganglion complex (CMGC). This complex even contains fibers of the parasympathetic *Truncus vagalis dorsalis*.

The CMGC is the only nervous structure clearly visible on gross examination of cattle mesentery and represents a diffuse mass of fibrous network surrounding the origin of the celiac and mesenteric arteries. Goshal and Getty (1970) describe a paired celiac and a single cranial mesenteric ganglia in cattle, which is more distinct on the left side, being closely related to the corresponding artery. They are connected to each other by means of several strong but short fibers. Parts of the cranial mesenteric ganglion also extend along the caudal aspect of the corresponding artery and establish a loose connection with the right celiac ganglion. According to Nickel et al. (1987), in ruminants the *Ggl. coeliacum* is a rounded to oval structure smaller in size as compared to the *Ggl. mesentericum craniale* which is a more longish structure. The whole CMGC is connected in a knobbly mass around the *Radix mesenterii*. Data concerning the weight or the exact size of this ganglion are not reported. For adult horses (ponies), a range of 35-40x18 mm on the right and 60x12 mm on the left side are described (Dyce, 1958).

Laterally on both sides of the *Plexus mesentericus cranialis* are the *Plexus renalis* and *suprarenalis* with the *Ganglia aorticorenalia* and the *Ganglia renalia*. These structures are localized along the trunk of the *A. renalis* and the smaller renal arteries.

More caudal around the trunk of the *A. mesenterica caudalis* is the *Plexus mesentericum caudale* with the *Ganglion mesentericum caudale.* In ruminants, this structure usually consists of two ganglia on both sides which are flat and of oval shape (Frewein, 1962).

All these plexi and ganglia are connected by the *Plexus aorticus abdominalis* which is localized on the ventral parts of the *Aorta abdominalis*. The parts localized between the trunks of the A. mesenterica cranialis and caudalis are also called *Plexus intermesentericus*.

Different plexuses arise from the *Plexus mesentericus caudalis*. These are the *Plexus testicularis* resp. *Plexus ovarica* which are localized around the trunks of the corresponding arteries as well as the *Plexus colicus sinister* and the *Plexus rectalis cranialis*. The so-called *N. hypogastricus* originates from the caudal parts of the *Ggl. mesentericum caudalis* and leads on both sides on its own to the pelvic cavity building up the *Plexus pelvinus* together with the parasympathetic *Nn. pelvini*. This plexus can be found along the walls of pelvic cavity and around the rectum.

From all the bigger plexuses/ganglia described above a network of several nerves arises, which also contains small ganglia and leads to the different organs in the abdominal and rectal cavity supporting all parts of the gastrointestinal tract, from the forestomach to the rectum. They are localized within the fat along the arterial vessels of the mesenterium and anastomize as the vessels do (Krediet, 1910).

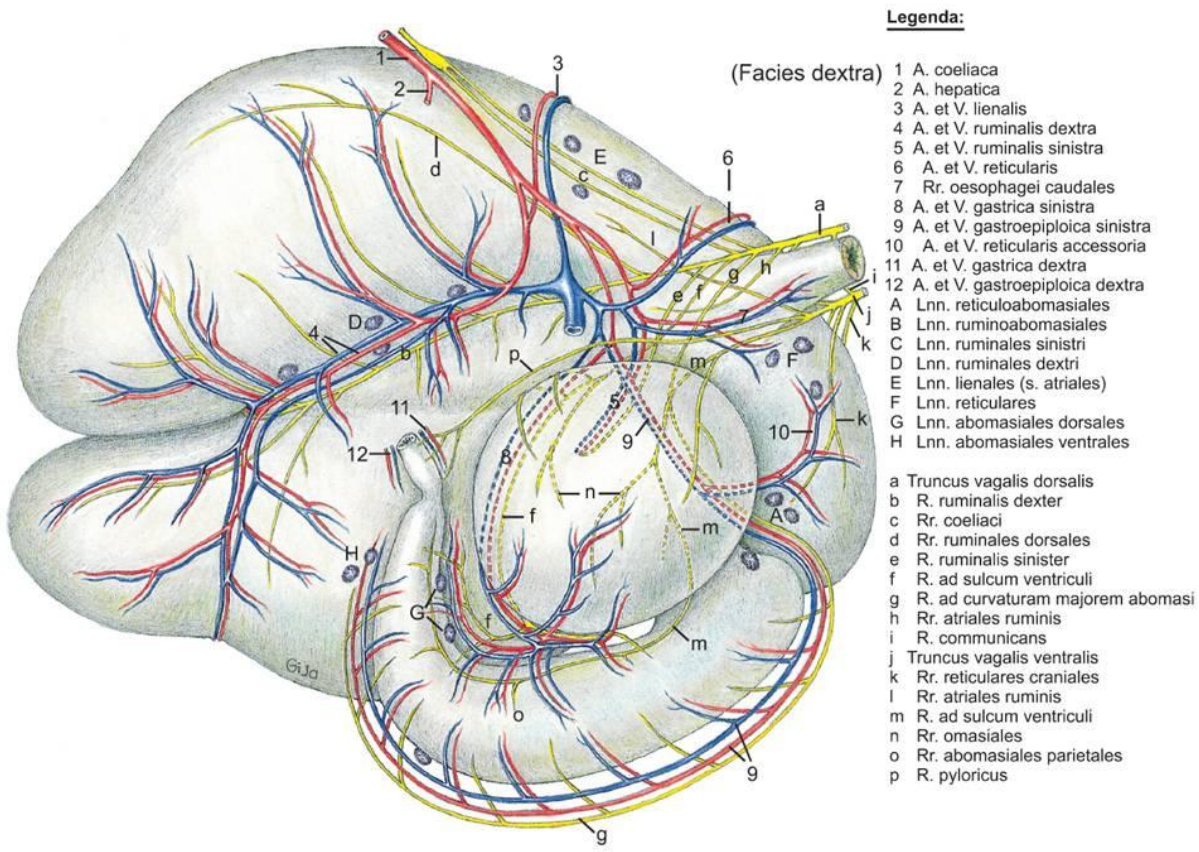

Figure 6: Schematic overview visualizing nerves, vessels and lymph nodes localised on the right side of the cattle forestomach. Modified from page 73 Budras and Wünsche (2007)

Unfortunately, no information is available with regard to the weight of the mesenteric nerves. The BIOHAZ Panel assumed that the mean weight of mesenteric nerves varies between 100 g to 200g depending on the age of the animals with a between animal variability represented by a minimum estimate ranging from 50 g to 100 g and a maximum estimate of 200 g to 500 g by age at slaughter.

A 90% correlation between the weight of CMGC and mesenteric nerves was also assumed.

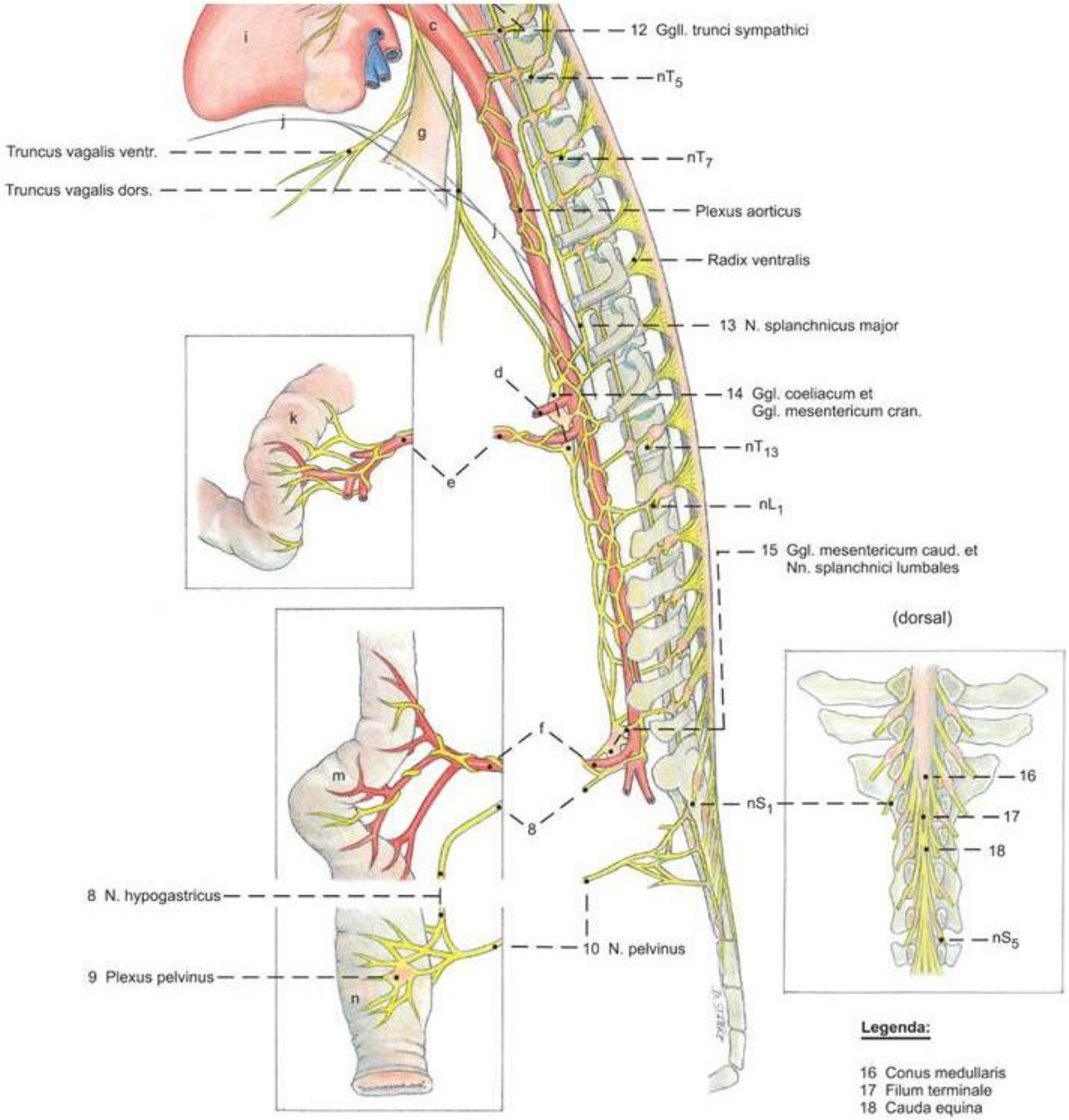

Figure 7: Schematic overview visualizing nerves and ganglia of the bovine spinal cord and the autonomic nervous system. Modified from page 57 Budras and Wünsche (2007).

5.2.2. Lymphoid tissue of the mesentery

The most important lymphatic tissues within the mesentery of cattle are the *Lymphocentrum coeliacum*, the *Lymphocentrum mesentericum craniale* and the *Lymphocentrum mesentericum caudale* with their different lymph nodes. 0summarises the most important lymph nodes.

A "normal size" of lymph nodes is not defined. In contrast there is a high variability regarding number and size of lymph nodes, even among individuals of one species. As a rule, the number and size of lymph nodes in one region are inversely proportional (Nickel et al., 1987).

Table 5: Characteristics of the most important lymph nodes in the mesenterium of cattle (Nickel et al., 1987; Koch and Berg, 1993).

Lymph Node	N° of nodes	Size (single lymph node)	Localisation	Remarks
Lymphocentrum coeliacum				
Lnn. ceoliaci et mesenterici craniales	2-5	--	Origin of the A. mesenterica cran and coeliaca	--
Lnn. Lienales	1-7	--	Between rumen and left parts of diaphragm, lying close to the dorsal edge of the spleen	Up to three additional nodes are seen on the right side of the rumen
Lnn. pancreaticoduodenales	few	small	Nearby the Flexura cranialis between pancreas and duodenum/colon transversum	--
Lymphocentrum mesentericum craniale				
Lnn. Jejunales	10-50	5-1200 mm	Mesojejunum	Either small number of long lymph nodes or numerous number smaller lymph nodes
Lnn. Ileales	0-4	--	Mesoileum	Inconstant lymph nodes
Lnn. Caecales	1-3	5-20mm	Plica ileocaecalis	--
Lnn. Colici	1-6	5-40mm	Ansa proximalis coli	--
	1-4		Between Ansae prox. et dist. coli	
	7-30		Ansa spiralis	
Lymphocentrum mesentericum caudale				
Lnn. mesenterici caudales	few	--	Close to colon descendens	--

In a thesis from 1911, the size and weight of several lymph nodes of cattle including *Lnn. lienales, Lnn. gastrici and Lnn. mesenterici* are reported (Jänicke, 1911). However, it is not quite clear which lymph nodes are summarized under these different groups as the nomenclature is different from the one used today. Healthy slaughtered cattle of different age groups, sex and condition status were regarded, in total 35 adult cattle and 25 calves. In doing so, the author concluded that the size of the lymph nodes is highly variable between the different types of the animals. For example, the mesenteric lymph nodes of bulls have a range of about 114-281g, in cows from 69-141g and in ox 140-294g. In calves, the range in the weight of these lymph nodes was about 55-101g. Overall, the author concluded that younger animals reveal bigger lymph nodes as compared to older animals. In estimating the relation of the weight of the lymph node to the slaughter weight, it could be shown that younger males without too much fat have the biggest weight of lymph nodes. Furthermore, several factors seem to influence the weight of the lymph node: (i) the age, the younger an animal the higher the weight of the lymph nodes, (ii) the condition, less fat is associated with higher weight and vice versa and (iii) sex, males reveal a higher weight of lymph nodes as compared to females. Tables 6-9 are modified from Jänicke (1911) and summarize the most important results of this thesis.

Table 6: Overview on weight and size of different lymph nodes in adult bovines. Modified from Jänicke (1911).

	Cattle type	Gender	Age	Live weight (Kg)	Slaughter weight (Kg)	Condition	Mesenteric lymph nodes				
							Weight (g)	Proportion (%) to slaughter weight	Length (cm)	Width (cm)	Height (cm)
1	Bull	NR	7 months	NR	68.5	III	164.7	0.24%	0.7 - 62	0.4 – 2.5	0.4 – 1
2	Bull	NR	13 months	NR	160	IIa	181.2	0.11%	0.6 – 45.5	0.5 – 3.1	0.4 – 1.9
3	Bull	NR	1.25 years	NR	158	IIb	157.5	0.10%	0.8 – 50	1.7 – 2.5	0.4 – 1.8
4	Bull	NR	1.5 years	NR	205	IIa	211.7	0.10%	0.6 – 55	0.5 – 3	0.4 – 2
5	Bull	NR	2 years	NR	240	II	204.2	0.09%	0.9 – 87	0.5 – 2.5	0.4 – 1.3
6	Ox	NR	4 years	620	355	IIa	283.0	0.08%	0.7 – 73	0.5 – 3	0.4 – 0.7
7	Veal	NR	1.5 years	NR	198	II	147.6	0.07%	0.7 – 60	0.4 – 2.3	0.4 – 1.4
8	Ox	NR	8.5 years	NR	342	IIa	236.0	0.07%	0.5 – 43	0.4 – 3	0.4 – 1.8
9	Cow	NR	7 years	NR	140	IIIb	89.0	0.06%	0.6 – 42	0.5 – 1.8	0.4 – 1.1
10	Cow	NR	5 years	390	201	IIb	141.0	0.07%	0.5 – 67	0.5 – 3	0.4 – 2
11	Bull	NR	2.5 years	NR	344	IIa	229.5	0.07%	0.5 – 64	0.4 – 2.7	0.4 – 1.6
12	Ox	NR	2.5 years	NR	354	Ib	213.3	0.06%	1.3 – 55	0.9 – 2.8	0.5 – 2.3
13	Cow	NR	NR	NR	215	II	122.0	0.06%	0.5 – 48	0.4 – 2.4	0.4 – 1.5
14	Bull	NR	3 years	NR	360	IIa	281.0	0.08%	1.1 – 76	0.4 – 4.5	0.4 – 2
15	Cow	NR	NR	NR	171	IV	105.0	0.06%	0.5 – 42	0.3 – 2.2	0.3 – 1.1
16	Bull	NR	3 years	NR	385	IIa	206.0	0.05%	1.2 – 22	1 – 3.2	1 – 2.5
17	Cow	NR	6 years	475	227.5	II	125.7	0.06%	0.8 – 71	0.5 – 2	0.4 – 1
18	Ox	NR	4 years	665	384	Ib	214.0	0.06%	1.5 – 46	1 – 3	0.6 – 2
19	Ox	NR	3.5 years	610	365	I	190.0	0.05%	1 – 22	0.4 – 3	0.4 – 1.8
20	Veal	NR	2.5 years	557.5	320	I	178.5	0.06%	1.8 – 67	1 – 3.2	0.6 – 1.8
21	Ox	NR	5 years	692.5	410	Ib	234.0	0.06%	0.6 – 32.5	0.5 – 3.8	0.4 – 1.8
22	Cow	NR	6 years	467.5	220	II	73.0	0.03%	0.7 – 16.5	0.6 – 2.5	0.4 – 1.2
23	Cow	NR	7 years	5995	270	II	126.1	0.05%	1.7 – 4.15	1 – 2.7	0.5 – 2
24	Ox	NR	4 years	645	376	I	155.0	0.04%	1 – 82	0.9 – 3	0.5 – 1.5
25	Bull	NR	2.5 years	632.5	375	Ib	170.0	0.05%	1 – 27	0.7 – 2	0.8 – 1.5
26	Bull	NR	2.5 years	710	401	I	188.5	0.05%	1.5 – 21	1 – 4.5	0.9 – 1.4
27	Cow	NR	4.75 years	NR	296	IIa	117.8	0.04%	1 – 37	0.6 – 1.6	0.4 – 0.9
28	Ox	NR	3.5 years	750	450	I	201.0	0.04%	1 – 35	0.9 – 2.5	0.6 – 1.5
29	Ox	NR	3.5 years	695	401	I	140.0	0.03%	1 – 59	1 – 3.5	0.6 – 1.5
30	Ox	NR	3 years	NR	340	I	160.8	0.05%	1.3 – 42	1 – 2.4	1 – 1.8
31	Bull	NR	3 years	NR	356	I	142.0	0.04%	0.6 – 40	0.5 – 2.7	0.4 – 1.3
32	Bull	NR	2.75 v	610	355.5	I	114.0	0.03%	0.8 – 63	1 – 2.5	1 – 1.5
33	Cow	NR	10 years	480	220	II	69.8	0.03%	1.4 – 105	0.7 – 1.3	0.4 – 0.8
34	Cow	NR	3 years	600	320	I	92.5	0.03%	0.6 – 46	0.5 – 2	0.4 – 1.4
35	Veal	NR	2.5 years	580	350	I	108.1	0.03%	1.8 – 48	1 – 2	0.3 – 0.8

Condition I: pretty good; Condition II: good; Condition III: moderate; Condition IV: bad
NR: Not reported

Table 7: Overview on weight and size of different lymph nodes in calves. Modified from Jänicke (1911)

	Cattle type	Gender	Age (weeks)	Live weight (Kg)	Slaughter weight (Kg)	Condition	Mesenteric lymph nodes				
							Weight (g)	Proportion (%) to slaughter weight	Length (cm)	Width (cm)	Height (cm)
1	Veal	Male	2	49	26.5	IIIa	76.5	0.29%	0.3 – 26	0.3 – 3	0.3 – 1.8
2	Veal	Male	2	52	30	II	92.0	0.31%	0.4 – 48	0.4 – 2	0.4 – 1.5
3	Veal	Female	2	52	27	IIIa	70.0	0.26%	0.4 – 50	0.3 – 1.8	0.3 – 1.2
4	Veal	Male	2.5	59	35	II	101.5	0.29%	0.3 – 52	0.3 – 2.1	0.2 – 1.3
5	Veal	Female	2	NR	35	IIa	82.5	0.24%	0.5 – 25	0.3 – 2	0.2 – 1.5
6	Veal	Male	2.75	64	39	IIa	93.8	0.24%	0.3 – 20.5	0.3 – 3.3	0.2 – 2
7	Veal	Male	3	57	33.5	II	76.2	0.23%	0.3 – 55	0.3 – 2.8	0.2 – 1.8
8	Veal	Female	2	55	34.5	IIa	70.7	0.20%	0.4 – 30	0.4 – 2	0.4 – 1.6
9	Veal	Female	2.5	58	34	IIa	68.0	0.20%	0.3 – 40	0.8 – 2	0.3 – 1.4
10	Veal	Female	2.75	58.5	30.5	IIa	55.5	0.18%	0.3 – 20	0.3 – 2.3	0.3 – 1.9
11	Veal	Female	2.5	61	40.5	Ib	77.2	0.19%	0.5 – 33	0.3 – 1.9	0.3 – 1.5
12	Veal	Male	3.5	72	48	IIa	85.0	0.18%	1 – 54	0.5 – 3	0.5 – 1.5
13	Veal	Female	6	NR	40	IIIa	64.0	0.16%	0.3 – 42	0.3 – 2	0.3 – 1.4
14	Veal	Male	4	NR	50.5	IIa	80.0	0.16%	0.6 – 60	0.5 – 2.8	0.4 – 1.3
15	Veal	Male	3.5	NR	43.5	IIa	70.2	0.16%	0.3 – 45	0.3 – 2.1	0.2 – 1.6
16	Veal	Female	9	NR	34	III	57.0	0.17%	0.3 – 34	0.3 – 1.8	0.3 – 1.2
17	Veal	Male	5	NR	49.5	II	64.0	0.13%	1 – 15.5	0.7 – 2.3	0.6 – 1.3
18	Veal	Male	6	NR	52	II	65.0	0.13%	1 – 9.5	0.6 – 2.3	0.5 – 1.5
19	Veal	Male	6	NR	52	IIa	66.5	0.13%	1 – 17	0.8 – 2.4	0.4 – 1.3
20	Veal	Female	8	NR	54.5	II	65.0	0.12%	0.5 – 22	0.3 – 3	0.3 – 1.9
21	Veal	Male	8	NR	62	Ib	83.07	0.13%	0.9 – 18.5	0.5 – 2.5	0.4 – 1.6
22	Veal	Male	8	NR	65.5	I	81.0	0.12%	0.5 – 38	0.4 – 2.8	0.3 – 1.6
23	Veal	Female	6	NR	58	Ib	63.0	0.11%	0.4 – 36	0.4 – 2	0.4 – 1.3
24	Veal	Female	8	NR	64	I	65.5	0.10%	0.5 – 29	0.3 – 3	0.3 – 1.8
25	Veal	Female	8	NR	63	I	63.5	0.10%	0.4 – 14.5	0.4 – 2	0.3 – 1.5

Condition I: pretty good; Condition II: good; Condition III: moderate; Condition IV: bad
NR: Not reported

Table 8: Average weight of lymph nodes in adult cattle and calves. Modified from Jänicke (1911)

Gastric and mesenteric lymph nodes			
Age	**Slaughter weight (Kg)**	**Weight of lymph nodes (g)**	**Proportion (%) to slaughter weight**
Adult cattle	295.2	182.06	0.0616
Calves	44.8	79.36	0.180

Table 9: Size of mesenteric lymph nodes of adult cattle and calves. The smallest and biggest measurements are mentioned. Number in brackets indicate the smallest and biggest value found in the group. Modified from Jänicke (1911)

Age	Length (cm)	Width (cm)	Height (cm)
Adult cattle	0.5-105 (0.5-1.8) (16.5-105)	0.3-4.5 (0.3-1) (1.3-4.5)	0.3-2.5 (0.3-1) (0.7-25.5)
Calves	0.3-60 (0.3-1) (9.5-60)	0.3-3.3 (0.3-0.8) (2.0-3.3)	0.2-2.0 (0.3-0.6) (1.3-2.0)

5.2.3. Elements retained to model the evolution of the weight of lymphoid and nervous tissue in mesentery

Very few elements are available to model the evolution of the weight of the nervous tissue in mesentery according to the age of the animal.

Although a lot of uncertainty remains, a reasonable amount of information is available to model the evolution of the weight of the lymphoid tissue in mesentery according to the age of the animal.

In order to develop a quantitative model, the Panel had to make several assumptions as regard to the quantity and evolution of nervous tissue in mesentery, in particular:

- to consider the data on the volume of CMGC in horse by Dyce as a proxy for bovine animals;
- to assume that the shape of CMGC in horse can be considered as cylindrical;
- to sum together the left and right part of CMGC in horse to obtain the total volume of this tissue in bovine;
- to assume that the specific weight of CMGC can be considered equivalent to water;
- to assume that that the mean weight of mesenteric nerves varies between 100 g to 200g depending on the age of the animals with a between animal variability represented by a minimum estimate ranging from 50 g to 100 g and a maximum estimate of 200 g to 500 g by age at slaughter.
- To assume a 90% correlation between the weight of CMGC and mesenteric nerves.

These assumptions can be considered as a worst case scenario, in particular because the resulting estimated weight for CMGC and the assumed weight for the nervous tissue are regarded as an overestimation of the reality, and can be revised in the future if new data would become available.

6. BSE Infectivity distribution and accumulation in bovine intestine and mesentery

6.1. Bovine intestine

The previous EFSA opinions on the same subject (EFSA Panel on Biological Hazards (BIOHAZ), 2009, 2011b) summarised the scientific information, available at that time in relation to the presence of infectivity and abnormal PrP in the intestine of BSE infected animals. Since then only a limited number of new elements has become available.

6.1.1. BSE agent in the ileum

The detection of BSE agent infectivity using bovine transgenic mouse models (Buschmann and Groschup, 2005) or abnormal PrP (Terry et al., 2003; Iwata et al., 2006) in the distal ileum of naturally affected cattle has been established. However, several studies report failure to transmit BSE in RIII mice using distal ileum in clinically naturally affected cattle (Wells et al., 1994a; Buschmann and Groschup, 2005) and suggest that infectious titre at terminal stage of the disease might be low in natural BSE cases.

In experimentally infected calves (100g oral dose), BSE infectivity in distal ileum was detected throughout much of the incubation (6-18 and 36-40 months post exposure) using bioassays in conventional mice lines (RIII and C57Bl) (Wells et al., 1994b; Wells et al., 1998; Wells et al., 2005).

Assays of distal ileum tissue (including Peyer's patches), pooled from the animals killed at 6, 10, 18, 26 and 32 months post-exposure were also conducted by the intracerebral inoculation of cattle (Wells et al., 2005). They confirmed infectivity presence in the ileum of cattle killed 6, 10 and 18 months post-exposure, but animals challenged with the samples collected in cattle killed at 26 and 32 months post-exposure were negative.

Arnold et al. (2009) estimated the titre of infectivity in the distal ileum from the incubation time found by bioassay in wild type mice; the pattern of evolution over time of infectivity in the distal ileum was that of an initial increase up to 14 months post exposure, followed by a decrease, which was likely to be highly variable between animals. These estimates, however, were based on mouse titration of brain material. The incubation period to dose relationship may differ between brain and intestines (Robinson et al., 1990). However, other studies reported an absence of effect of the tissue on the incubation period/dose response for a given TSE agent and reporting bioassay model (Kimberlin and Walker, 1977; Andreoletti et al., 2012).

Espinosa et al. (2007) reported infectivity detection using a transgenic bovine mouse model (Tg110) following inoculation with pooled distal ileum from BSE inoculated calves (between 4 and 6 months of age) in the UK (100g single oral dose) and culled at ages between 24 and 39 months. In a recently published study, Hoffmann et al. (2011) investigated the infectivity presence in the distal ileum of individual cattle orally challenge with 100g of a previously titrated BSE inoculum ($10^{6.1}$ ID50/g in Tgbov XV)(Buschmann and Groschup, 2005). The results obtained from those individual samples confirmed the presence of infectivity in distal ileum at all the tested ages (8, 12,16 and 20 mpi) although with some inter-individual variations.

Despite their limitations (number of individuals, experimental challenge) the data collected in the German study published by Hoffmann et al. (2011) probably represents the best data-set to estimate:

- the BSE infectious titre in the ileum in cattle at different stages of the incubation period; and
- the inter-individual variation of the BSE infectious titre in this organ.

The systematic examination of distal ileum by immunohistochemistry in a proportion of the animals involved in the UK BSE pathogenesis study revealed the presence of PrP^{Sc} deposition in lymphoid follicles of the ileal Peyer's patches in a large majority of the animals examined (killed between 4

months post challenge and 60 months post challenge). Whereas some abnormal PrP deposition was observed in the myenteric plexus these types of deposits were rarely observed in this study (Simmons personal communication, Terry et al., 2003). These observations roughly concur with those reported by Hoffmann et al. (2011) where PrP^{Sc} presence in the follicles of ileal Peyer's patches was observed in the majority of the BSE challenged (100 g oral route) animals killed between 4 months and 44 months post challenge. However, the first clear PrP^{Sc} deposit in the ENS was seen in a single myenteric plexus neurone of one animal killed 16 months post infection. Between 24 and 44 months post challenge, a slight increase in the number of plexus ganglia (mucosal and myenteric) accumulating PrP^{Sc} are detectable including in areas where no PrP^{Sc} was identified in the lymphoid follicles. In addition, labelling of myenteric plexus in the absence of PrP^{Sc} detection in the lymphoid follicles was also detected in 9/29 field cases of BSE examined in the UK (Terry et al., 2003).

Despite their limitations (number of individuals, experimental challenge) these elements indicate that in the ileum:

- the BSE agent accumulates from the first months post exposure and persists till clinical onset;
- the dynamics of the BSE infectivity can be estimated using the Hoffmann et al. (2011) and Arnold et al. (2009) studies;
- abnormal PrP associated with BSE infection mainly accumulates:
  - in the lymphoid follicles of the ileocaecal plate and to a lesser extent in the myenteric and mucosal plexus during the first half of the incubation period;
  - in both the ileocaecal plate and myenteric and mucosal plexus during the second half of the incubation period.

However, since bioassays performed on distal ileum used homogenates from the whole organ as inoculum, the infectious titre obtained encompasses the infectivity associated with both lymphoid and nervous structures.

6.1.2. BSE agent in the duodenum and jejunum

Data related to the presence of the BSE agent in the small intestine remain sparse. Most of the published studies failed to detect abnormal PrP presence or infectivity in jejunum samples collected in natural (Iwata et al., 2006) and experimental (Wells et al., 1998; Terry et al., 2003; Hoffmann et al., 2007) BSE cases. A Japanese study reported the presence of abnormal PrP in the myenteric plexus of duodenum and jejunum of a BSE case (94 months of age) (Kimura and Haritani, 2008). However, its results (based on immunohistochemistry only) remain debated.

In the study published by Hoffmann et al. (2011) infectivity presence was also investigated in the jejunum of individual cattle orally challenged with 100g of a previously titrated inoculum ($10^{6.1}$ ID_{50}/g in Tgbov XV)(Buschmann and Groschup, 2005).

The results demonstrated unequivocally the presence of a low but consistent amount of infectivity in the proximal and central portion of the jejunum in some (seven out of sixteen) of the animals killed at 8, 12, 16 and 20 months post challenge. No clear PrP^{Sc} deposition was observed in the Peyer's patches or Enteric Nervous System (ENS) of the jejunum from these animals.

However, in a UK study (Stack, 2009; Stack et al., 2011) examination of small intestine samples collected from orally inoculated calves (100g dose) by immunohistochemistry revealed the presence of a limited amount of abnormal PrP in a proportion of follicles in the jejunal Peyer's patches in animals culled between the age of 4 and 30 months post challenge. According to the authors of this study (Simmons et al personal communication) no PrP^{Sc} deposits were observed in the jejunal ENS from the same animals.

Together these data indicate that in the jejunum:

- the BSE agent can be detected as early as 4 months post-challenge and persists until clinical onset;
- the BSE agent is probably mainly associated with lymphoid follicles;
- BSE infectivity remains limited and can be estimated (using an incubation period/attack rate derived model) from the data reported by Hoffmann et al. (2011) study.

No specific data are currently available with regards to BSE agent presence/ infectivity/level in the duodenum.

6.1.3. BSE agent in the caecum and colon

In addition to the ileocaecal plate and the jejunal Peyer`s patches Hoffmann et al. (2011) examined the lymphoreticular tissue within the caecum, taking samples from the ileocaecal-junction. In doing so BSE-infectivity was shown in seven cows culled 8, 12, 16 and 20 months post infection. A $PrP^{Sc}$ accumulation was seen in Peyer`s patches and/or ENS in five animals, the oldest at 44 months post challenge.

Concerning the colon only a limited amount of data has been reported. In 2010, a Japanese group (Okada et al., 2010) conducted a study to investigate the BSE agent presence in various areas of the intestine in natural BSE cases. The authors reported the detection of PrP^{Sc} in the most distal part of jejunum (with continuous Peyer's patches), in ileum and in colon of respectively 3 out of 3, 4 out of 4 and 1 out of 4 naturally occurring confirmed BSE cases. The positive results were obtained by both immunohistochemistry and Western Blot. In the colon PrP^{Sc} deposition was observed (IHC) in the myenteric plexus. In addition, the authors reported the presence of infectivity in terminal ileum and in colon after inoculation of bovine transgenic mice. However, because of the experimental design the reported results do not allow the estimation of an infectious titre associated with the colon.

At this stage there is no data available concerning:

- the possible accumulation of the BSE agent in lymphoid follicles in the colon;
- the moment of the incubation period from which the colon can accumulate BSE agent;
- the range of infectious titre in the colon of BSE infected cattle.

6.1.4. Elements retained to model the BSE infectivity accumulation in the intestine

Whereas a reasonable amount of data is available concerning the age dependant evolution of the BSE infectious titre in the ileum and to a lesser extent in the jejunum and caecum in cattle, little is known concerning the other parts of the intestine (duodenum and colon). Similarly, there are no elements that would allow estimation of how the infectivity is split between the different histological structures in the gut wall (myenteric plexus system and lymphoid formation).

In order to allow the development of a quantitative model the Panel had to make several assumptions/approximations:

1. infectivity in duodenum, jejunum, ileum and caecum is mainly associated with lymphoid formation (Peyer's patches and isolated lymphoid follicles) and the model will not consider the infectivity that could be present outside these structures;
2. the duodenum displays similar features, in terms of BSE infectivity accumulation, to the proximal jejunum[15];

3. the caecum displays similar features, in terms of BSE infectivity accumulation, to the ileum;

4. in the absence of specific data related to the colon, the QRA model will consider that the colon has similar features to the proximal jejunum[15].

These assumptions can be considered as a worst case scenario and can be revised in the future if new data would become available.

6.2. Bovine mesentery

The collection of the mesentery at the slaughterhouse (see section 8.2) will result in the possible inclusion of the different tissues listed below. As for the intestine, the amount of data related to BSE agent presence in these structures is relatively limited.

6.2.1. BSE and TSE agents in fatty tissue

In conventional mice inoculated with adipose tissue (white and brown fat) from murine adapted scrapie agent (RML) TSE infectivity has been reported. Infectivity in adipose tissue seems to be associated with nerves and the direct involvement of fatty cells remains uncertain (Race et al., 2008).

In BSE affected cattle adipose tissue failed to transmit infection in conventional RIII mice (Fraser and Foster, 1993).

However, it is important to remember that, due to the species barrier phenomenon, RIII mice have a limited sensitivity for BSE detection and that no inoculation of adipose tissue in Tgbov mice or cattle has been reported. Therefore a low level of infectivity (below RIII mice model sensitivity) might be associated with adipose tissue.

6.2.2. Infectivity in mesenteric lymph nodes

Bioassay in RIII mice failed to detect infectivity in spleen, thymus (cervical), tonsil, submandibular lymph node, retropharyngeal lymph node, bronchial-mediastinal lymph node, hepatic lymph node, mesenteric lymph node, prescapular lymph node and popliteal lymph node (SSC, 2002), indicating a low (if any) involvement of lympho-reticular structures in BSE pathogenesis.

The apparent lack of BSE infectivity in the mesenteric lymph node was further supported by some bioassay in Tgbov mice (Buschmann and Groschup, 2005) in which inoculation of mesenteric lymph node homogenate from a natural BSE affected cow failed to transmit the disease (0 out 12 inoculated mice).

However, lingual tonsil from orally challenged cattle at preclinical stage of the disease (10 mpi) (Wells et al., 2005) and nictitating membrane pool from field BSE cases (WHO, 2010) intracerebrally inoculated into cattle resulted in positive transmission (low attack rate). This result was later confirmed by inoculation of lingual tonsil collected in orally challenged cattle and killed at different time points of the incubation period (20 to 33 month post inoculation) in transgenic bovine mouse line (Tg110) (Espinosa et al., 2007). These elements support the contention that low infectivity titre can accumulate in lymphoid formations outside the intestine.

Very recently Franz et al. (2012) demonstrated that BSE agent could be detected after Protein Misfolding Cyclic Amplification (PMCA) in mesenteric lymph nodes (ileal and jejunal) collected in one orally challenged cow (36 mpi). Interestingly the same tissues collected from three other cattle (killed at respectively 40, 44, and 50 mpi) remained negative after PMCA.

Together these results suggest that a very low level of BSE infectivity can be present in mesenteric lymph nodes in certain cattle at preclinical stage of the disease.

6.2.3. Infectivity in mesenteric nerves and autonomic ganglia

The accumulation of the BSE agent in the abdominal autonomic nervous system and the putative role of these pathways in the dissemination of the agent towards the central nervous system has been documented by Hoffmann et al. (2007).

More recently a second study (Kaatz et al., 2012) reported bioassay results in Tgbov XV mice inoculated with autonomic ganglia (*Celiac and Mesenteric Ganglion Complex* (CMGC) and *Ganglium mesenteriale caudale*) and autonomic nerves (vagal nerve) collected in seventeen cattle orally challenged with BSE and killed at different time points of the incubation period (between 16 and 44 months post inoculation). These bioassays clearly demonstrated that infectivity can be present in these structures from 16 mpi and persist until clinical onset.

Unfortunately, no result is available in younger animals and it is not possible from this study to determine how early these structures accumulate BSE agent during the incubation period. Similarly mesenteric nerve roots were not tested. However, results obtained from vagal nerve (cervical and thoracic portion) inoculation provide a likely pertinent picture of the infectivity level that could be found in the mesenteric nerve roots.

6.2.4. Elements retained to model BSE infectivity accumulation in the mesentery

Only a few elements are available concerning BSE agent distribution and infectious titre in the structures that constitutes the mesentery.

In order to develop a quantitative model the Panel had to make different assumptions with regards to BSE agent presence and accumulation in these anatomical structures:

- infectivity in the mesentery, if present is likely to be associated with nerve roots, autonomic nervous system ganglia and lymph nodes. Fatty cells by themselves are unlikely to contain BSE infectivity;

- in infected animals, the BSE agent could accumulate in the mesenteric lymph nodes, mesenteric nerves and autonomic ganglia as early as the ileal Peyer's Patches become positive;

- the infectivity level that can be found in the nerves and autonomic ganglia can be approached using data from Kaatz et al. (2012);

- if present in the mesenteric lymph nodes, BSE infectivity could range up to the upper limit of the confidence interval that can be derived from the negative transmission results obtained by Buschmann and Groschup (2005) (0 out of 12 inoculated animals).

These assumptions can be considered as a worst case scenario and can be revised in the future if new data would become available.

7. BSE epidemiological situation in the bovine population of the European Union

7.1. Introduction

The epidemiological knowledge of the spatial distribution and the temporal evolution of BSE epidemics in EU is based on data obtained through surveillance activities. The characteristics of animal TSEs (heterogeneous clinical presentation, long incubation period ranging in years, extremely low incidence, no ante mortem tests available) make their monitoring complex. Before 2001 BSE surveillance exclusively relied on passive surveillance (i.e. the mandatory reporting of clinically suspect animals and the confirmation of BSE).

Since 2001 an EU wide active surveillance system has been implemented. The active system is based on post mortem rapid testing for the presence of abnormal PrP in the brainstem and involves animals above a certain age defined according the assigned risk stream (e.g. healthy animals at abattoir or fallen stock). It revealed the poor sensitivity of passive surveillance (Ducrot et al., 2008).

The BSE surveillance system in the EU clearly surpasses the OIE BSE monitoring requirements (World Organisation for Animal Health, 2013). It allows the evolution of the epidemics to be followed country by country.

7.2. Analysis of the trend of BSE in the 27 EU Member States

7.2.1. Approach data source and general assumptions

The data needed to run the Cattle TSE Monitoring Model (C-TSEMM) (Adkin et al., 2012) were used (see section 10.2.1 for further details). As those data do not cover BSE surveillance in 2001, data related to this particular year were retrieved from the European Commission BSE monitoring database. Detailed epidemiological information on BSE monitoring in the EU can be found in the TSE annual reports released by the EC, available at: http://ec.europa.eu/food/food/biosafety/tse_bse/monitoring_annual_reports_en.htm

When interpreting the data presented in this section, unless otherwise specified, the term "BSE" on its own refers to Classical BSE.

Some minor differences may be found between the data presented in this opinion and those presented in previous EFSA opinions. This is due to updates/corrections that the MSs provide to the databases of the European Commission and EUROSTAT. In addition, the few BSE cases that were detected when implementing BSE eradication measures (i.e. cohort-culling) are not included in the calculations presented, as they come from a stream other than the epidemiological surveillance. However, it has to be noted that their exclusion does not affect the trends of the BSE epidemic.

In order to make the analysis presented in this chapter valid, two key assumptions were made:

- it is assumed that all 27 EU MSs have implemented a BSE surveillance system and control measures as set out in Reg. (EC) 999/2001;
- it is assumed that all 27 EU MSs will continue to implement the current measures aimed at controlling and reducing BSE as set out in Reg. (EC) 999/2001.

Beside this, it is also assumed that the sensitivity of rapid tests for BSE surveillance is 100% in cattle reaching the very late stages of the incubation period**.** The likely point in the incubation period at which PrP^{Sc} is detectable with the rapid BSE tests depends on the infective dose. While the range of doses of exposure of field cases of BSE is not known, an oral attack rate study has shown that the mean incubation period arising from doses in the range 0.1-1g fits with that estimated for field cases (Wells et al., 2007). For a 1g dose, it was found that PrP^{res} was detectable only after 97% of the length of the incubation period. This degree of under-detection has to be taken into account when estimating

infection prevalence from surveillance data (Arnold et al., 2007).

When interpreting the results presented in this section, the following points should also be considered:

- the dynamics of the age distribution of BSE cases is directly impacted by the age distribution of the cattle population and the level of BSE transmission in the past;
- out of the BSE cases found in the EU27, only 73 cases were related to animals born after the start of the total feed ban in 2001.

7.2.2. Trends of BSE in the EU27 during the period 2001 to 2012

From 2001 till the end of 2012, more than 107 million tests were carried out in the framework of BSE Active Surveillance in the EU27. 5,326 of the tested animals were positive; including 1,334 healthy slaughtered cattle (14.3 per million tested), and 3,992 at risk animals (255.8 per million tested).

In 2012 the 11 BSE cases were detected in France, Ireland, Poland, Portugal, Spain and the UK.

The overall picture of BSE cases, detected through the BSE Active and Passive Surveillance in EU27 from 2001 to 2012, is reported in Table 10.

Table 10: Number of Classical BSE cases detected through the BSE Active and Passive Surveillance and eradication measures in EU27 during the period 2001 – 2012 per target group.

Target Group	**No of detected BSE cases per testing year**												
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	**Total**
Active Surveillance													
Healthy slaughtered	280	292	264	177	115	81	39	29	28	15	9	5	1 334
At risk animals													
Emergency slaughter	321	509	316	172	123	31	8	7	3	0	0	0	1 490
Fallen stock	400	610	406	313	219	165	95	75	32	29	19	6	2 369
Presenting Clinical signs at *ante mortem* inspection	35	24	31	11	16	9	4	2	0	0	0	0	133
Total Active Surveillance	1 036	1 435	1 017	648	446	273	133	107	57	41	27	11	5 326
Passive Surveillance													
Suspects subject to lab	1121	674	304	172	74	37	15	8	2	0	0	0	2 407
Eradication Measures	9	10	3	5	16	1	1	3	0	0	0	0	48
Total	2 166	2 119	1 324	850	564	324	162	124	65	44	28	11	7 781

Both the crude incidence rates and the adjusted prevalence rates of the disease are declining exponentially in the EU27 (Figure 8). The trend observed in the absolute number of cases indicates that the control measures in place against BSE have been effective.

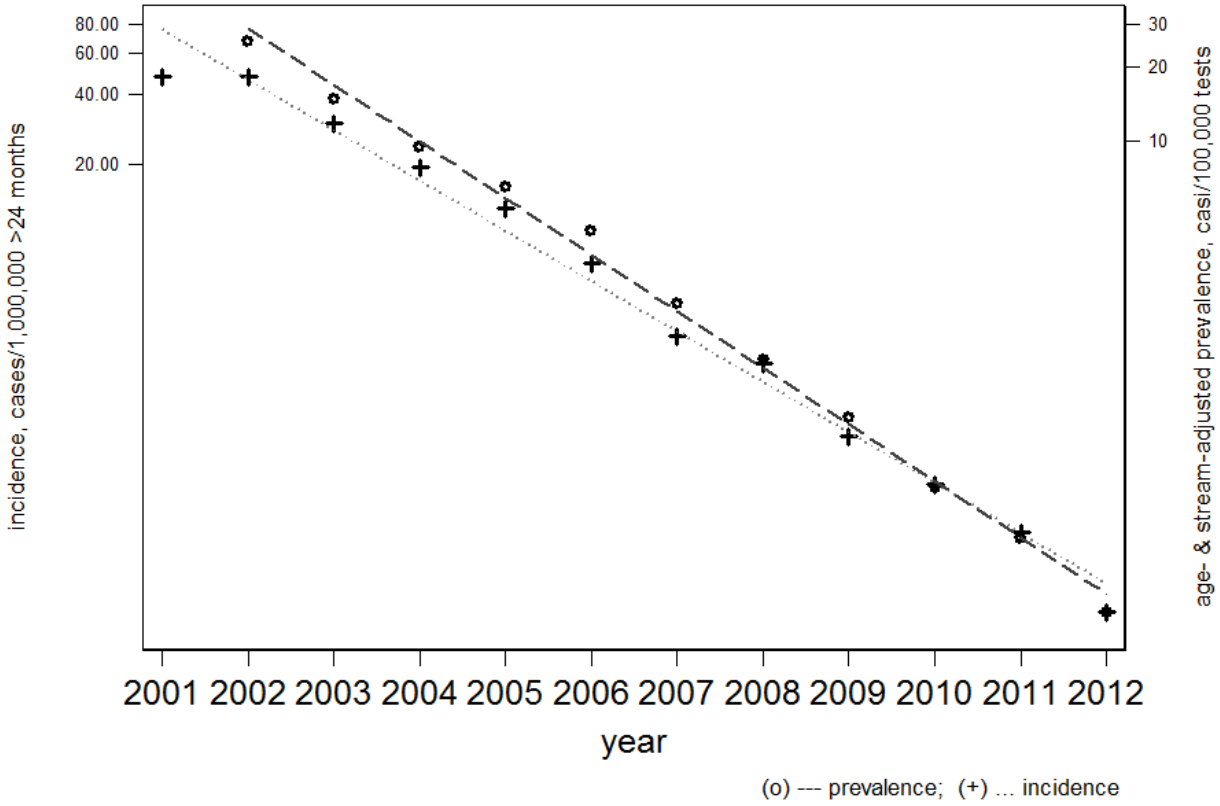

Figure 8: EU27-wide crude incidence (cases per million cattle aged over 24 months, log scale) and standardized prevalence (cases per 100,000 tests, log scale) by year between 2001 and 2012. For the direct standardization the overall age- and stream-distribution over the 2002-2012 period has been used as standard. Prevalence rates referred to 2001 were excluded as the breakdown by age was not available.

When fitting regression models using year of testing as the independent variable, and the $\log_{10}$ incidence or the $\log_{10}$ adjusted prevalence as the dependent variables, both the EU27 declining trends are statistically significant ($p<0.0001$).

The decrease in the frequency of the disease was paralleled by the increase in the average age of the cases that shifted e.g. from 7 years in 2001 to 14.7 years in 2011. The age-specific prevalences by two-year periods of monitoring are provided in Figure 9a. These show a clear reduction in the prevalence of BSE over time. When looking at the same data by birth cohort (Figure 9b, where e.g. the label 1998 stands for the birth cohorts of cattle born from January 1996 to December 1999; consecutive cohorts are overlapping for two-year periods), the effectiveness of the measures against BSE taken over the 90's is evident: the risk was progressively increasing from the 1990 birth cohort up to the 1994 one. After that the risk has been decreasing cohort by cohort leading to an exposure to the BSE agent currently being very low.

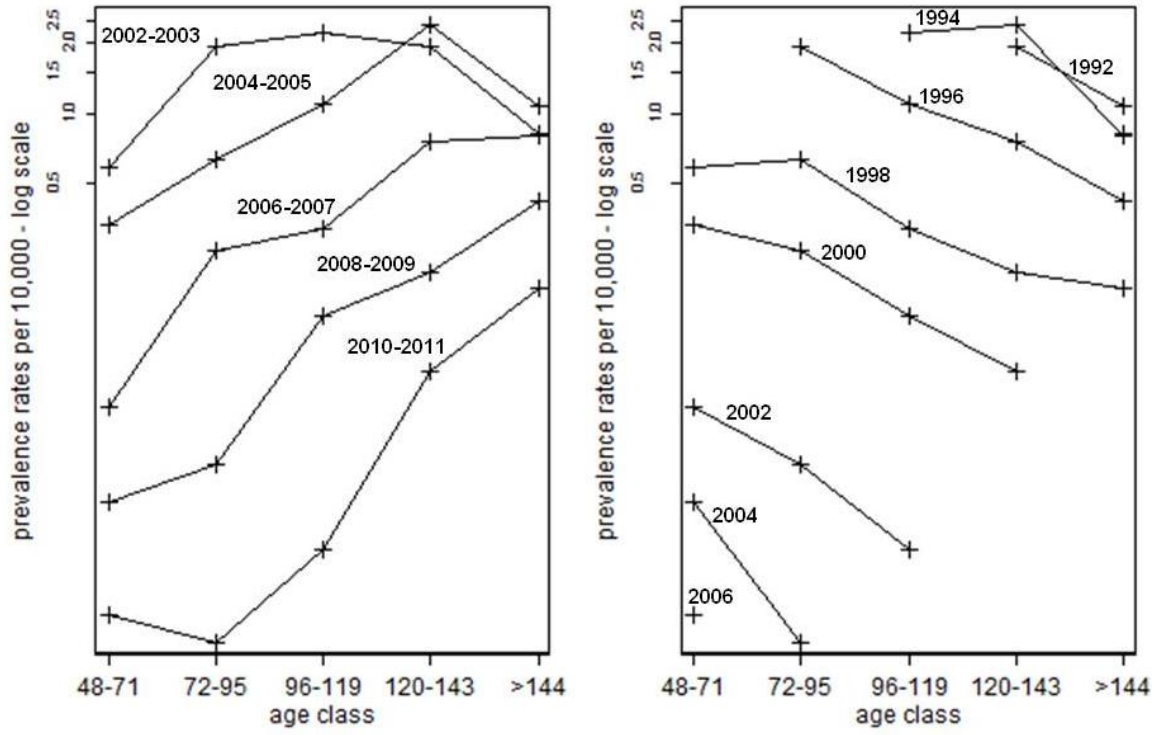

Figure 9: (a) Observed age-specific prevalences of BSE by two-year periods of diagnosis. The ages (before 144 months) are 24 months wide. (b) Observed age-specific prevalences of BSE by birth cohort. The labelled year indicates a four-year birth cohort e.g. 1998 stands for a birth cohort from January 1996 to December 1999; consecutive cohorts are overlapping for two-year periods. Data from 2012 are not considered.

To account for country-specific heterogeneities in the monitoring by stream, the geographical distribution of the disease within the EU may be shown by mapping the national stream-standardized prevalences (Figure 10). The impact and therefore the risk of BSE was very heterogeneous among the countries: e.g. over the 2002-2012 ten-year period the national crude prevalences ranged between 41.7 to 0.01 cases per 10,000 animals tested. However, differences may be in part due to the availability of data or to a different application of the active surveillance: e.g. as indicated in the map a group of countries enforced the monitoring some years later and finally UK (on the basis of the so called Over Thirty Months cattle scheme[16]) and Sweden were allowed to carry out special monitoring activities.

[16] Fresh Meat (Beef Controls) Regulations 1996 (S.I. 1996/1743). http://www.legislation.gov.uk/uksi/1996/1743/made

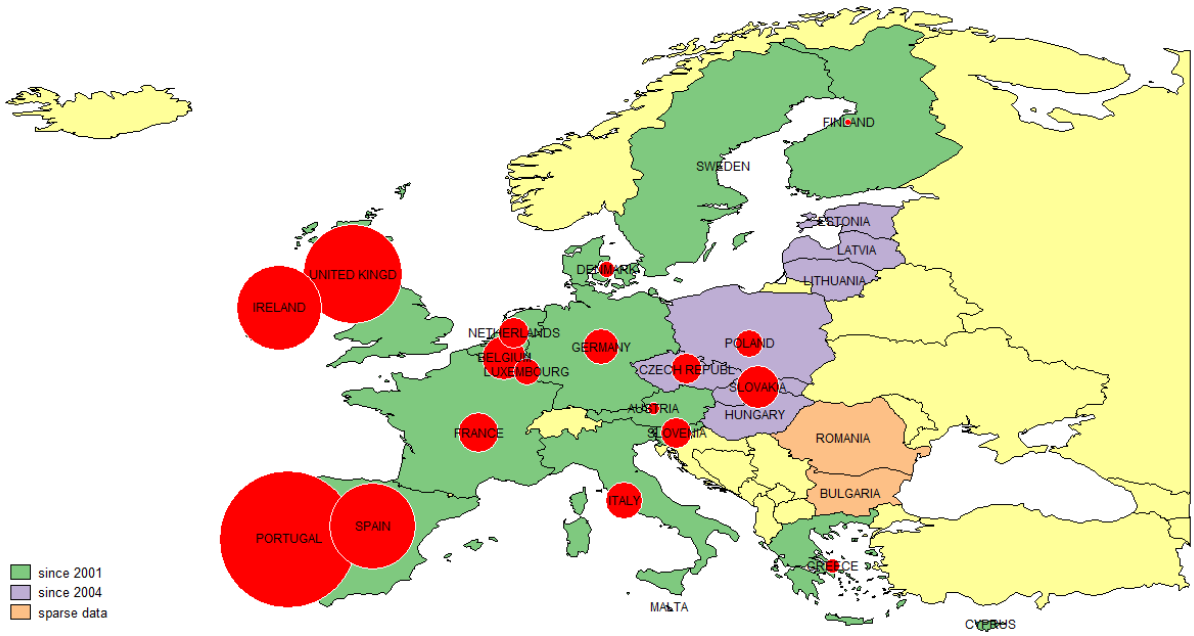

Figure 10: Geographical distribution of BSE within EU. Prevalence by country (in terms of cases/10,000 tests) standardized on stream i.e. healthy slaughtered animals vs. risk animals (fallen stock combined with emergency slaughtered animals) of surveillance and calculated over the 2001-2012 period. The red markers are proportional to the prevalence. The green indicates the 17 countries in which the monitoring data refer to the entire period whereas the violet indicates the 8 countries where 2004-2011 data are available. Data related to the UK might be underestimated due to the application of the Over Thirty Months scheme.

The temporal peaking varied between countries. For instance, Portugal and the UK experienced the peak in the epidemic before the year 2000, Belgium, Denmark, France, Germany, Ireland, Italy, the Netherlands and Spain in the first two years of the 2000s, whereas the Czech Republic, Poland and Slovakia experienced a later peak, suggesting a different evolution of the human exposure to the BSE agent.

However, as already stated in a previous Opinion (EFSA Panel on Biological Hazards (BIOHAZ), 2011a), the European countries shared a similar evolution of the BSE epidemic leading at present to an extremely low prevalence of the disease, allowing the EU27 to be considered as a single epidemiological entity.

Finally, as previously reported by EFSA (EFSA, 2012), in the EU27 the Atypical BSE does not show any particular trend as evident by the list of cases by year of detection (Table 11).

Table 11: Number of Atypical BSE cases (combining L- with H- types) detected through the BSE Active surveillance in EU27 during the period 2001 – 2012.

N° of detected Atypical BSE cases per testing year												
2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	**Total**
2	8	6	5	4	5	8	5	7	8	6	7	71

7.2.3. Conclusions

- The epidemiological knowledge of BSE in cattle relies on the EU wide TSE monitoring system.
- Before 2001, BSE monitoring in animals relied on passive surveillance, which is of limited sensitivity.
- A constant decline in the total number of Classical BSE cases per year (coming from both Active and Passive surveillance) has been recorded in the EU 27, from 2,166 cases in 2001 to 11 cases in 2012, as seen both for incidence and prevalence with statistical significance.
- In the past, the BSE epidemic was heterogeneous between the EU27 MSs in terms of level of prevalence and temporal evolution.
- Currently, the tailing of the BSE epidemic allows the EU27 MSs to be considered as a single epidemiological entity.

8. Collection of bovine intestine and mesentery for processing purpose

Since currently in the EU bovine intestine and mesentery are classified as SRM, there is no validated standard operating procedure available concerning the collection for processing purposes of this material.

8.1. Collection of intestine for casings production

A procedure for the collection of small intestine for processing into sausage casings was described by Wijnker et al. (2008) (including an addendum). Briefly, when post-mortem inspection is completed, the intestines are separated from the stomach and transferred to the gut room for processing. According to the article, the small intestines are hand-pulled from the mesentery and cut off with a knife approximately 2m proximal to the ileocaecal junction. However, it should be noted that in a document provided by ENSCA[12], a figure of 1m proximal to the ileocaecal valve was given as the amount of intestine removed and destroyed. The remainder of the small intestines, comprising the entire ileum and a small part of the jejunum are removed as SRM. According to the ENSCA "Community Guide to Good Practice for Hygiene and the application of the HACCP principles in the production of natural sausage casings"[17] large intestines are collected in a way similar to small intestines i.e. they are pulled from the mesentery and the intestinal contents stripped out.

8.1.1. Elements to be retained to model the collection of bovine intestines for casings production

In order to develop a quantitative model the Panel assumed that:

- the anatomical ileum is removed and does not enter into the processing phase;
- all tissues potentially harbouring BSE infectivity, i.e. the lymphoid tissue, as identified under Section 6.1.4, could enter into the processing phase.

8.2. Collection of mesentery for mesenteric fat production

According to information provided by European Fat Processors and Renderers Association (EFPRA)[18], the collection of mesenteric fat involves, in brief, separation of the gastrointestinal convolution at the proximal end by cutting through the duodenum, and separation at the distal end by cutting through the rectum. The mesentery is then separated from the small and large intestine using manual and mechanical means. The fat obtained in this way is checked visually for the presence of residual parts of the intestine. Provided that no residual parts of the intestine are still present, the fat is collected in suitably labelled containers.

8.2.1. Elements to be retained to model the collection of bovine mesentery for mesenteric fat production

In order to develop a quantitative model the Panel assumed that all the structures identified under Section 6.2.4 as potentially containing BSE infectivity (i.e. nerves roots, autonomic nervous system ganglia and lymph nodes) would remain attached to the mesentery and could enter into the processing phase.

[17] http://ec.europa.eu/food/food/biosafety/hygienelegislation/guidelines_good_practice_haccp_en.pdf
[18] Information received from the European Fat Processors and Renderers Association (EFPRA) on 19 September 2012.

9. Processing of the material

9.1. Processing intestine into casings

Wijnker et al. (2008) carried out a study describing a procedure for the processing of small intestines into sausage casing. However, it is important to note that the study was performed on cattle from South America that did not match the main breeds and the mean age of the animals slaughtered in the EU. Moreover, it cannot be guaranteed that the protocol used for the study would be equivalent to the protocol eventually used by the industry. After separation from the mesentery and removal of the ileum, the outside of the small intestines are scraped manually to remove any remaining mesentery, adipose tissue and loose serosa. The intestines are moved to another station and the intestinal content stripped from the stomach-end towards the distal-end. This can either be done manually or mechanically, according to the set-up of the processing line.

The small intestines are transferred to another holding tank filled with water at around 30 ºC and turned inside-out, bringing the mucosal side to the surface. This is done by pouring water into the small intestine which is fixed to the rim of the tank. Gravity and weight of the water will invert the intestine.

The small intestines are then transferred to a Stridh-type cleaning machine (built by Mecanica Primitiva Ltda – Salto Grande, São Paulo, Brazil). This machine scrapes and removes the outer tissue layers of the intestines. As the intestine is turned inside-out before cleaning, the tissue layers removed consist of mucosa and lymphoid tissue, leaving the submucosa, muscularis layer and serosa intact after cleaning.

After the cleaning process, the beef casings are bundled and moved to the salting department. The bundles are salted manually by rubbing salt over the entire length of the casings, extracting water and starting the preservation process. The entire cleaning process of the cattle intestines into beef casings is done within 30 minutes. After salting, the beef casings are packed into plastics casks with new plastic liners (volume 250 litre), sealed, labelled and shipped to the selecting facility. At the selecting facility, the beef casings are desalinated in water and divided into different categories, depending on quality, length and calibre.

After selecting, the beef casings are re-salted, stored and subsequently shipped to the customer.

In the study carried out by Wijnker et al. (2008), the processing resulted in a significant decrease in the thickness and weight of the intestine. The thickness decreased from 3.01 mm (SD 0.96) to 1.15 mm (SD 0.28). The weight decreased from 3,900g (SD 874) to 2,264g (SD 596), a weight reduction of 42%. This was achieved mainly due to the removal of mucosa. The average length of both unprocessed and processed small intestine was 35m (+- 4m). All samples from the processed intestines also contained residual amounts of mucosa but there was a 90% reduction in mucosa present in the processed intestine compared to the fresh intestine. Lymphoid tissue was found in 40 (10%) of the processed intestinal samples (n = 400) compared to 60 (15%) of the fresh intestine samples (n= 400). The authors assumed that the cleaning process would not remove any of the neural tissue present in the small intestine.

According to Wijnker et al. (2008), several different cleaning procedures are in place within the casings industry for the processing of bovine intestine into beef casings. However, the difference in these procedures relate mostly to the level of automation, whereas the general principle remains the same throughout. They concluded that all cleaning processes will lead to a similar quantitative reduction of mucosa and lymphoid tissue.

The results of the experimental study published by Wijnker et al. (2008) were already assessed by the EFSA Scientific opinion on Quantitative histological studies and the re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings (EFSA, 2007). According to the opinion, after processing into casings there is no reduction in the quantity of enteric neural tissue and

the reduction in quantity of lymphoid tissue is in the order of 50% while the total weight reduction is about 40%.

No similar studies are reported as regard to large intestine. However, according to information received from ENSCA[19] the total weight reduction after processing of caecum and colon is in the range respectively of 30 – 70% and 30 – 50%. As regards the reduction in the quantity of neural and lymphoid tissue, it can be considered that the casing processing for large intestine has the same effectiveness as for small intestine.

9.1.1. Elements to be retained to model the effect of casings processing on BSE infectivity

There is considerable uncertainty as regards the effectiveness of casings processing on the reduction of BSE infectivity in intestine.

In order to develop a quantitative model, the Panel assumed that the efficacy of casing processing on BSE infectivity reduction ranges between 0 and 50%.

Moreover, the Panel concluded that since the intestine collected from one single infected bovine would be consumed by a limited number of people the processing of intestine would not bring any dilution effect.

9.2. Rendering fat from mesentery

Fat may be rendered in the same slaughterhouse in which it is produced. In that case, it is generally collected in bins or conveyors and is transferred to a particular room (the fat room) for processing. If it is processed in another establishment, it must be transported and stored until rendering, in hygienic conditions and at an internal temperature of not more than 7°C. However, raw materials may be stored and transported without active refrigeration if rendered within 24 hours after the day on which they are obtained.

Prior to processing, the fat is minced. There is no specific requirement of the particle size, but according to an EFPRA Working document[20], it is ground to a particle size of < 30 mm (average 10mm). Following mincing, the material is put into the fat melting machine where it is heated to a temperature of 90-95°C for approximately 20 minutes. The processing procedure produces greaves as well as fat; the former is produced from connective tissue and the other non-fat material present in the mesentery. Fat cleaning can be one of the following procedures or combinations thereof a) decantor (horizontal centrifuge), b) separator (vertical centrifuge), c) filter (filter techniques) and d) sedimentation (sedimentation /gravitation in tanks). The tallow is pumped into silos where it is kept in liquid form.

The processing conditions must comply with Regulation (EC) No 852/2004[21] (the food hygiene Regulation) and the specific rules on the hygiene of food of animal origin laid down in Regulation (EC) 853/2004[22].

There is a requirement in the legislation that the insoluble impurities in the rendered fat must be less than 0.15%. In addition, a number of other characteristics of the rendered fat are measured in order to determine the quality of the product. These include percentage of free fatty acids (FFA), peroxide value (POV) and moisture. Typically freshly melted edible animal fats have the following commercial specifications: FFA < 0.50%, POV < 4.0%, moisture < 0.2% and insoluble impurities < 0.02% (Woodgate and Van Der Veen, 2004).

[19] Information received from the European Natural Casings Association (ENC) on 19 September 2012.
[20] Information received from the European Fat Processors and Renderers Association (EFPRA) on 4 September 2012
[21] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs OJ L 139, 30.4.2004, p. 1-54.
[22] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin OJ L 139, 30.4.2004, p. 55-205.

Detailed information on the processing methods for animal fats and greaves can be found in the paper by Woodgate and Van Der Veen (2004).

According to information provided by the European Livestock and Meat Trading Union (UECBV), the average mass of mesenteric fat in Europe is variable: 2 kg for a calf, 4.1 kg for a bovine animal less than 24 months and between 5.2 and 5.5 kgs for cattle over 24 months of age[23].

According to information provided by EFPRA, the estimated yield of mesenteric fat is as follows:

- Calves < 7 months: 1.6 kg
- Young male/female < 20 months: 2.5 kg
- Cows: 5-7 kg
- Limousin cow: 12 kg
- Heavy bulls: 9-15 kg

9.2.1. Elements to be retained to model the effect of rendering mesenteric fat on BSE infectivity

In order to develop a quantitative model the Panel assumed that:

- due to the high resistance of prions to inactivation, the tallow production method does not have any impact in reducing the infectivity level in the processed material;
- the processing of mesenteric tissues collected in a batch of several animals would dilute the infectivity associated with mesenteric tissues from one BSE infected cow. This dilution effect cannot presently be quantified.

10. Modelling TSE infectivity level in bovine intestine and mesentery

10.1. Introduction

In order to quantitatively assess the BSE risk associated with the bovine intestine and mesentery, the information reported in the previous sections of this document was used to feed a model on "TSE infectivity level in animal tissues" (TSEi) that was specifically developed for EFSA by a contractor selected through an open call for tender (CFT/EFSA/BIOHAZ/2012/02). Detailed information on the model is reported in the External Scientific Report TSE infectivity model (TSEi) in animal tissues: Bovine intestines and mesenteries (Adkin et al., 2014). Available on-line at the following URL: http://www.efsa.europa.eu/en/supporting/pub/559e.htm

All the parameters and the related data and assumptions used to model the BSE infectivity in bovine intestine and mesentery are detailed in Appendix A. Moreover, the main assumptions and limitations related to the development of the model are narratively described in Table 16 in section 10.3.

10.2. Methodology

The model was developed in Microsoft Excel with the use of the stochastic add-in Palisade @Risk version 6.2.1. This allows:

- the comparison of the level of TSE infectivity in animal tissues;

[23] Information received from the European Livestock and Meat Trading Union (EUCBV) on 29 June 2012.

- the estimation of the impact of risk management options (SRM removal) at single animal level and at animal population (country) level;

- the estimation of the impact of processing on the TSE infectivity level.

The model is divided into five data components: (1) surveillance, (2) abattoir, (3) SRM, (4) infectivity, (5) processing and a central component which scales up the estimates based on a single random infected animal to the cumulated infectivity in one year in a selected country or in a group of countries.

The model takes into account both uncertainty and variability. An indication on whether the parameters used are representative of uncertainty or variability is given in Appendix A.

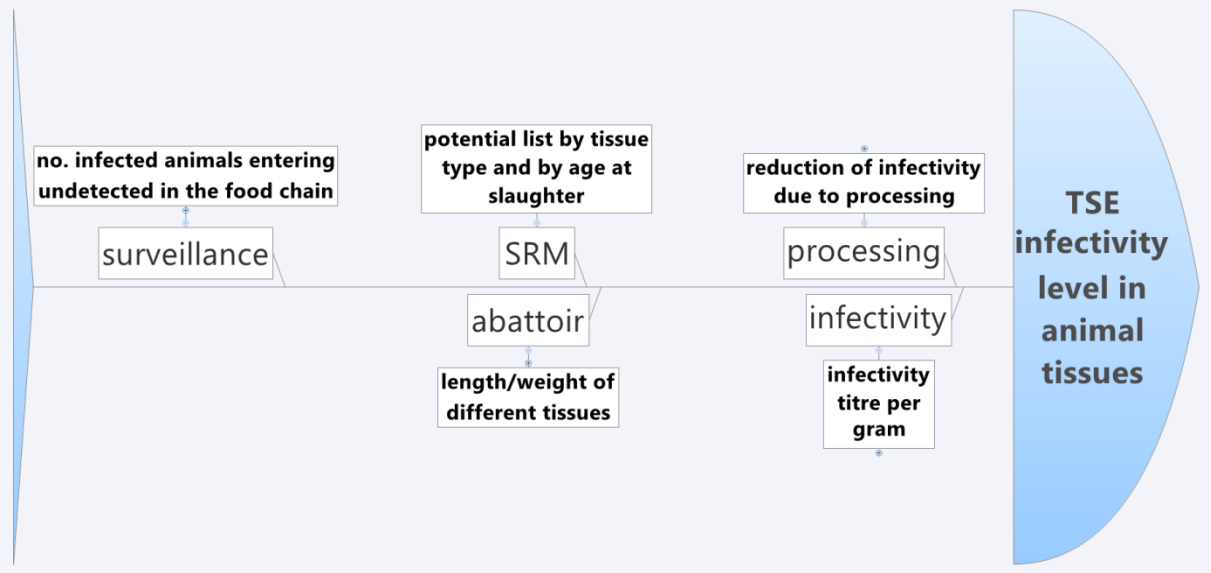

Figure 11: Schematic overview of the model with the main components and their specific contribution

10.2.1. Surveillance component

The surveillance component is based on a mathematical model (C-TSEMM) that has been developed to estimate the BSE prevalence trend in the European Member States (MSs) (Adkin et al., 2012).

C-TSEMM estimates the number of BSE infected cattle slaughtered in a given year by birth cohort, including those testing positive if a BSE test was applied, and the number of BSE infected animals entering undetected into the food and feed chain.

The age intervals within C-TSEMM are <24 months, 24-29 months, 30-35 months, and in 12 monthly intervals to those >204 months. In order to model young animals less than 24 months old it is assumed that the number of infected animals can be estimated as the proportion of the total number slaughtered multiplied by the estimated total number infected < 24 months.

The age intervals start with <6 months, then in 6 monthly intervals up to 36 months, then in 12 monthly intervals to the final band of >204 months producing 21 intervals in total.

The data collected in the framework of the development of C-TSEMM were updated till 2011 and did not consider Romania and Bulgaria; moreover, the number of animals slaughtered between 0 and 24 months of age was not requested. In order to supplement these data a questionnaire was sent to the EU 27 MS asking them to provide the relevant information for 2012 to run C-TSEMM and the number of

animals slaughtered between 0 and 24 months of age by age category. The data were received until 18 September 2013.

From 1 March 2013[24] the EU MS (except Bulgaria and Romania) are no longer required to test healthy slaughtered animals. However, emergency slaughtered and animals showing clinical signs at *ante mortem* inspection need to be tested before entering into the food and feed chain. In any case EU MSs may decide to apply stricter schemes. Table 12 below summarises the current EU legislative context. For the purpose of this assessment it is assumed that healthy slaughtered animals are not tested in the whole EU MS.

Table 12: Age at testing in the EU per target group according to Regulation (EC) 999/2001[25] and Commission Decision 2009/719/EC[26]as amended.

Target Group	Age at testing (months)	
	EU25	**Romania, Bulgaria**
Healthy slaughtered (HR)	No test foreseen	30
At risk animals (AR)		
Emergency slaughtered	48	24
Fallen stock	48	24
Presenting clinical signs at ante mortem inspection	48	24

10.2.2. Abattoir component

The abattoir component simulates the length and/or weight of the different segments of intestine and components of the bovine mesentery (nervous tissues, lymphoid tissue and fat). It also provides correlation coefficients between the weight/size of the tissues under consideration.

In the intestine, the lymphoid tissue is assumed to harbour all of the BSE infectivity. Therefore, the amount of infectivity in intestinal tissues is estimated from the weight of the lymphoid tissue in the duodenum, jejunum, ileum, caecum and colon.

In the mesentery, the infectivity is assumed to be associated with the lymph nodes, celiac and mesenteric ganglion complex (CMGC) and mesenteric nerves.

[24] 2013/76/EU: Commission Implementing Decision of 4 February 2013 amending Decision 2009/719/EC authorising certain Member States to revise their annual BSE monitoring programmes. Official Journal L 035 , 06/02/2013 P. 0006 – 0007.

[25] Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 147, 31.5.2001, p. 1-40.

[26] 2009/719/EC: Commission Decision of 28 September 2009 authorising certain Member States to revise their annual BSE monitoring programmes (notified under document C(2009) 6979) (Text with EEA relevance) OJ L 256, 29.9.2009, p. 35-37.

10.2.2.1. Infectivity distribution in the intestine

Figure 12 below shows a schematic representation of the intestinal tissues considered for modelling purposes according to Section 6.1.

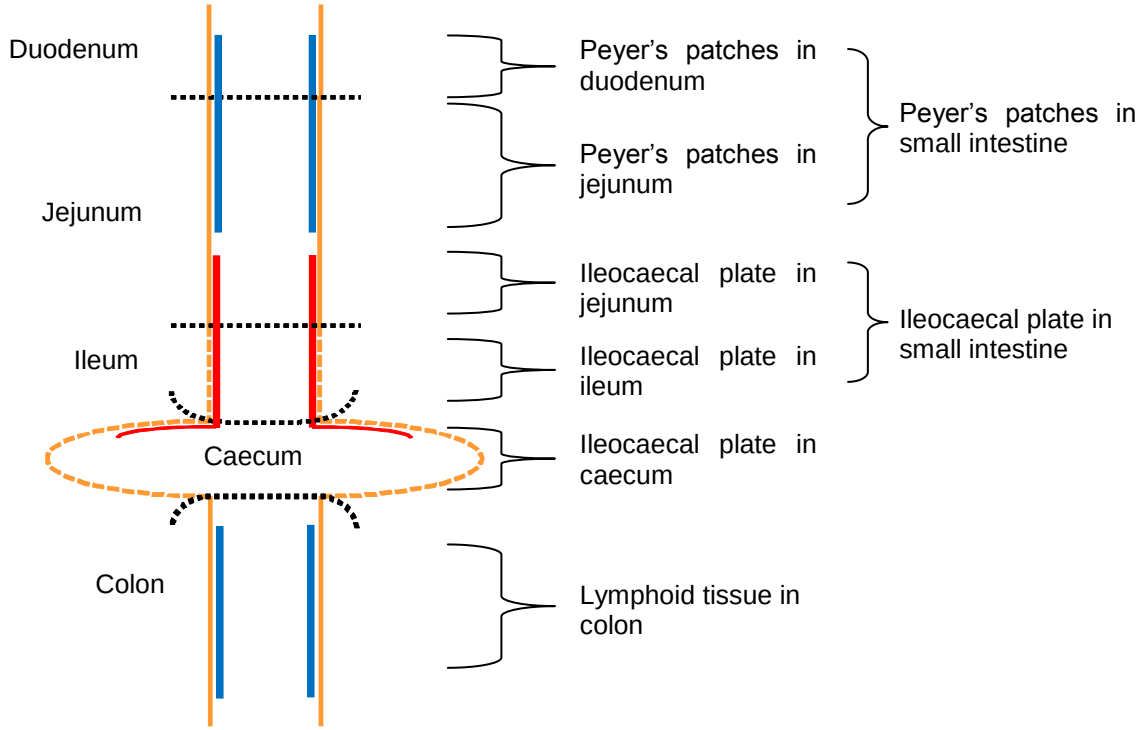

Figure 12: Simplified diagram of bovine intestines indicating location of ileocaecal plate (red line) in jejunum, ileum and caecum, and Peyer's patches PP (blue line) in duodenum, jejunum and colon.

According to the information received from ENSCA intestines derived from calves still on a milk diet are not used for casings production[27]. The age at slaughter categories within the surveillance component starts at <6 months. Due to a lack of data in the model it is assumed that the weight of tissues from infected animals slaughtered <6 months old can be approximated by the 4-6 months data age intervals. This is an overestimate as intestines from those animals < 4 months old would not be processed into sausage casings.

10.2.2.2. Length of intestine

Section 5.1 provides the detailed information used in the model.

The minimum and maximum length of the entire small intestine (duodenum, jejunum and ileum) in bovine animals at different ages was estimated to be respectively 28 and 56 m. The variability is described in the model by a uniform distribution between the minimum and maximum estimate by age category.

The length of ileum is estimated to range from 0.5 to 1 m. Estimates for the length of duodenum range from 0.9 to 1.2 m depending on the age of the animal. To account for animals slaughtered at different ages, it is assumed that the lowest estimate applies to animals slaughtered before 6 months, and the

[27] Information received from the European Natural Sausage Casings Association (ENSCA) on 14 June 2012.

highest estimate to those animals aged over 24 months at slaughter. Between these ages, it is assumed that there is a linear increase in length.

The jejunum length by age at slaughter is estimated from the total length of small intestine by age at slaughter minus the length of ileum and duodenum.

The length of caecum was estimated to range from 0.5 to 0.75 m depending on the age of the animal.

For the colon, a minimum value of 6 m, average of 10 m to a maximum of 12 m has been recorded. To account for animals slaughtered at different ages, it is assumed that the lowest estimate applies to animals slaughtered before 6 months, and the highest estimate to those animals aged over 24 months at slaughter. Between the ages classes from <6 m to <24 m there is a linear increase in length. For animals aged over 24 months, the colon is assumed to vary between 10 and 12 m. This variability has been described in the model by a uniform distribution.

Within a randomly selected animal, a 90% correlation is assumed between the lengths of the different parts of the intestine.

10.2.2.3. Weight of lymphoid tissue in intestine

Section 5.1 describes the information used in the model.

The total weight of PP in duodenum and jejunum plus the ileocaecal plate in jejunum and ileum and, separately the weight of PP in duodenum and jejunum for cattle slaughtered at different ages were available.

For each individual animal identified by age, the weight of the ileocaecal plate in jejunum and ileum (thus not including the caecum) was estimated from the total weight of the PP, including the ileocaecal plate, minus the weight of non-ileocaecal plate PP in the small intestine.

For each age category, the between animal variability of the total weight of the ileocaecal plate in ileum and jejunum and the PP (excluding the ileocaecal plate) in duodenum and jejunum is described in the model by the estimated minimum, maximum and fitted 'most likely' weight by a Pert distribution with values shown in Table 13 below.

Table 13: Weight of ileocaecal plate PP and non-ileocaecal plate PP, mean, minimum and maximum values by age at slaughter adapted from Carlens (1928)

Age at slaughter (months)	Ileocaecal plate PP			Non-ileocaecal plate PP in duodenum and jejunum		
	Minimum weight (g)	Mean weight (g)	Maximum weight (g)	Minimum weight (g)	Mean weight (g)	Maximum weight (g)
< 6	274.90	369.00	439.80	69.00	105.00	142.00
6 - 11	276.00	386.00	497.00	102.00	134.00	206.00
12 - 17	252.00	350.00	461.00	160.00	196.00	252.00
18 - 23	30.20	210.00	472.30	155.00	189.00	242.00
24 - 59	0.00	1.25	4.00	111.00	158.75	207.00
60 - 119	0.00	1.25	3.00	66.00	110.73	149.00
> 120	0.00	0.29	1.00	40.00	66.71	96.00

Within a randomly selected animal, a 90% correlation is assumed between the weight and the length of lymphoid tissues (see Table 2 and Table 4 in section 5.1.1).

Since the total ileocaecal plate measurements include the anatomical structures of the jejunum and ileum (the measurement in the caecum is not included), it is assumed that the proportion of the ileocaecal plate located in the ileum can be estimated by dividing the length of ileocaecal plate in the

ileum by the total length of the ileocaecal plate (in ileum and jejunum). Moreover, it is assumed that the proportion of ileocaecal plate in ileum and jejunum evolves proportionally according to the age. In this way, the proportion of ileocaecal plate in each of these tissues can be estimated using the data from those animals slaughtered under 6 months of age. Since at this age the ileocaecal plate covers the whole length of the ileum, it can be assumed that the length of the ileocaecal plate in the ileum is equal to the length of the ileum.

The proportion of the weight of the non-ileocaecal plate PP that is located in the duodenum and jejunum is assumed to be equal to the length of duodenum and jejunum respectively divided by the total length of duodenum and jejunum by age at slaughter.

As regards the part of ileocaecal plate that is located into the caecum, it is assumed that it has the same weight per surface area as the part located into the ileum. Its surface is assumed to range from 60 to 80 cm^2 and its variability is described in the model by a uniform distribution.

The model assumes that the weight of lymphoid tissue in the colon can be approximated by the concentration of Peyer's patches (weight per metre length) that is estimated to be present in the proximal jejunum[15].

10.2.2.4. General information on modelling infectivity in mesentery

Section 6.2 and Figure 13 below indicate the mesenteric structures that are assumed to accumulate BSE infectivity in mesentery (Coeliac ganglia, nerves and lymph nodes). The infectivity is assumed to be homogenously distributed in these different structures.

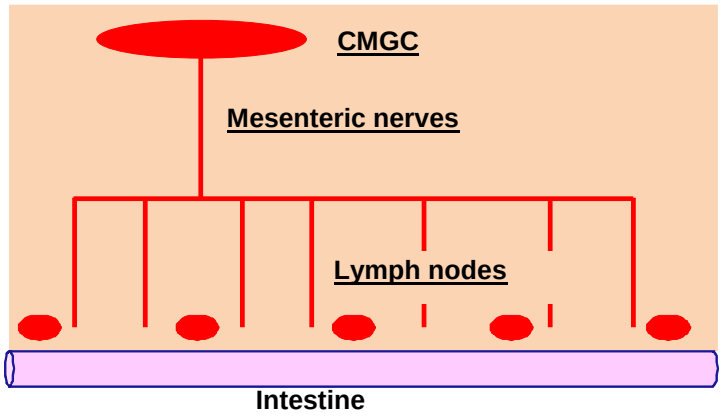

Figure 13: Simplified diagram of the mesenteric tissues (in red) considered by the model (i.e. mesenteric lymph nodes, celiac and mesenteric ganglion complex and mesenteric nerves).

10.2.2.5. Weight of mesenteric lymph nodes

Section 5.2 describes in detail the information used in the model.

Calves under 6 months of age are described in the model using a pert distribution of fitted most likely weight of 70.9 g with minimum value of 55.5 g and maximum of 101.5 g. For those animals older than 6 months of age there is a high degree of variability between animals with no trends associated with age. Therefore, mesenteric lymph nodes from animals older than 6 months are described using a pert distribution of fitted most likely weight of 163.4 g, with minimum value of 69.8 g and maximum value of 283.0 g.

10.2.2.6. Weight of mesenteric nerves

Section 5.2 details the information used in the model.

There is no data related to the weight of mesenteric nerves. In this context, the model used expert opinion only. The mean weight of mesenteric nerves is assumed to range from 100 g for animals less than 6 months old to 200 g for those older than 24 months at slaughter. Between animal variability is represented by a minimum estimate ranging from 50 g to 100 g by age at slaughter, and a maximum estimate of 200 g to 500 g by age at slaughter. This variability is described in the model using a pert distribution and the assumption that there is a linear weight increase with the aging for those animals less than 24 months old. The values used in the pert distribution are the estimated minimum and maximum weight and a fitted most likely weight to yield a distribution with the same mean weight as given in Table 14.

Table 14: Expert opinion estimates for the weight of mesenteric nerves with minimum, mean and maximum values.

Age at slaughter (months)	Mesenteric nerves		
	Minimum weight (g)	Mean weight (g)	Maximum weight (g)
< 6	50.00	100.00	200.00
6 - 11	62.50	125.00	275.00
12 - 17	75.00	150.00	350.00
18 - 23	87.50	175.00	425.00
24 >	100.00	200.00	500.00

10.2.2.7. Weight of celiac and mesenteric ganglion complex (CMGC)

Section 5.2 provides the information used in the model.

There is no data available as regards to the weight of CMGC in cattle. This was estimated by extrapolating data collected from a pony. The weight of CMGC was estimated to be 50.5 g in adult cattle. A mean weight ranging from 25.25 g for animals less than 6 months old to 50.50 g for those greater than 24 months at slaughter is used in the model. It is assumed that there is a linear weight gain between these age intervals. Between animal variability is represented by a minimum estimate ranging from 12.50 g to 25 g by age at slaughter, and a maximum estimate of 50 g to 100 g by age at slaughter. This variability is described in the model using a pert distribution. The values used in the pert distribution are the estimated minimum and maximum weight and a fitted most likely weight to yield a distribution with the same mean weight as given in Table 15. Within a randomly selected animal a 90% correlation is assumed between the weight of CMGC and mesenteric nerves.

Table 15: Expert opinion estimates for the weight of CMGC with minimum, mean and maximum values.

Age at slaughter (months)	Mesenteric nerves		
	Minimum weight (g)	Mean weight (g)	Maximum weight (g)
< 6	12.50	25.25	50.00
6 - 11	15.63	31.56	62.50
12 - 17	18.75	37.87	75.00
18 - 23	21.88	44.18	87.50
24 >	25.00	50.50	100.00

10.2.3. SRM component

The SRM component permits the user to define a list of SRM by tissue type and by age at slaughter to investigate the impact of this risk management measure on the amount of infectivity that could enter the food and feed chain.

10.2.4. Infectivity component

The Infectivity component estimates the infectivity titre per gram of tissue using the months post infection or months before clinical onset and age of the random animal at slaughter.

The titre of infectivity estimated for the ileum is based on the infectivity of the ileocaecal plate within the ileum. Since the ileoacaecal plate extends from jejunum to caecum, this estimate is used in the model to estimate the RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g for the ileocaecal plate present in the ileum, jejunum and caecum.

Section 6.1.1 provides the information used for the model.

There is no discernible pattern to the infectivity titres in the ileum. Therefore, it is assumed that infectivity in ileum is essentially random with high between-animal variability.

A normal distribution with a mean titre of 0.37 $\log_{10}$ RIII i.c. i.p ID_{50}/g and a standard deviation of 0.81 $\log_{10}$ RIII i.c. i.p ID_{50}/g is used in the model to parameterise the infectivity titre for the ileum.

10.2.4.1. Infectivity titre in jejunum

Section 6.1.2 provides the information used for the model.

There is no discernible pattern to infectivity titres in the Peyer's patches in the jejunum. However, there is a significant difference associated with the titre recorded in the ileocaecal plate in the distal ileum. Therefore, it is assumed that infectivity in the Peyer's patches in the jejunum can be modelled as an estimated $\log_{10}$ lower value than the ileocaecal plate in the ileum.

The estimated mean difference between the Peyer's patches in the jejunum and the ileocaecal plate in the ileum is estimated as a mean of 3.735 $\log_{10}$ RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g, with a 95% uncertainty range of 3.072 to 4.384 $\log_{10}$ RIII mouse i.c. i.p. log10 ID50/g. This is described in the model using a modified pert distribution.

10.2.4.2. Infectivity titre in duodenum, caecum and colon

Sections 6.1.2 and 6.1.3 provide the information used for the model.

No data are available as regards to the infectivity in the duodenum, caecum and colon.

The Peyer's patches in the duodenum and the lymphoid tissue in the colon have similar characteristics to the Peyer's patches in the jejunum. In order to quantitatively model the BSE infectivity in these tissues it is assumed that the infectious tissue in duodenum and colon has an equal titre to that estimated for Peyer's patches in the proximal jejunum[15].

As regards the caecum, the model assumes that any infectivity accumulating will be the same as that estimated for the ileocaecal plate in the ileum.

10.2.4.3. Infectivity in mesenteric lymph nodes

Section 6.2.2 provides the information used for the model.

The model assumes that infectivity in mesenteric lymph nodes ranges from 0 to -6.7 $\log_{10}$ RIII mouse i.c. i.p. ID_{50}/g (i.e. the upper confidence interval established from 0 positive out to 12 TgBov XV mice). The uncertainty associated with this range is accounted for using a uniform distribution.

10.2.4.4. Infectivity in mesenteric nerves and CMGC

Section 6.2.3 provides the information used for the model.

The model assumes that infectivity in these structures can accumulate as early as the ileum becomes positive and that there is a $\log_{10}$ linear increase by months post infection until a plateau is reached. Moreover, it assumes that the pattern and titre of infectivity detected in the vagus nerve can be used to represent the mesenteric nerves.

The maximum titre reached is assumed to be equal to respectively 0.013 RIII i.c. i.p. $\log_{10}$ ID_{50}/g for mesenteric nerves and -0.01 RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g for CMGC.

A common growth rate for mesenteric nerves and CMGC of 0.224 with a 95% credibility interval of 0.20 to 0.25 with constant terms -8.40 (95% CrI: -9.22, -7.47) for mesenteric nerves and -7.70 (95% CrI: -8.53, -6.82) for CMGC respectively are used. These parameters are described in the model using pert distributions.

10.2.5. Processing component

The processing component simulates the reduction, if any, of the weight of infectious materials or infectivity resulting from the processing of tissues. According to information received from industry, the ileum is not suitable for casing production and therefore processing it into casing is not considered in the model[28].

10.2.5.1. Reduction of infectivity due to processing intestines for sausage casing

According to section 8.1 the model considers that all tissues potentially harbouring BSE infectivity in intestine (i.e. the lymphoid tissue) except the anatomical ileum, could enter into the processing phase.

Section 9.1 provides the information used to estimate the reduction of infectivity due to processing.

The model assumes that the reduction of infectivity per animal could be described by a minimum of 0 to a maximum of 50% with this variability described using a uniform distribution.

10.2.5.2. Reduction of infectivity due to processing mesentery for mesenteric fat production

According to section 8.2 the model considers that all tissues potentially harbouring BSE infectivity in mesentery, i.e. mesenteric lymph nodes, CMGC and mesenteric nerves, could enter into the processing phase.

Section 9.2 provides the information used to estimate the reduction of infectivity due to processing.

The model assumes that processing mesentery for mesenteric fat production does not have any impact in reducing the infectivity level in the output material.

10.3. Main assumptions and limitations related to the development of the model

Table 16 provides a narrative overview of the main assumptions and limitations encountered during the development of the model.

[28] Information received from the European Natural Sausage Casings Association (ENSCA) on 14 June 2012

Table 16: Overview of the main assumptions and limitations encountered during the development of the model.

Model component	Assumptions	Limitations
Surveillance	• Healthy slaughtered animals are not tested in the whole EU MS. • In animals less than 24 months, the number of infected can be estimated from the proportion of the total slaughtered. E.g. the number of animals infected aged <6 months is estimated as the number slaughtered <6 months, divided by the total slaughtered under 24 months, multiplied by the total infected under 24 months.	• UK animals born before August 1996 (>209 months in 2013) are not allowed to enter into the food and feed chain and are not tested. However, due to the small proportion of the total this represents, all UK animals have been included in the analysis.
Abattoir	• Lymphoid tissue harbours the whole BSE infectivity in intestine. • The weight of tissues from infected animals slaughtered <6 months old can be approximated by the 4-6 data age intervals. • Within a randomly selected animal there is a 90% correlation between the length of the different parts of the intestine. • Within a randomly selected animal there is a 90% correlation between the weight and the length of lymphoid tissues in small intestine. • The proportion of ileocaecal plate in duodenum and jejunum can be estimated using the data from those animals slaughtered under 6 months of age. • The proportion of the Peyer's patches weight that is located in the duodenum and jejunum is equal to the length of duodenum and jejunum respectively divided by the total length of duodenum and jejunum by age at slaughter. • The ileocaecal plate in caecum has the same weight per surface area as the ileocaecal plate in ileum. • Colon has the same quantity per metre of lymphoid tissue as proximal jejunum. • The entire weight of mesenteric lymph nodes, celiac and mesenteric ganglion complex and mesenteric nerves may be infectious. • The weight of mesenteric nerves ranges from 100 g for animals less than 6 months old to 200g for those greater than 24 months at slaughter with minimum and maximum estimates ranging respectively from 50 g to 100 g and from 200 g to 500 g by age at slaughter. • The volume of the CMGC in a horse is used as a proxy for bovine animals, its shape can be considered as cylindrical and its left and right parts can be summed together in order to obtain the total volume of this tissue in bovine animals. The specific weight of CMGC is considered equivalent to water. • The weight of CMGC ranges from 25.25 g for animals less than 6 months old to 50.50 g for those greater than 24 months at slaughter with minimum and maximum estimates ranging respectively from 12.50 g to 25 g and from 50 g to 100 g by age at slaughter. • Within a randomly selected animal there is a 90% correlation between the weight of CMGC and mesenteric nerves.	• Very limited data available as regards to the length and weight of the tissues considered.

Model component	Assumptions	Limitations
Processing	• The ileum is not suitable for casing production and therefore processing it into casing is not considered in the model. • The infectivity reduction due to processing of bovine intestines into casing ranges from 0 to 50% with this variability described in the model using a uniform distribution. • Processing mesentery for mesenteric fat production does not have any impact in reducing the infectivity level in the output material.	• There is considerable uncertainty related to the processing of bovine intestines into casings.
Infectivity	• Infectivity in ileum is essentially random with high between animal variability. • Infectivity in the Peyer's patches in the jejunum can be modelled as an estimated $\log_{10}$ lower value than the ileocaecal plate in the ileum. • The infectious tissue in duodenum and colon has an equal titre to that estimated for Peyer's patches in the proximal jejunum. • The infectious tissue in caecum has an equal titre to that estimated for the ileocaecal plate in ileum. • Infectivity in mesenteric lymph nodes ranges from 0 to the upper confidence interval established from 0 positive out to 12 TgBov XV mice. • Infectivity in mesenteric nerves and CMGC could accumulate as early as the ileum become positive and persists until clinical onset. • The pattern and titre of infectivity detected in the vagus nerve can be used to represent the mesenteric nerves. • A $\log_{10}$ linear increase of infectivity in mesenteric nerves and CMGC by months post infection until reaching a plateau.	• No data are available as regards to the infectivity in duodenum, caecum and colon. • Few data are available as regards to the infectivity in mesenteric nerves and CMGC.

10.4. Results

Both variability and uncertainty are considered in the model and are represented by 2.5th and 97.5th percentiles (within parentheses), which indicate the range within which 95% of the results lie. The greater the range between the percentiles, the greater the total uncertainty. The baseline age at slaughter model was run for 150,000 iterations using Latin Hypercube sampling. Convergence to 4% of the mean value of each output parameter was achieved at approximately 100,000 iterations. The baseline EU27 annual model was run for 300,000 iterations using Latin Hypercube sampling. Convergence to 4% of the mean value of each output parameter was achieved between 250,000 to 300,000 iterations. It should be emphasised that not all variability and uncertainty has been estimated in the calculations, as not all can be quantified. Therefore the 2.5th and 97.5th percentiles describe the amount of *quantified* variability and uncertainty included in the model.

Results are provided for the infectivity associated with a single infected animal by age at slaughter and also that associated with a random infected animal drawn from the EU27 healthy slaughter and emergency slaughter stream destined for use in the food and feed chain. Results are stratified by age at slaughter, tissue type, by metre length, and the infectivity remaining in processed tissues. Annual results are provided for the total amount of infectivity from bovine intestines and mesenteries. These results are stratified by tissue type and provided for the annual infectivity remaining in processed tissues.

10.4.1. Infectivity over time in bovine intestines and mesenteries from an infected animal by age at slaughter

The estimation of the infectivity over time in bovine intestines and mesenteries from an infected animal by age at slaughter is based on the abattoir, SRM, infectivity and processing components (as shown in Figure 14).

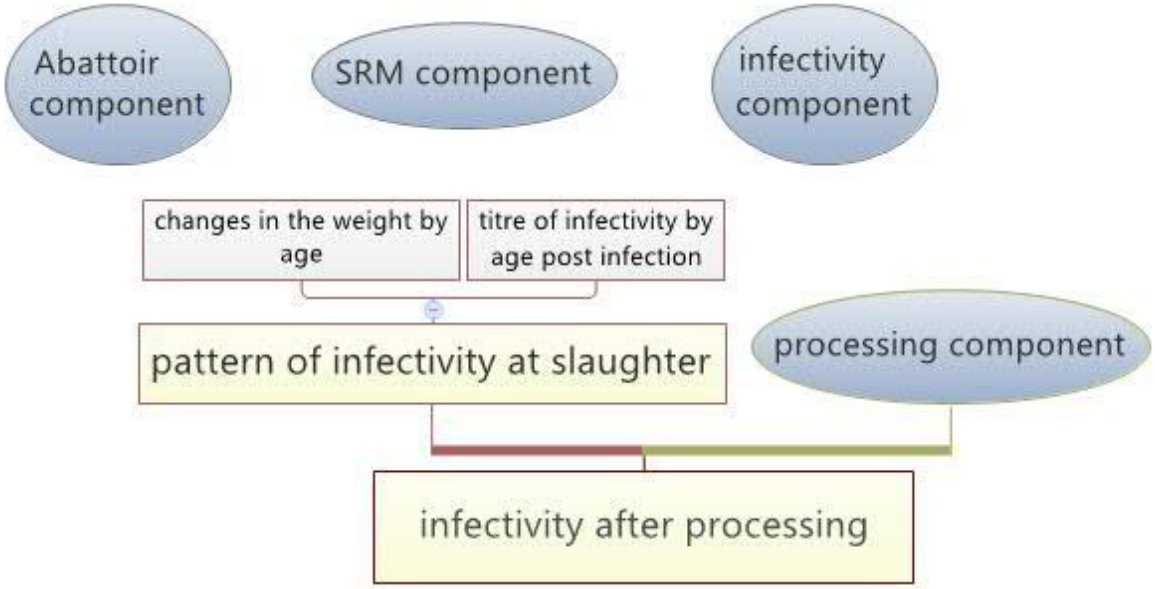

Figure 14: Schematic overview of the components of the model used to obtain the estimation of the infectivity over time in bovine intestines and mesenteries from an infected animal by age at slaughter.

The level of BSE infectivity by age at slaughter for each tissue type included in the risk assessment is shown in Figure 15 based on estimated mean values. It can be seen that several distinctive patterns of infectivity are evident. These patterns are the result of changes in the weight of infectious tissue by age combined with the titre of infectivity varying by age post infection. The infectivity in jejunum dominates, with ileum and caecum contributing significantly at the early stages of disease. The infectivity in jejunum, ileum and caecum is estimated to peak before 18 months, with an estimated high of approximately 15 $BoID_{50}$ per animal and decline to low levels, less than one $BoID_{50}$ by 60 months. After this point there is a tailing of infectivity to very low levels where mesenteric nerves, CMGC and jejunum contribute the most to the low total.

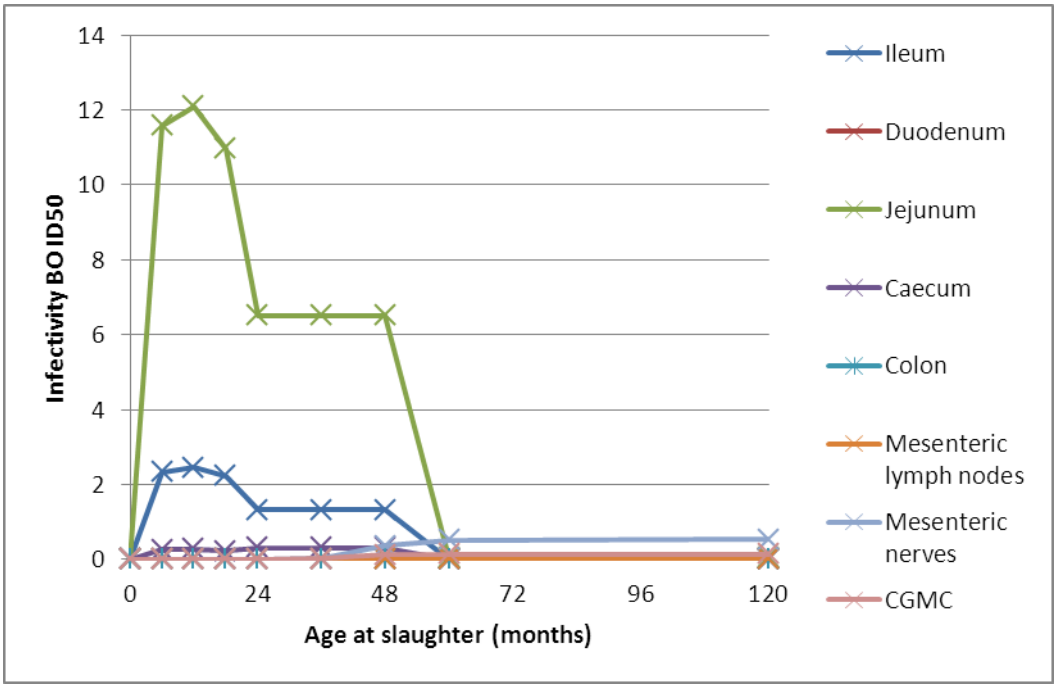

Figure 15: Summary graph of estimated mean infectivity by intestine and mesentery tissue type ($BoID_{50}$ per infected animal) by age at slaughter

All the results generated are associated with uncertainty and variability. Appendix B. provides infectivity distributions for each by tissue type at slaughter with 95% uncertainty and variability range.

Table 17 displays the mean percentage contribution made to the total infectivity load per infected animal by each of the tissue types. It can be seen that the jejunum, ileum, and caecum contribute for younger infected animals aged less than 36 months. Despite the mean values seem suggesting that the above mentioned tissues contribute significantly to the overall infectivity also for animals at 48 months of age, it is important to highlight that there is a substantial inter-individual variability in the relative contribution of intestinal and mesenteric structures to the total infectivity. Mesenteric nerves dominate the load in older animals (from 60 months of age), with CMGC contributing significantly but to a low total infectivity. Duodenum, colon and mesenteric lymph nodes contribute less than 0.1% regardless of the age at slaughter viewed. The estimated mean amount of infectivity for two subsets of tissues: the total ileocaecal plate (ileocaecal plate in the ileum and the jejunum) and the ileocaecal plate that is confined to the jejunum, are also provided.

Table 17: Mean percentage contribution by each tissue type to total infectivity per infected animal by age at slaughter

	Age at slaughter (months)							
	6	**12**	**18**	**24**	**36**	**48**	**60**	**120**
Tissue type								
Ileum	16%	17%	17%	16%	16%	15%	1%	1%
Duodenum	0%	0%	0%	0%	0%	0%	0%	0%
Jejunum	82%	82%	82%	80%	80%	76%	6%	6%
Caecum	2%	2%	2%	4%	4%	4%	0%	0%
Colon	0%	0%	0%	0%	0%	0%	0%	0%
Mesenteric lymph nodes	0%	0%	0%	0%	0%	0%	0%	0%
Mesenteric nerves	0%	0%	0%	0%	0%	4%	74%	74%
CMGC	0%	0%	0%	0%	0%	1%	19%	19%
Total ($BoID_{50}$)	**14.16**	**14.81**	**13.41**	**8.15**	**8.17**	**8.62**	**0.70**	**0.72**
Total ileocaecal plate ($BoID_{50}$)	13.91	14.54	13.18	7.83	7.83	7.83	0.05	0.05
Jejunal ileocaecal plate ($BoID_{50}$)	11.58	12.09	10.96	6.51	6.51	6.51	0.04	0.04

10.4.1.1. Pattern of infectivity in intestines by length ($BoID_{50}$/m)

For each intestinal tissue type (ileum, duodenum, jejunum, colon and caecum), the estimated mean, 2.5th and 97.5th percentiles of infectivity per metre length by age at slaughter is provided in Table 18. It can be seen that the ileum has the highest amount of infectivity per metre with a mean estimate of 4.65 $BoID_{50}$/m at age 6 months. This reduces to less than one $BoID_{50}$/m by age 60 months. The caecum and jejunum also contain significant infectivity per metre, which peaks at 6 months with a mean of 0.5 and 0.4 $BoID_{50}$/m respectively. The duodenum and colon exhibit much lower infectivity per metre, in the order of approximately 4 x 10^{-5} ID_{50}/m. The ileocaecal plate running through the ileum and jejunum is estimated to have the highest infectivity per length in the intestines with a peak of 5.8 $BoID_{50}$/m between 24 and 48 months, before declining to approximately 0.03 $BoID_{50}$/m.

Table 18: Mean infectivity per length ($BoID_{50}$/m) for intestinal tissues by age at slaughter (2.5th and 97.5th percentiles in brackets)

	Infectivity per length ($BoID_{50}$/m)					
	Ileum	**Duodenum**	**Jejunum**	**Caecum**	**Colon**	**Ileocaecal plate***
Age at slaughter (months)						
6	4.6 ($1x10^{-2}$, 31)	$3.4x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	$3.9x10^{-1}$ ($8x10^{-4}$, 3)	$5.1x10^{-1}$ ($1x10^{-3}$, 4)	$3.3x10^{-5}$ ($4x10^{-8,}$ $2x10^{-4}$)	4.7 ($1x10^{-2}$, 31)
12	3.9 ($8x10^{-3}$, 28)	$4.0x10^{-5}$ ($5x10^{-8}$, $3x10^{-4}$)	$3.8x10^{-1}$ ($8x10^{-4}$, 3)	$4.8x10^{-1}$ ($1x10^{-3}$, 3)	$3.9x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	5.0 ($1x10^{-2}$, 34)
18	3.0 ($6x10^{-3}$, 21)	$5.5x10^{-5}$ ($6x10^{-8}$, $4x10^{-4}$)	$3.2x10^{-1}$ ($7x10^{-4}$, 2)	$3.6x10^{-1}$ ($1x10^{-3}$, 3)	$5.4x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	4.2 ($9x10^{-3}$, 28)
24	1.6 ($3x10^{-3}$, 11)	$4.7x10^{-5}$ ($5x10^{-8}$, $3x10^{-4}$)	$1.7x10^{-1}$ ($3x10^{-4}$, 1)	$4.6x10^{-1}$ ($1x10^{-3}$, 4)	$4.6x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	5.8 ($1x10^{-2}$, 39)
36	1.4 ($2x10^{-3}$, 9)	$4.4x10^{-5}$ ($5x10^{-8}$, $3x10^{-4}$)	$1.5x10^{-1}$ ($3x10^{-4}$, 1)	$4.2x10^{-1}$ ($1x10^{-3}$, 4)	$4.0x10^{-5}$ ($4x10^{-8}$, $3x10^{-4}$)	5.8 ($1x10^{-2}$, 39)
48	1.4 ($2x10^{-3}$, 9)	$4.4x10^{-5}$ ($5x10^{-8}$, $3x10^{-4}$)	$1.5x10^{-1}$ ($3x10^{-4}$, 1)	$4.2x10^{-1}$ ($1x10^{-3}$, 4)	$4.0x10^{-5}$ ($4x10^{-8}$, $3x10^{-4}$)	5.8 ($1x10^{-2}$, 39)
60	$7.9x10^{-3}$ ($1x10^{-5}$, $6x10^{-2}$)	$3.4x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	$8.9x10^{-4}$ ($1x10^{-6}$, $6x10^{-3}$)	$2.3x10^{-3}$ ($5x10^{-6}$, $3x10^{-2}$)	$3.3x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	$3.2x10^{-2}$ ($6x10^{-5}$, 0.2)
120	$8.1x10^{-3}$ ($1x10^{-5}$, $6x10^{-2}$)	$2.4x10^{-5}$ ($3x10^{-8}$, $2x10^{-4}$)	$9.0x10^{-4}$ ($1x10^{-6}$, $6x10^{-3}$)	$2.4x10^{-3}$ ($6x10^{-6}$, $3x10^{-2}$)	$2.3x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	$3.3x10^{-2}$ ($7x10^{-5}$, 0.2)

* Results for jejunal ileocaecal plate are the same as those estimated for the ileocaecal plate

10.4.1.2. Pattern of infectivity for processed products, infectivity load ($BoID_{50}$) per infected animal

Results have also been provided for the infectivity of processed products (duodenum, jejunum, colon, caecum and mesenteric tissues) as shown in Figure 16. Reductions in infectivity have been applied to the duodenum, jejunum, caecum, and colon post processing equating to an average 25% reduction in infectivity by tissue type and in total. This results in the largest reduction of approximately 3 $BoID_{50}$ per infected animal slaughtered before 18 months and processed, with animals aged greater than 120 months estimated to reduce any infectivity present by a mean of 0.01 $BoID_{50}$ by processing. Table 19 presents the mean infectivity of processed tissues by age at slaughter.

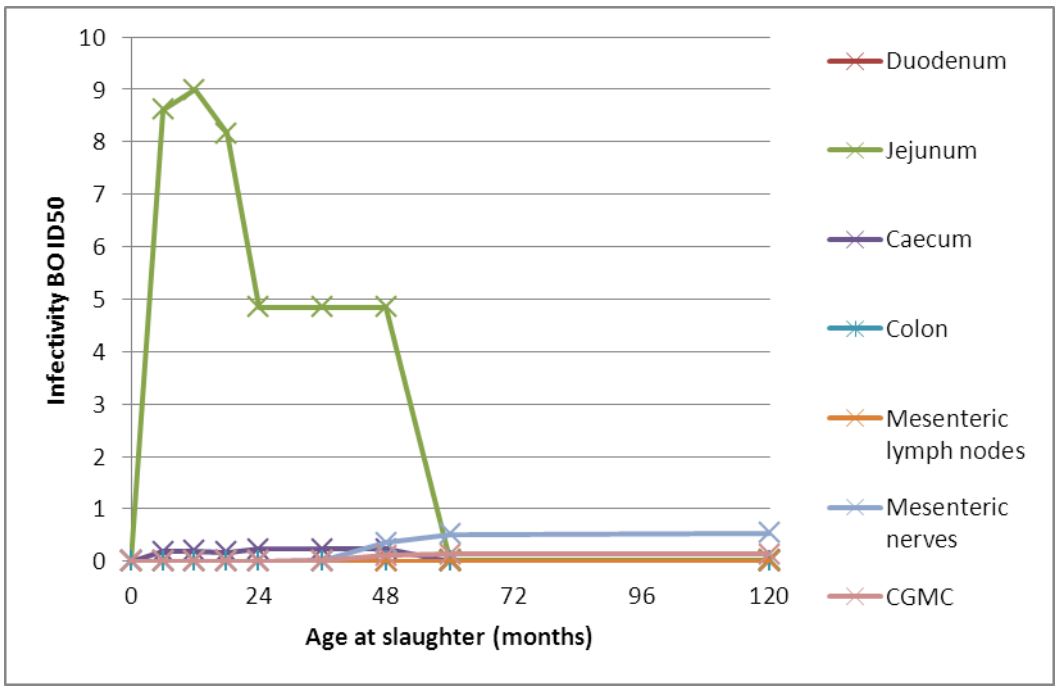

Figure 16: Summary graph of estimated mean infectivity of processed products ($BoID_{50}$ per infected animal) by age at slaughter

Table 19: Mean percentage contribution by processed tissues to total infectivity per infected animal by age at slaughter

	Age at slaughter (months)							
	6	**12**	**18**	**24**	**36**	**48**	**60**	**120**
Processed tissue type								
Duodenum	0%	0%	0%	0%	0%	0%	0%	0%
Jejunum	98%	98%	98%	95%	95%	87%	4%	4%
Caecum	2%	2%	2%	5%	5%	4%	0%	0%
Colon	0%	0%	0%	0%	0%	0%	0%	0%
Mesenteric lymph nodes	0%	0%	0%	0%	0%	0%	0%	0%
Mesenteric nerves	0%	0%	0%	0%	0%	6%	76%	76%
CGMC	0%	0%	0%	0%	0%	2%	19%	19%
Total ($BoID_{50}$)	**8.83**	**9.22**	**8.35**	**5.14**	**5.16**	**5.59**	**0.68**	**0.70**
Jejunal ileocaecal plate ($BoID_{50}$)	8.64	9.02	8.18	4.91	4.90	4.89	0.03	0.03

* Ileum and total ileocaecal plate are classed as tissues not processed for consumption and therefore not included in the analysis

All the results generated are associated with uncertainty and variability. Appendix B. provides infectivity distributions for each by tissue type at slaughter with 95% uncertainty and variability range.

10.4.1.3. Pattern of infectivity in processed intestines by length ($BoID_{50}$/m)

For processed intestinal tissue types (duodenum, jejunum, colon, caecum and jejunal ileocaecal plate), the estimated mean, 2.5th and 97.5th percentiles of infectivity per metre length by age at slaughter after processing is provided in Table 20. Comparing these results from those before processing in Table 18, it can be seen that the mean results reflect, on average a 25% reduction in infectivity per metre.

Table 20: Mean infectivity per length ($BoID_{50}$/m) for processed tissues by age at slaughter (2.5th and 97.5th percentiles in brackets)

	Infectivity per length ($BoID_{50}$/m)*				
	Duodenum	**Jejunum**	**Caecum**	**Colon**	**Jejunal ileocaecal plate**
Age at slaughter (months)					
6	$2.5x10^{-5}$ ($3x10^{-8}$, $2x10^{-4}$)	$2.9x10^{-1}$ ($6.x10^{-4}$, 2)	$3.8x10^{-1}$ ($8x10^{-4}$, 3)	$2.5x10^{-5}$ ($3x10^{-8}$, $2x10^{-4}$)	3.5 ($7x10^{-3}$, 24)
12	$3.0x10^{-5}$ ($3x10^{-8}$, $2x10^{-4}$)	$2.8x10^{-1}$ ($6x10^{-4}$, 2)	$3.6x10^{-1}$ ($7x10^{-4}$, 3)	$3.0x10^{-5}$ ($3x10^{-8}$, $2x10^{-4}$)	3.7 ($8x10^{-3}$, 25)
18	$4.1x10^{-5}$ ($5x10^{-8}$, $3x10^{-4}$)	$2.4x10^{-1}$ ($5x10^{-4}$, 2)	$2.7x10^{-1}$ ($6x10^{-4}$, 2)	$4.1x10^{-5}$ ($5x10^{-8}$, $3x10^{-4}$)	3.1 ($7x10^{-3}$, 21)
24	$3.5x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	$1.3x10^{-1}$ ($2x10^{-4}$, $9x10^{-1}$)	$3.4x10^{-1}$ ($7x10^{-4}$, 2)	$3.5x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	4.3 ($9x10^{-3}$, 29)
36	$3.3x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	$1.1x10^{-1}$ ($2x10^{-4}$, $8x10^{-1}$)	$3.1x10^{-1}$ ($6x10^{-4}$, 2)	$3.3x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	4.3 ($9x10^{-3}$, 30)
48	$3.3x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	$1.1x10^{-1}$ ($2x10^{-4}$, $8x10^{-1}$)	$3.2x10^{-1}$ ($6x10^{-4}$, 2)	$3.3x10^{-5}$ ($4x10^{-8}$, $2x10^{-4}$)	4.3 ($9x10^{-3}$, 30)
60	$2.5x10^{-5}$ ($3x10^{-8}$, $2x10^{-4}$)	$6.7x10^{-4}$ ($1x10^{-6}$, $5x10^{-3}$)	$2.3x10^{-3}$ ($2x10^{-6}$, $2x10^{-2}$)	$2.5x10^{-5}$ ($3x10^{-8}$, $2x10^{-4}$)	$2.4x10^{-2}$ ($4x10^{-5}$, 0.2)
120	$1.8x10^{-5}$ ($2x10^{-8}$, $1x10^{-4}$)	$6.7x10^{-4}$ ($1x10^{-6}$, $5x10^{-3}$)	$2.3x10^{-3}$ ($3x10^{-6}$, $2x10^{-2}$)	$1.8x10^{-5}$ ($2x10^{-8}$, $1x10^{-4}$)	$2.4x10^{-2}$ ($5x10^{-5}$, 0.2)

* Ileum and total ileocaecal plate are classed as tissues not processed for consumption and therefore not included in the analysis

10.4.1.4. Conclusions

- The total amount of infectivity associated with intestine and mesentery in one infected animal dramatically evolves with the age of the animal:
  - it peaks to an average value of 15 $BoID_{50}$ in animals younger than 18 months of age;
  - it decreases to around 8 $BoID_{50}$ between the age of 24 and 48 months; and
  - it finally decreases to 0.7 $BoID_{50}$ in animals older than 60 months of age.
- The amount of infectivity in a BSE infected animal associated with the different segments of intestine and with mesentery also evolves with the age of the animal:
  - the ileocaecal plate (distal part of jejunum plus ileum) and the caecum are on average the main contributors (more than 90%) to the overall amount of infectivity in animals up to 36 months of age;
  - in the age category of animals older than 36 months and younger than 60 there is a substantial inter-individual variability in the relative contribution of intestinal and mesenteric structures to the total infectivity;
  - the mesenteric tissue is on average the main contributor (more than 90%) to the overall amount of infectivity in animals from 60 months of age;
  - duodenum, colon and mesenteric lymph nodes contribute less than 0.1% to the total infectivity in an infected animal regardless of the age at slaughter.

10.4.2. Infectivity in bovine intestines and mesenteries per year in EU27

The previous section has focused on the infectivity associated with a single animal. The results in this section are the total amount of infectivity resulting from the slaughter of infected animals in the healthy and emergency slaughter streams in the baseline year (2012) that are not detected, and is the summed result of each infected animal expected (Figure 17).

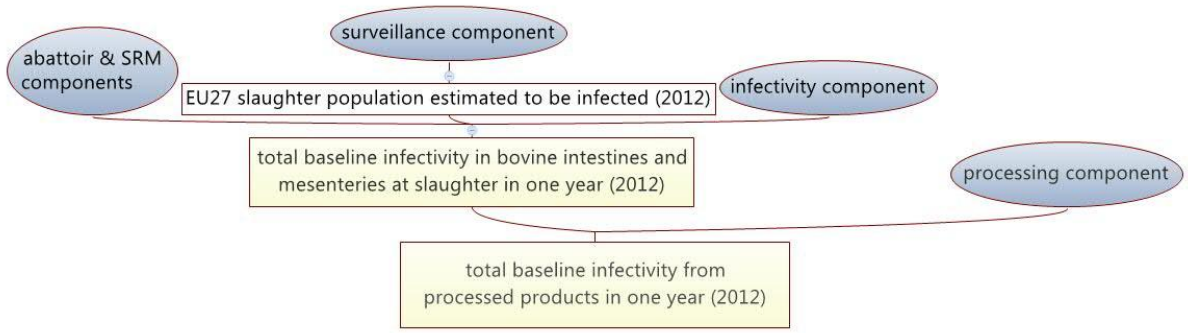

Figure 17: Schematic overview of the components of the model used to obtain the estimation of the total infectivity associated with intestine and mesentery of undetected infected cattle from the healthy and emergency slaughter streams in 2012.

The number of infected animals in the healthy and emergency slaughter streams in one year that are not detected has been estimated by a previous EFSA contracted model, C-TSEMM (Adkin et al., 2012). The estimate includes all strain types of BSE (classical, H type, L type and unclassified strains). A mean of 613 infected animals are estimated to be slaughtered in the EU27 in the baseline year 2012, ranging from 566 to 661 at the 2.5th and 97.5th percentiles representing uncertainty and variability, assuming an exponential decline in the prevalence of BSE.

Using the estimated number of infected animals, an estimated mean amount of infectivity of 1,985 $BoID_{50}$/year is present within bovine intestines and mesenteries in infected animals at slaughter, with 2.5th and 97.5th percentiles this varies between 1,170 and 4,557 $BoID_{50}$/year accounting for uncertainty and variability as shown in Figure 18. The distributions by tissue type before and after processing are provided in Appendix B.

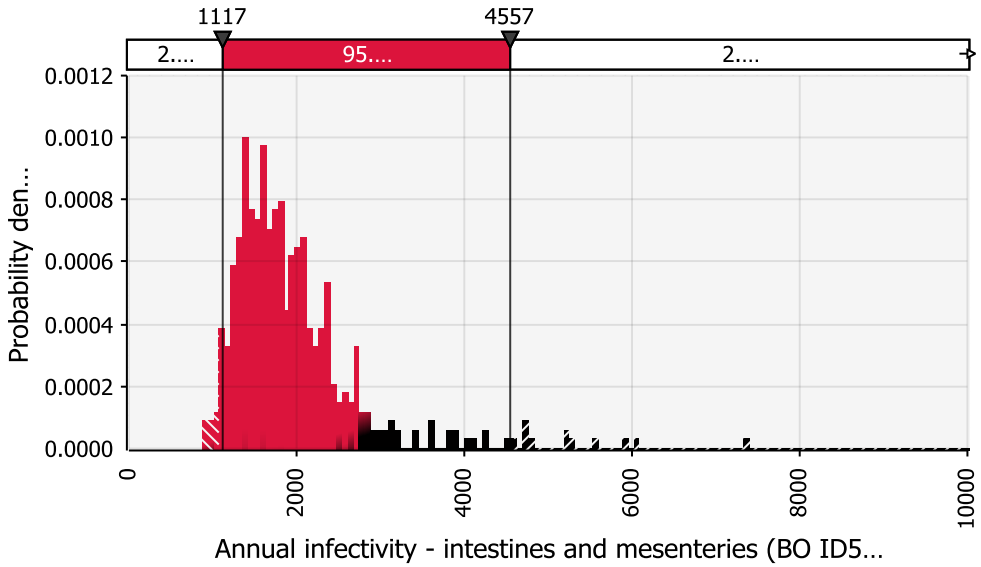

Figure 18: Probability density function describing the total baseline infectivity in bovine intestines and mesenteries in the slaughter stream for the EU27 in one year ($BoID_{50}$/year) considering uncertainty and variability (95% percentiles indicated by top bar)

The contribution by tissue type to the annual total is shown in Table 21 with the estimated mean and 2.5th and 97.5th percentiles. For slaughtered animals it can be seen that jejunum tissues dominate the infectivity estimate, with ileum and mesenteric nerves making a smaller but significant contribution.

Table 21: Baseline mean total infectivity, contribution by tissue type and total length per year for intestinal tissues at slaughter from infected animals in the EU27 (2.5th and 97.5th percentiles in brackets).

	Infectivity per year ($BoID_{50}$/yr) Mean (2.5th and 97.5th)	**Mean % contribution to infectivity**	**Total length per year (m/yr) Mean (2.5th and 97.5th)**	**Infectivity per length ($BoID_{50}$/m) before processing Mean (2.5th and 97.5th)**
Tissue type				
Ileum	271 (130, 687)	14%	595 (548, 642)	0.55 (8 x 10^{-6}, 4)
Duodenum	0.02 (0.01, 0.04)	0%	726 (668, 782)	3.1 x 10^{-5} (3 x 10^{-8}, 2 x 10^{-4})
Jejunum	1,350 (644, 3484)	68%	27,370 (25141, 29474)	5.7 x 10^{-2} (1 x 10^{-6}, 0.4)
Caecum	50 (24, 116)	3%	451 (415, 486)	0.12 (3 x 10^{-6}, 0.8)
Colon	0.2 (0.12, 0.4)	0%	6,534 (6002, 7044)	3.1 x 10^{-5} (3 x 10^{-8}, 2 x 10^{-4})
Mesenteric lymph nodes	3 x 10^{-5} (2.6 x 10^{-5}, 3.3 x 10^{-5})	0%	NA	NA
Mesenteric nerves	249 (218, 284)	13%	NA	NA
CMGC	64 (57, 73)	3%	NA	NA
Total per year	**1,985 (1117, 4557)**			
Total ileocaecal plate	1,620 (767, 4116)	[82%]	968 (883, 1056)	1.5 (4 x 10^{-5} , 10)
Jejunal ileocaecal plate	1,349 (643, 3482)	[68%]	805 (736, 878)	1.5 (4 x 10^{-5} , 10)

10.4.2.1. Pattern of infectivity for processed products, infectivity load ($BoID_{50}$) per year in EU27

Figure 19 displays the estimated distribution of infectivity from processed products (duodenum, jejunum, colon, caecum and mesenteric tissues) per year in the EU27. An estimated mean amount of infectivity of 1,362 $BoID_{50}$ arises from processed products per year in the EU27, with 2.5th and 97.5th percentiles this varies between 796 and 2,791 $BoID_{50}$/animal accounting for uncertainty and variability. This represents a reduction in the amount of infectivity of, on average, of 352 $BoID_{50}$ per year, approximately 21% of the unprocessed total (excluding ileum tissues).

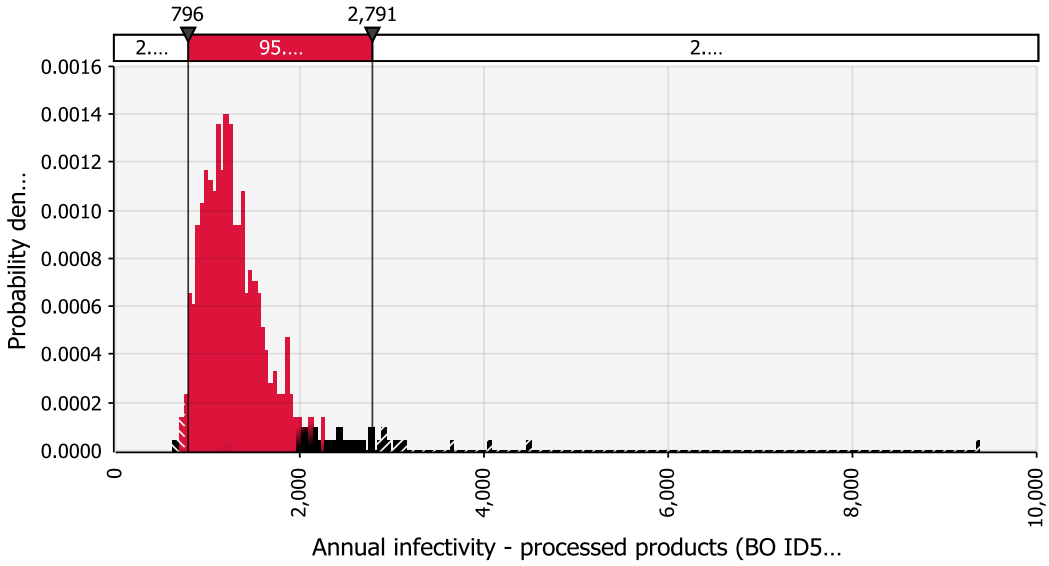

Figure 19: Probability density function describing the total infectivity in processed tissues (duodenum, jejunum, caecum, colon and mesenteries) for the EU27 ($BoID_{50}$/year) considering uncertainty and variability (95% percentiles indicated by top bar)

The contribution by tissue type after processing to this yearly total is shown in Table 22 with the estimated mean and 2.5th and 97.5th percentiles. It can be seen that the largest contributor is jejunum (about 75%) with mesenteric nerves making a significant contribution (about 18%).

Table 22: Mean total infectivity per year for processed products in the EU27 (2.5^{th} and 97.5^{th} percentiles in brackets) and percentage contribution

	Infectivity post processing* ($BoID_{50}$) Mean (2.5^{th} and 97.5^{th})	**Mean % contribution**	**Infectivity per length ($BoID_{50}$/m) after processing* Mean (2.5^{th} and 97.5^{th})**
Tissue type			
Ileum	NA	NA	NA
Duodenum	0.02 (0.01, 0.03)	0%	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})
Jejunum	1006 (487, 2455)	74.2%	4.2×10^{-2} (7×10^{-7}, 0.3)
Caecum	37 (18, 85)	2.7%	0.1 (2×10^{-6}, 0.6)
Colon	0.15 (0.09, 0.3)	0%	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})
Mesenteric lymph nodes	3×10^{-5} (2.6×10^{-5}, 3.3×10^{-5})	0%	NA
Mesentery nerves	249 (218, 284)	18.4%	NA
CMGC	64 (57, 73)	4.7%	NA
Total per year	**1,362 (796, 2791)**		
Total ileocaecal plate	NA	NA	NA
Jejunum ileocaecal plate	1005 (486, 2454)	[74.1%]	1.1 (3×10^{-5} , 8)

* Ileum and total ileocaecal plate are classed as tissues not processed for consumption and therefore not included in the analysis

10.4.2.2. Infectivity in bovine intestines and mesenteries from an infected animal drawn from EU27 slaughter population

In section 10.4.1, the infectivity per infected animal was sampled by each age at slaughter to demonstrate the estimated pattern of infectivity. Here the age at slaughter of the infected animal is drawn at random from the EU27 slaughter population estimated to be infected in the baseline year of 2012.

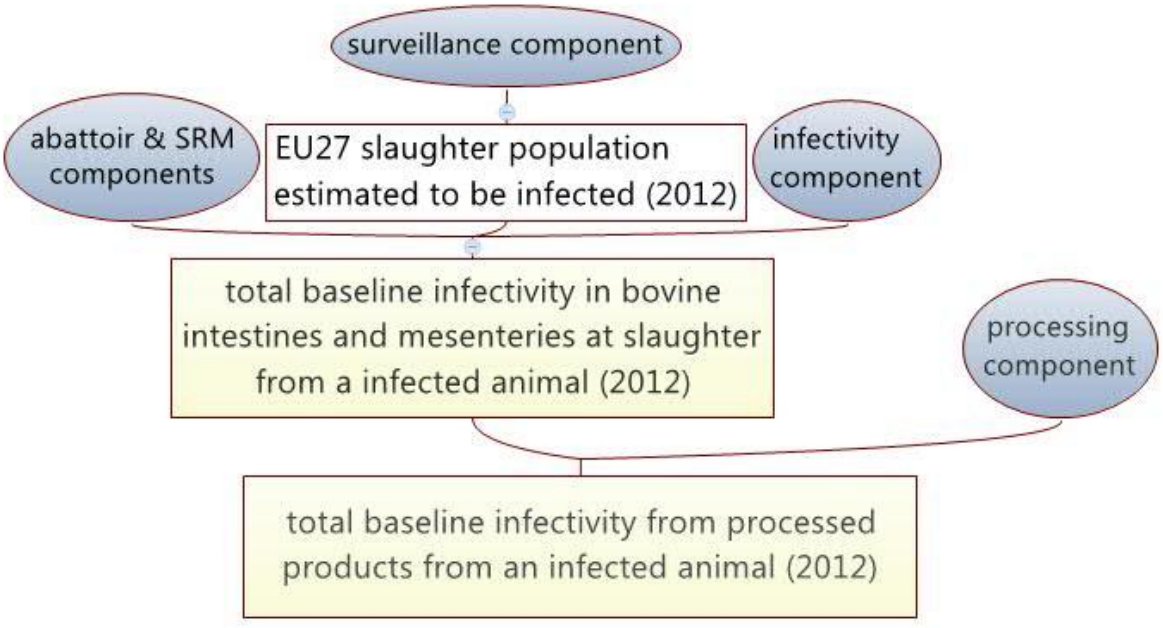

Figure 20: Schematic overview of the components of the model used to obtain the estimation of the infectivity associated with intestine and mesentery of an infected animal drawn at random from the EU27 slaughter population in 2012

Therefore, the mean infectivity estimated by tissue type reflects the average infected animal destined for the food and feed chain in 2012. Figure 21 displays the estimated distribution of total baseline infectivity from intestines and mesenteries per infected animal. An estimated mean amount of infectivity of 3 $BoID_{50}$ arises from bovine intestines and mesenteries in a single animal, with 2.5th and 97.5th percentiles this varies between 0.02 and 18 $BoID_{50}$/animal accounting for uncertainty and variability. The distributions by tissue type before any processing are provided in Appendix B.

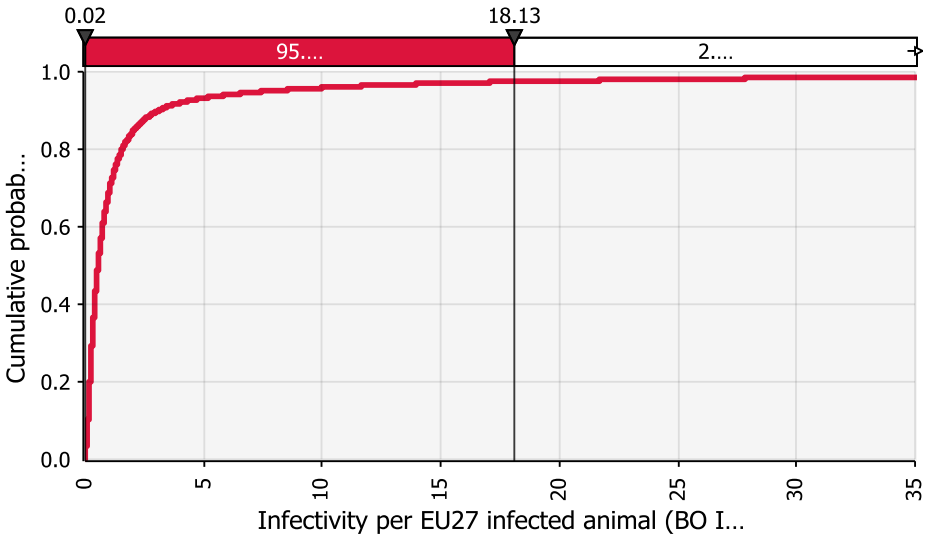

Figure 21: Cumulative probability function describing the total baseline amount of infectivity in bovine intestines and mesenteries at slaughter ($BoID_{50}$) for an EU27 infected animal considering uncertainty and variability (95% percentiles indicated by top bar).

It can be seen that the mean total infectivity at slaughter (3 $BoID_{50}$/animal) for the average EU27 infected animal equates in Table 17 (also shown in Figure 15) to the total infectivity from animals slaughtered above 48 months of age. The estimated number of infected animals in the EU27 by age slaughtered peaks between 72 and 120 months. There are more infected animals in these age intervals as a result of the multiplication of cohort prevalence and the number infected from those cohorts surviving and subsequently being slaughtered.

The estimated mean results for each tissue type before and after any processing and contribution to the total infectivity per animal are shown in Table 23. The estimated amount of infectivity for two subsets of tissues: the total ileocaecal plate (ileocaecal plate in the ileum and the jejunum) and the ileocaecal plate that is confined to the jejunum, are also provided. It can be seen from Table 23 that jejunum, ileum and mesenteric nerves contribute significantly to the total of an average EU27 infected slaughtered animal.

Table 23: Baseline mean infectivity ($BoID_{50}$) by tissue type in an infected EU27 slaughter animal and post processing (2.5^{th} and 97.5^{th} percentiles in brackets).

	Infectivity ($BoID_{50}$) Mean (2.5^{th} and 97.5^{th})	**Mean % contribution**	**Infectivity post processing* ($BoID_{50}$) Mean (2.5^{th} and 97.5^{th})**
Tissue type			
Ileum	0.44 ($8x10^{-6}$, 3)	14%	NA
Duodenum	3.6 x 10^{-5} ($4x10^{-8}$, $2x10^{-4}$)	0%	2.7 x 10^{-5} ($3x10^{-8}$, $2x10^{-4}$)
Jejunum	2.2 ($4x10^{-5}$, 14)	68%	1.6 ($3x10^{-5}$, 11)
Caecum	8.1 x 10^{-2} ($2x10^{-6}$, 0.6)	3%	6.1 x 10^{-2} ($2x10^{-6}$, 0.4)
Colon	3.2 x 10^{-4} ($3x10^{-7}$, $2x10^{-3}$)	0%	2.4 x 10^{-4} ($2x10^{-7}$, $2x10^{-3}$)
Mesenteric lymph nodes	4.8 $x10^{-8}$ ($1x10^{-9}$, $2x10^{-7}$)	0%	4.8 $x10^{-8}$ ($1x10^{-9}$, $2x10^{-7}$)
Mesentery nerves	0.4 ($1x10^{-9}$, 2)	13%	0.4 ($1x10^{-9}$, 2)
CMGC	0.1 ($2x10^{-9}$, 0.5)	3%	0.1 ($2x10^{-9}$, 0.5)
Total	**3.2 (0.02, 18)**		**2.2 (0.01, 11)**
Total ileocaecal plate	2.6 ($5x10^{-5}$, 17)	[82%]	NA
Jejunum ileocaecal plate	2.2 ($4x10^{-5}$, 14)	[68%]	1.6 ($3x10^{-5}$, 11)

* Ileum and total ileocaecal plate are classed as tissues not processed for consumption and therefore not included in the analysis

10.4.2.3. Pattern of infectivity in intestines by length ($BoID_{50}$/m) from an infected animal drawn from EU27 slaughtered population

For each intestinal tissue (ileum, duodenum, jejunum, colon and caecum) and the subset of the ileocaecal plate (ileocaecal plate in ileum and jejunum) and the ileocaecal plate that is confined to the jejunum, the estimated mean, 2.5^{th} and 97.5^{th} percentiles of infectivity per metre length for an average EU27 infected slaughter animal is provided in Table 24. It can be seen that the infectivity per length is most similar to those animals with a slaughter age older than 48 months.

Table 24: Baseline mean infectivity per length ($BoID_{50}$/m) for intestinal tissues within an infected EU27 slaughter animal (2.5^{th} and 97.5^{th} percentiles in brackets).

	Infectivity unprocessed per length ($BoID_{50}$/m) Mean (2.5^{th} and 97.5^{th})
Tissue type	
Ileum	0.55 (8×10^{-6}, 4)
Duodenum	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})
Jejunum	5.7×10^{-2} (1×10^{-6}, 0.4)
Caecum	0.12 (3×10^{-6}, 0.8)
Colon	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})
Total ileocaecal plate	1.5 (4×10^{-5} , 10)
Jejunal ileocaecal plate	1.5 (4×10^{-5} , 10)

10.4.2.4. Pattern of infectivity for processed products, infectivity load ($BoID_{50}$) from an infected animal drawn from EU27 slaughtered population

Figure 22 displays the estimated distribution of infectivity from processed products (duodenum, jejunum, colon, caecum and mesentery tissues) from an infected animal from the EU27. An estimated mean amount of infectivity of 2.2 $BoID_{50}$ arises from processed products from a single animal, with 2.5^{th} and 97.5^{th} percentiles this varies between 0.01 and 11 $BoID_{50}$/animal accounting for uncertainty and variability. This equates to an overall reduction in total infectivity of 20% after processing. The distributions by tissue type before and after processing are provided in Appendix B.

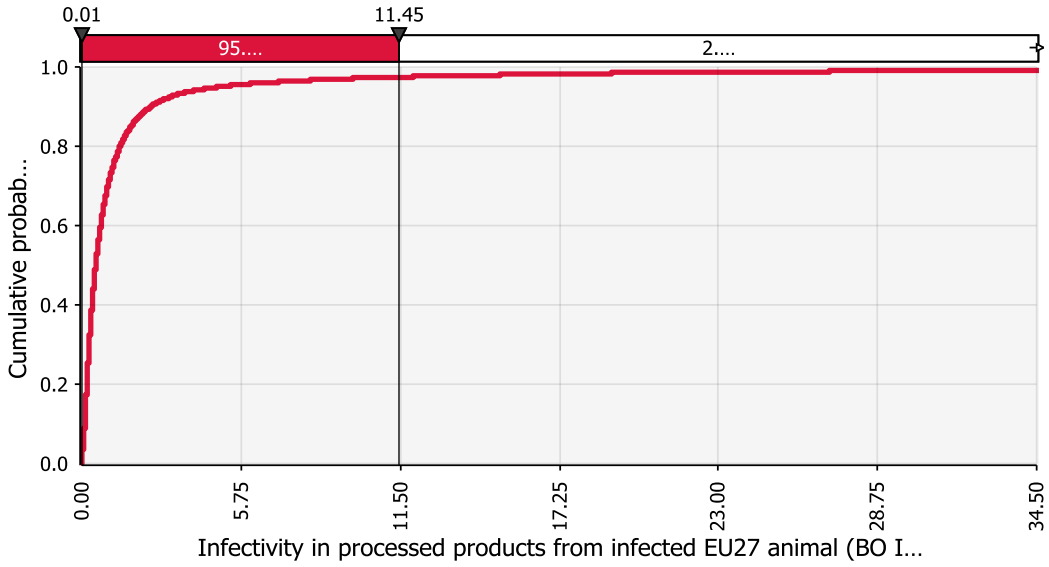

Figure 22: Cumulative probability function describing the total baseline amount of infectivity from processed products (duodenum, jejunum, colon, caecum and mesentery tissues) from an infected animal from the EU27 considering uncertainty and variability (95% percentiles indicated by top bar).

10.4.2.5. Pattern of infectivity in processed intestines by length ($BoID_{50}$/m) from an infected animal drawn from EU27 slaughtered population

For each intestinal tissue (ileum, duodenum, jejunum, colon and caecum) and the subset of the ileocaecal plate (ileocaecal plate in ileum and jejunum) and the ileocaecal plate that is confined to the

jejunum, the estimated mean, 2.5th and 97.5th percentiles of infectivity per metre length for processed products from an average EU27 infected slaughter animal is provided in Table 25. Also in this case, it can be seen that the infectivity per length is most similar to those animals with a slaughter age older than 48 months.

Table 25: Baseline mean infectivity per length ($BoID_{50}$/m) for intestinal tissues within an infected EU27 slaughter animal (2.5th and 97.5th percentiles in brackets).

	Infectivity post processing* per length ($BoID_{50}$/m) Mean (2.5th and 97.5th)
Tissue type	
Ileum	NA
Duodenum	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})
Jejunum	4.2×10^{-2} (7×10^{-7}, 0.3)
Caecum	0.1 (2×10^{-6}, 0.6)
Colon	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})
Total ileocaecal plate	NA
Jejunal ileocaecal plate	1.1 (3×10^{-5} , 8)

* Ileum and total ileocaecal plate are classed as tissues not processed for consumption and therefore not included in the analysis

10.4.2.6. Conclusions

According to TSEi model in EU27 in 2012:

- A mean of 613 BSE infected and undetected cattle (CI95% 566 – 661) entered into the food and feed chain.
- The total amount of BSE infectivity associated with intestine and mesentery from these animals was 1,985 $BoID_{50}$ (CI95% 1,170 – 4,557).
- Intestine accounted for about 1,670 $BoID_{50}$ (about 85% of the total amount of infectivity) and a total of about 35,000 metres of intestine were sourced from infected animals.
- Mesentery accounted for about 310 $BoID_{50}$ (about 15% of the total amount of infectivity).
- If intestine from those BSE infected animals had entered the food and feed chain it would have been consumed by a small number of individuals (since it would have been distributed in a small number of serving portions). The average infectivity per metre associated with intestine ranged between 3.1×10^{-5} and 1.5 $BoID_{50}$ according to the type of intestine considered. However, this value could have reached 10 $BoID_{50}$ per metre in the most infectious part of the intestine of certain individuals.
- The processing of intestine would have reduced the infectivity present in the material by about 21%.
- The removal of ileum[29] (as a SRM measure) would have reduced the total amount of infectivity in the whole intestinal and mesenteric unprocessed material by about 14%.

[29] As defined under section 5.1.1 for the purpose of this document the ileum is defined as the part of small intestine contained within the *Plica ileocaecais* since this is the operational identification of ileum used by industry for casing production.

- The removal of the last 4 metres of small intestine (i.e. the ileocaecal plate) and the caecum would have reduced the amount of infectivity in the whole intestinal and mesenteric unprocessed material by about 85%.

10.4.3. Case study on estimated historical levels of infectivity

As described in section 10.2.1, a mathematical model (C-TSEMM) has been developed to estimate the trend in prevalence of BSE in European Member States (MSs). This model estimates the number of BSE infected cattle slaughtered in a year and can be used to estimate the retrospective total number of infected animals in the healthy and emergency slaughter stream from 2007 to 2011 by age at slaughter.

C-TSEMM requires annual historical information on the numbers of cattle slaughtered each year from 2007 to 2011. This information for the EU27 is available retrospectively for those animals > 30 months for years; however, there are no data for 2007 for the age intervals 0-23 months and 24-29 months. It is assumed, at the EU27 level, that the average number slaughtered for these age intervals between 2008 and 2012 can be used in 2007.

During 2007 to 2011 some infected animals slaughtered in the healthy slaughter and emergency slaughter streams were found test positive by active surveillance. The actual number of test positive animals by age interval at slaughter has been subtracted from the number infected thereby estimating the number of animals infected and missed.

In cases where there is an estimated low prevalence of BSE in certain year/age at slaughter combinations (less than one infected animal), and there is an occurrence of a test positive animal from surveillance, the estimated number of infected animals is set to 0 (rather than a negative figure). A summary of the estimated number of infected animals missed in the EU27 (estimated number infected minus test positive cases) for each year is shown in Table 26. It can be seen that the number of infected animals missed in the EU27 steadily decreases to the baseline year.

Table 26: Estimated total number of infected animals in the healthy slaughter and emergency slaughter streams from 2007 to 2011, and the baseline year 2012, minus the number of test positive animals (output from C-TSEMM 28/11/13).

	Infected cattle missed (EU27) in HS and ES Mean (2.5^{th} and 97.5^{th})
Year	
2007	6816 (6438, 7214)
2008	4382 (4253, 4511)
2009	2533 (2436, 2630)
2010	1665 (1586, 1744)
2011	1031 (970, 1093)
2012	613 (566, 661)

Given the estimated number of EU27 infected animals, the estimated total amount of infectivity within bovine intestines and mesenteries designated as SRM and for each separate tissue type is provided in Table 27.

Table 27: Historical mean total infectivity per year (2007 to 2011) for intestinal and mesenteric tissues at slaughter in the EU27 (2.5^{th} and 97.5^{th} percentiles in brackets)

	Infectivity per year ($BoID_{50}$/yr) Mean (2.5^{th} and 97.5^{th})				
	2007	**2008**	**2009**	**2010**	**2011**
Tissue type					
Ileum	3,194 (2384, 4689)	1,931 (1434, 2816)	1,158 (803,1782)	727 (439,1397)	450 (246,949)
Duodenum	0.25 (0.21, 0.3)	0.16 (0.13, 0.22)	0.09 (0.07,0.14)	0.06 (0.04,0.10)	0.04 (0.03,0.06)
Jejunum	15,773 (11900, 23351)	9,629 (6830, 14194)	5,772 (3968,9158)	3,620 (2150,6583)	2,246 (1227,4330)
Caecum	586 (441, 832)	357 (264, 559)	216 (152,343)	136 (81,251)	84 (46,167)
Colon	2 (1.9, 3)	1.40 (1.14, 1.89)	0.82 (0.6,1.2)	0.53 (0.39,0.86)	0.33 (0.22,0.55)
Mesenteric lymph nodes	3.2×10^{-4} (3×10^{-4}, 3.4×10^{-4})	2.1×10^{-4} (2×10^{-4}, 2.2×10^{-4})	1.2×10^{-4} (1×10^{-4}, 1×10^{-4})	7.9×10^{-5} (7×10^{-5}, 9×10^{-5})	4.9×10^{-5} (4×10^{-5}, 5×10^{-5})
Mesentery nerves	2,734 (2622, 2848)	1,779 (1707, 1869)	1,017 (952,1096)	680 (626,736)	420 (381,470)
CMGC	705 (677, 730)	458 (439, 479)	262 (247,281)	175 (163,190)	108 (98,120)
Total per year	**22,994 (18,223, 31,986)**	**14,156 (10,886, 19,548)**	**8,426 (6,184,12,568)**	**5,338 (3,502,9,050)**	**3,308 (2,059,5,896)**
Total ileocaecal plate	18,958 (14,261, 27,814)	11,555 (8,278, 16,964)	6,927 (4,760, 11,037)	4,345 (2,584, 7,973)	2,695 (1,477, 5,265)
Jejunal ileocaecal plate	15,764 (11,892, 23342)	9,623 (6,824, 14,187)	5,769 (3,965, 9,154)	3,618 (2,148, 6,579)	2,244 (1,226, 4,328)

For each intestinal tissue type (ileum, duodenum, jejunum, colon and caecum) and the combined ileocaecal plate (ileocaecal plate in ileum and jejunum), the estimated mean, 2.5^{th} and 97.5^{th} percentiles of infectivity per metre length for an average EU27 infected slaughter animal for each historical year is provided in Table 28. Table 29 provides the estimated total length of these tissues with the addition of jejunal ileocaecal plate length for each historical year.

Table 28: Historical mean infectivity per length ($BoID_{50}$/m) for intestinal tissues within an infected EU27 slaughter animal in 2007 to 2011 (2.5^{th} and 97.5^{th} percentiles in brackets).

	Infectivity per length ($BoID_{50}$/m) Mean (2.5^{th} and 97.5^{th})				
	2007	**2008**	**2009**	**2010**	**2011**
Tissue type					
Ileum	0.58 (8×10^{-6}, 4)	0.55 (8×10^{-6}, 4)	0.57 (8×10^{-6}, 4)	0.53 (8×10^{-6}, 3)	0.54 (8×10^{-6}, 4)
Duodenum	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	3.0×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})
Jejunum	6.0×10^{-2} (1×10^{-6}, 0.4)	5.7×10^{-2} (1×10^{-6}, 0.4)	5.9×10^{-2} (1×10^{-6}, 0.4)	5.6×10^{-2} (1×10^{-6}, 0.4)	5.6×10^{-2} (1×10^{-6}, 0.4)
Caecum	0.12 (3×10^{-6}, 0.9)	0.12 (3×10^{-6}, 0.8)	0.12 (3×10^{-6}, 0.9)	0.12 (3×10^{-6}, 0.8)	0.12 (3×10^{-6}, 0.8)
Colon	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	3.0×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})
Total ileocaecal plate*	1.6 (4×10^{-5} , 11)	1.5 (4×10^{-5} , 10)	1.6 (4×10^{-5} , 11)	1.5 (4×10^{-5} , 10)	1.5 (4×10^{-5} , 10)

* Results for jejunal ileocaecal plate are the same as those estimated for the total ileocaecal plate

Table 29: Historical total length of intestinal tissues from infected animals in the EU27 per year from 2007 to 2011 (m/yr) (2.5th and 97.5th percentiles in brackets)

	Total length per year (m/yr) Mean (2.5th and 97.5th)				
	2007	**2008**	**2009**	**2010**	**2011**
Tissue type					
Ileum	6,604 (6470, 6746)	4,250 (4103, 4387)	2,460 (2366,2550)	1,618 (1540,1687)	1,002 (944,1060)
Duodenum	8,053 (7886, 8220)	5,180 (4998, 5346)	2,998 (2885,3107)	1,971 (1878,2056)	1,220 (1149,1290)
Jejunum	303,735 (297531,309965)	195,450 (188684,201782)	113,128 (108874,117463)	74,400 (70749,77668)	46,049 (43347,48695)
Caecum	5,006 (4902, 5112)	3,221 (3108,3324)	1,864 (1794,1932)	1,226 (1167,1279)	759 (714,803)
Colon	72,502 (71010, 74018)	46,663 (45076,48159)	27,008 (25985,28010)	17,770 (16888,18515)	10,996 (10367,11638)
Total ileocaecal plate	10,762 (10502, 11011)	6,903 (6660,7158)	3,999 (3844,4158)	2,611 (2477,2748)	1,620 (1522,1731)
Jejunal ileocaecal plate	8,953 (8735, 9164)	5,742 (5542,5956)	3,327 (3198,3456)	2,172 (2062,2285)	1,348 (1266,1440)

10.4.3.1. Conclusions

According to TSEi model in the EU27 between 2007 and 2012:

- The average number of BSE infected and undetected cattle entering the food and feed chain per year dropped from 6,816 (CI95% 6,438 – 7,214) in 2007 to 613 (CI95% 566 – 661) in 2012.
- The total amount of infectivity associated with intestine and mesentery collected from BSE infected and undetected cattle dropped from 22,994 $BoID_{50}$ (CI95% 18,223 – 31,986) in 2007 to 1,985 $BoID_{50}$ (CI95% 1,170 – 4,557) in 2012.
- In 2007 intestine and mesentery accounted for about 85% and 15% respectively of the total amount of infectivity versus 86% and 14% respectively in 2012.
- The total length of intestines collected each year from BSE infected and undetected cattle dropped from 395,900 metres (CI95% 387,799 – 404,061) in 2007 to 35,676 metres (CI95% 34,393 – 40,362) in 2012.
- The average infectivity titre per metre in the ileo-caecal plate (the most infectious part of the intestine) was estimated to be about 1.5-1.6 $BoID_{50}$ along the entire period.
- Consequently, over this time period:
  - the BSE infectivity that was associated with the intestine and mesentery (eliminated as SRM) from infected but undetected animals entering the food and feed chain was reduced by a factor of 10;
  - the potential maximum level of exposure to the BSE agent for an individual consumer in 2007 and 2012 would have remained stable.

10.4.4. Case study on infectivity in a re-emergence scenario

A re-emergence scenario has been implemented in C-TSEMM extending the exponential trend as estimated by the baseline model into future years (Adkin et al., 2012). A 10% increase in the BSE prevalence between successive birth cohorts has been used to represent the rate of the emergence

starting in the baseline year 2012. The surveillance of cattle for BSE was limited to all fallen stock and emergency slaughtered animals tested over the age of 48 months and all clinical suspects tested, i.e. those conditions in place in the majority of EU27 member states in 2013. After discussion with the European Commission, an age at slaughter window of 48 to 72 months was selected for observing cases with results shown for observing 1 case or 3 cases between these ages.

It was assumed that the number of cattle born each year and the age at which cattle are slaughtered in the future healthy slaughter and fallen stock stream remained steady based on the baseline year. The future percentage of infected animals exiting by stream (healthy slaughter, emergency slaughter, fallen stock, and clinical suspects) was assumed to be the average estimated between 2002 and 2011.

Given a 10% increase in prevalence by birth cohort across the EU27, detection of one case between the age window of 48 to 72 months in tested streams (emergency slaughter, fallen stock and clinical suspects) is estimated to occur, on average, after 16 years (11 years, 25 years), with a mean of 3 cases observed after 36 years (23 years, 44 years). Table 30 provides the estimated mean cumulative number of infected and missed animals and resulting infectivity until detection together with 2.5th and 97.5th percentiles depending on the number of cases observed between 48 and 72 months.

Within the first 16 years of the theoretical re-emergence an estimated mean of 38,874 $BoID_{50}$ would arise from bovine intestines and mesenteries in infected animals at slaughter, with 2.5th and 97.5th percentiles that varies between 27,626 and 52,554 $BoID_{50}$ accounting for uncertainty and variability. Following a slow build-up of infected animals, and given no changes in control of the disease, the estimated number of infected and missed animals, and therefore the amount of infectivity in these tissues, increases exponentially as shown in Figure 23.

Table 30: Re-emergence mean estimated cumulative number of infected missed animals in the EU27 slaughter stream and resulting infectivity until year of detection of 1 to 5 cases between 48 to 72 month age at slaughter (2.5th and 97.5th percentiles in brackets)

		Slaughter population Mean (2.5th and 97.5th)		
		Number infected and missed before detection (head)	**Cumulative total infectivity (unprocessed) ($BoID_{50}$)**	**Cumulative total infectivity (processed*) ($BoID_{50}$)**
Number of cases observed†	**Years until detection**			
1 case	16 (11, 25)	4,607 (3475, 5908)	38,874 (27626, 52554)	25,199 (17932,37520)
2 cases	27 (13, 37)	13,293 (8359,18967)	123,823 (76391,178495)	78,674 (51499,117727)
3 cases	36 (23, 44)	28,482 (18867, 35349)	268,486 (161031, 337792)	168,155 (110764, 220474)
4 cases	41 (30, 48)	43,279 (32,223, 54,627)	418,761 (320883,518696)	260,198 (191957, 338763)
5 cases	45 (36, 51)	61,212 (47699, 73068)	595,250 (465939, 730711)	380,293 (288727, 465073)

* Excludes ileum

† Cases observed in emergency slaughter >48 months, fallen stock >48 months and clinical suspects of any age, assumed no testing of healthy slaughter

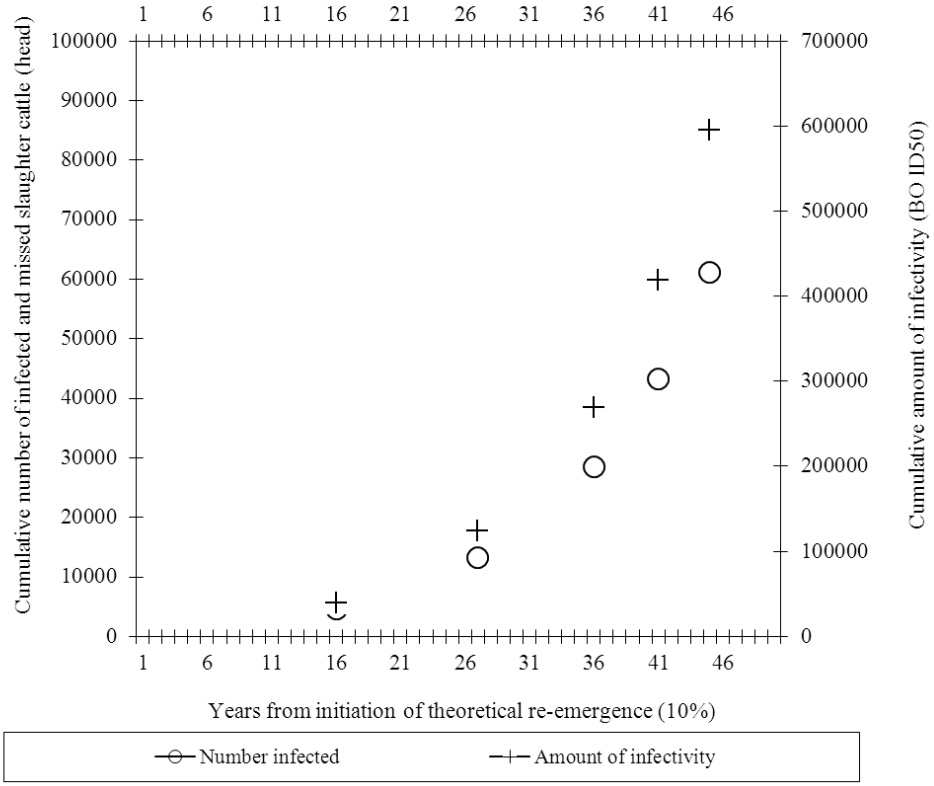

Figure 23: Estimated mean cumulative number of BSE infected and missed cattle slaughtered in EU27 and resulting cumulative infectivity from bovine intestines and mesenteries following a theoretical 10% increase in prevalence by birth cohort from 2012. Points indicate time period of detection of 1 case, 2 cases etc in the age window 48 to 72 month age at slaughter.

Table 31 provides the estimated cumulative total infectivity stratified by each tissue type before detection of 1 case and 3 cases within the surveillance window. Table 32 highlights the changes in the contribution of each tissue type to the total infectivity. As the number of years increases from the initiation of the theoretical emergence from the baseline year 2012, the contribution of those tissues most infectious in younger animals (less than 60 months) contribute more to the total infectivity including the ileum and jejunum, with those tissues contributing to total infectivity from older animals (greater than 60 months), for example, mesentery tissues becoming less important to the overall total.

Table 31: Re-emergence mean estimated cumulative infectivity for intestinal and mesenteric tissues in the EU27 slaughter stream until detection of 1 or 3 cases between 48 to 72 month age at slaughter (2.5th and 97.5th percentiles in brackets).

	Infectivity ($BoID_{50}$) Mean (2.5th and 97.5th)			
	Cumulative infectivity (unprocessed) 1 case detected	**Cumulative infectivity (processed*) 1 case detected**	**Cumulative infectivity (unprocessed) 3 cases detected**	**Cumulative infectivity (processed*) 3 cases detected**
Tissue type				
Ileum	6,210 (4379,8610)	NA	43275 (26179, 54844)	NA
Duodenum	0.20 (0.13,0.30)	0.15 (0.10,0.22)	1.26 (0.73,1.57)	0.94 (0.59,1.20)
Jejunum	30,827 (21824,41640)	23,583 (16810,35350)	214,778 (128395, 270144)	159,635 (104892, 209657)
Caecum	1,014 (700,1423)	776 (535,1118)	7,036 (4246, 8686)	5,212 (3460, 6716)
Colon	1.65 (1.11,2.43)	1.26 (0.86,1.80)	10 (6, 13)	7.78 (4.91,9.96)
Mesenteric lymph nodes	2.1×10^{-4} (2×10^{-4}, 3×10^{-4})	2.1×10^{-4} (2×10^{-4}, 3×10^{-4})	1.3×10^{-3} (9×10^{-4}, 2×10^{-3})	1.3×10^{-3} (9×10^{-4}, 2×10^{-3})
Mesentery nerves	646 (460,797)	646 (460,797)	2,640 (1719, 3275)	2,640 (1719, 3275)
CMGC	175 (124,215)	175 (124,215)	742 (485, 919)	742 (485, 919)
Total per year	**38,874 (27626, 52554)**	**25,199 (17932,37520)**	**231,568 (186296,283462)**	**168,155 (110764, 220474)**
Total ileocaecal plate	37,030 (26281,50239)	NA	258,008 (154548, 324933)	NA
Jejunal ileocaecal plate	30,820 (21819,41630)	23,578 (16807, 35341)	214,733 (128369, 270088)	159,601 (104871, 209612)

* Ileum and total ileocaecal plate are classed as tissues not processed for consumption and therefore not included in the analysis

Table 32: Re-emergence estimated mean percentage contribution to infectivity by intestinal and mesenteric tissues at slaughter in the EU27 by year of detection of 1 or 3 cases between 48 to 72 month age at slaughter (2.5th and 97.5th percentiles in brackets).

	Mean percentage contribution to infectivity per animal		
	Baseline 2012	**Year of 1 case detected (unprocessed)**	**Year of 3 cases detected (unprocessed)**
Tissue type			
Ileum	14%	16%	16%
Duodenum	0%	0%	0%
Jejunum	68%	80%	80%
Caecum	3%	3%	3%
Colon	0%	0%	0%
Mesenteric lymph nodes	0%	0%	0%
Mesentery nerves	13%	1%	1%
CMGC	3%	0%	0%
Total per animal ($BoID_{50}$)	**3.2 (0.02, 18)**	**8.5 (0.02, 59)**	**9.7 (0.02, 67)**
Total ileocaecal plate	[82%]	[96%]	[96%]
Jejunal ileocaecal plate	[68%]	[80%]	[80%]

The age shift of the average infected animal during the theoretical re-emergence can also be seen in the increasing infectivity per metre length of an EU27 animal as shown in Table 33 for unprocessed tissues and Table 34 for processed tissues.

Table 33: Re-emergence mean infectivity per length ($BoID_{50}$/m) for unprocessed intestinal tissues within an infected EU27 slaughter animal by year of detection of 1 or 3 cases between 48 to 72 month age at slaughter (2.5^{th} and 97.5^{th} percentiles in brackets)

	Unprocessed infectivity per length ($BoID_{50}$/m) Mean (2.5^{th} and 97.5^{th})		
	Baseline 2012	**Year of 1 case detected (unprocessed)**	**Year of 3 cases detected (unprocessed)**
Tissue type			
Ileum	0.55 (8×10^{-6}, 4)	2.1 (1×10^{-4}, 15)	2.1 (1×10^{-4}, 15)
Duodenum	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	4.1×10^{-5} (5×10^{-8}, 3×10^{-4})	4.1×10^{-5} (5×10^{-8}, 3×10^{-4})
Jejunum	5.7×10^{-2} (1×10^{-6}, 0.4)	0.2 (2×10^{-5}, 1)	0.2 (2×10^{-5}, 2)
Caecum	0.12 (3×10^{-6}, 0.8)	0.4 (5×10^{-5},3)	0.4 (5×10^{-5},3)
Colon	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	4.1×10^{-5} (5×10^{-8}, 3×10^{-4})	4.1×10^{-5} (5×10^{-8}, 3×10^{-4})
Total ileocaecal plate*	1.5 (4×10^{-5} , 10)	4.7 (7×10^{-4} , 33)	4.7 (7×10^{-4} , 33)

* Results for jejunal ileocaecal plate are the same as those estimated for the total ileocaecal plate

Table 34: Re-emergence mean infectivity per length ($BoID_{50}$/m) for processed intestinal tissues within an infected EU27 slaughter animal by year of detection of 1 or 3 cases between 48 to 72 month age at slaughter (2.5^{th} and 97.5^{th} percentiles in brackets)

	Processed* infectivity per length ($BoID_{50}$/m) Mean (2.5^{th} and 97.5^{th})		
	Baseline 2012	**Year of 1 case detected (processed)**	**Year of 3 cases detected (processed)**
Tissue type			
Ileum	-	-	-
Duodenum	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})	3.0×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})
Jejunum	4.2×10^{-2} (7×10^{-7}, 0.3)	0.16 (1×10^{-5}, 1)	0.16 (1×10^{-5}, 1)
Caecum	0.1 (2×10^{-6}, 0.6)	0.29 (4×10^{-5}, 2)	0.29 (4×10^{-5}, 2)
Colon	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})	3.0×10^{-5} (3×10^{-8}, 2×10^{-4})	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})
Jejunal ileocaecal plate	1.1 (3×10^{-5} , 8)	3.5 (5×10^{-4}, 25)	3.5 (5×10^{-4}, 25)

* Ileum and total ileocaecal plate are classed as tissues not processed for consumption and therefore not included in the analysis

The estimated mean cumulative length of intestinal tissues by tissue type from infected animals in the EU27 from the initiation of the re-emergence in 2012 to detection of 1 case and 3 cases in the surveillance window is provided in Table 35.

Table 35: Re-emergence cumulative length of intestinal tissues from infected animals in the EU27 until year of detection of 1 or 3 cases between 48 to 72 month age at slaughter (m) (2.5th and 97.5th percentiles in brackets).

	Total length until detection (m) Mean (2.5th and 97.5th)	
	Cumulative length 1 case detected	**Cumulative length 3 cases detected**
Tissue type		
Ileum	3,933 (2857,4919)	24,025 (15992, 29635)
Duodenum	5,086 (3695,6362)	31,419 (20915, 38755)
Jejunum	183,769 (133065,229710)	1,126,255 (750451, 1389427)
Caecum	3,102 (2254,3881)	19,099 (12713, 23558)
Colon	42,850 (31053,53592)	261,372 (174066, 322468)
Total ileocaecal plate	9,026 (6591,11355)	58,340 (38840, 72074)
Jejunal ileocaecal plate	7,511 (5491,9448)	48,550 (32316, 59983)

10.4.4.1. Conclusions

- The detection of either one or three cases per year in the 48 – 72 age category by the current BSE surveillance system[30] in the EU27 was defined by the European Commission as the trigger that would allow the identification of the re-emergence of BSE.

- The TSEi model was used to simulate a re-emergence of cattle BSE in the EU27. The simulation assumed i) that the current BSE surveillance system would be maintained (passive surveillance, and systematic testing of all at-risk animals older than 48 months) and ii) a 10% increase of the prevalence of the disease by birth cohort.

- According to TSEi model in the EU27:

  - 16 years (CI95% 11 - 25 years) would be needed for the current BSE surveillance system to detect a case in the 48 – 72 months age category.

  - 36 years (CI95% 23 - 44 years) would be needed for the current BSE surveillance system to detect three cases in the 48 – 72 months age category.

- Under the hypothesis of identifying such a re-emergence after 16 years:

  - the estimated mean number of infected slaughtered animals would be about 4,600 (CI95% 3,475 - 5,908), while the estimated mean number of infected slaughtered animals in the year in which the re-emergence would be detected would be about 430.

  - a cumulative estimated mean infectivity of 38,053 $BoID_{50}$ (CI95% 26,904 – 51,676 $BoID_{50}$) would be associated with the unprocessed intestines and 821 $BoID_{50}$ (95% CI 584 – 1,012 $BoID_{50}$) would have been associated with the mesentery of these animals.

  - Intestine would account for about 99% the total amount of infectivity.

[30] The testing of all at risk animals above 48 months of age and of all clinical suspects of any age.

  - The total estimated mean length of intestine collected from undetected BSE infected cattle would be 283,740 metres (CI95% 172,924 – 298,464).

- As the re-emergence of the disease would be associated with the infection of young animals, the mean contribution of the ileocaecal plate and caecum to the total amount of infectivity associated with intestine and mesentery would be prominent (about 99%).

- In an infected animal that would enter the food and feed chain, the average level of infectivity per metre of ileocaecal plate (used to estimate the potential maximum level of exposure for an individual consumer) would be about 4.7 $BoID_{50}$/m (CI95% 7 x 10^{-4} – 33). In comparison with 2012, this value would represent about a 3 fold increase.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Currently the intestine and the mesentery collected in healthy slaughtered cattle in the EU27 are classified as Specified Risk Material (SRM).

- A model (named TSEi) was developed that estimates the infectivity associated with the intestine and the mesentery of BSE infected cattle born in the EU and that enter undetected into the food and feed chain.

- In the absence of data related to L- and H- type BSE agent distributions in bovine tissues, the model cannot at this stage be applied to these diseases.

- This model was also developed for future estimation of the TSE infectivity associated with tissues other than intestine and mesentery and can be applied to species other than bovine in case data is available.

- The model relies on a combination of experimental data and assumptions that might have an impact on its final accuracy.

- To deliver its outputs, the model combines five components:

  - A surveillance component that simulates (on the basis of the C-TSEM model) the number of BSE infected animals entering undetected into the food and feed chain each year.

  - An abattoir component that estimates the length and/or the weight of the intestines and mesenteric tissues collected yearly from BSE infected animals that enter the food and feed chain undetected.

  - A SRM component that allows the user to define a list of SRM by tissue type and by age at slaughter.

  - An infectivity component that estimates the infectivity (expressed as Bovine oral Infectious Dose 50: $BoID_{50}$) in mesenteric and intestinal tissues of BSE infected animals according to their age at slaughter.

  - A processing component that simulates the impact in terms of infectivity reduction of the post harvesting processing of intestines and mesentery.

- The model results are particularly sensitive to changes in four parameters: (1) infectivity titre of the ileum, (2) age at slaughter, (3) ileocaecal plate weight in small intestines, and (4) conversion of the infectivity titre as measured by bioassays in conventional mice (log_{10} RIII ic ip ID_{50}/g) and in cattle ($BoID_{50}$/g).

Infectivity

- In a BSE infected bovine, the relative distribution of the infectivity in the different portions of the intestines and in the mesenteric tissues varies with the age of the animal, reflecting the stage of incubation of the disease.

- In a BSE infected healthy slaughtered bovine:

  - up to 36 months of age, the infectivity in intestine and mesentery is mainly (on average more than 90%) associated with the ileocaecal plate (distal part of jejunum plus ileum) and the caecum;

  - over 36 and under 60 months of age, there is a substantial inter-individual variability in the relative contribution of intestinal and mesenteric structures to the total infectivity;

  - from 60 months of age, the infectivity in intestine and mesentery is mainly (on average more than 90%) associated with the mesenteric nerves and the Celiac and Mesenteric Ganglion Complex.

  - duodenum, colon and mesenteric lymph nodes contribute less than 0.1% to the total infectivity in an infected animal regardless of the age at slaughter.

- The total infectivity that is associated with the intestines and the mesentery varies with the age of the infected animal. On average, it peaks at about 15 $BoID_{50}$ in animals younger than 18 months before progressively declining to 8-9 $BoID_{50}$ (in animals between 24 and 48 months of age) and dropping to 0.7 $BoID_{50}$ in animals older than 60 months.

Evolution of the situation since 2007

- The TSEi model allows a comparison between the level of BSE infectivity associated with the intestine and the mesentery collected from infected and undetected cattle in different years.

- In a scenario where the mesentery would no longer be considered as SRM, the processing of mesenteric tissues collected in a batch of several animals would dilute the infectivity associated with mesenteric tissues from one BSE infected animal. This dilution effect cannot presently be quantified.

- In a scenario where the intestines would no longer be considered as SRM, the intestine collected from one single infected bovine would be consumed by a limited number of people without any dilution effect. Given the available data, the infectivity titre per metre in the ileocaecal plate (the most infectious part of the intestine) may be used to estimate the potential maximum level of exposure for an individual consumer.

- According to the TSEi model:

  - In 2007, about 6,800 infected cattle were estimated to enter undetected the food and feed chain in the EU27. The total infectivity in the intestine and mesentery of those animals was estimated to be about 23,000 $BoID_{50}$ (about 19,500 $BoID_{50}$ in intestine and 3,500 $BoID_{50}$ in mesentery). The total length of intestines coming from those infected animals was estimated to be about 395,000 metres. The average infectivity titre per metre in the

ileo-caecal plate, the most infectious part of the intestine, was estimated to be about 1.6 $BoID_{50}$.

- In 2012, about 610 infected cattle were estimated to enter undetected the food and feed chain in the EU27. The total infectivity in the intestine and mesentery of those animals was estimated to be about 2,000 $BoID_{50}$ (about 1,700 $BoID_{50}$ in intestine and 300 $BoID_{50}$ in mesentery). The total length of intestines coming from those infected animals was estimated to be about 36,000 metres. The average infectivity titre per metre in the ileo-caecal plate, the most infectious part of the intestine, was estimated to be about 1.5 $BoID_{50}$.

- Based on these estimates:

  - between 2007 and 2012, the BSE infectivity that was associated with the intestine and mesentery (eliminated as SRM) from infected but undetected animals entering the food and feed chain in the EU27 was reduced by a factor of 10;

  - if the intestines of these animals would have entered into the food and feed chain in the EU27, the potential maximum level of exposure to the BSE agent for an individual consumer in 2007 and 2012 would have remained stable.

BSE re-emergence scenario

- The TSEi model was used to simulate a re-emergence of cattle BSE in the EU27. The simulation assumed i) that the current BSE surveillance system would be maintained (passive surveillance, and systematic testing of all at-risk animals older than 48 months) and ii) a 10% increase of the prevalence of the disease by birth cohort. It also considered that the detection of 1 or 3 BSE cases in EU27 cattle aged between 48 to 72 months would be regarded as a threshold for BSE re-emergence.

- Under such a scenario it was estimated that, on average, 16 years and 36 years would be needed to identify one or three BSE cases respectively.

- Under the hypothesis of identifying such a re-emergence after 16 years, over this period:

  - the mean of the estimated total number of infected slaughtered animals is about 4,600 and the mean of the estimated total number of infected slaughtered animals in the year in which the re-emergence would be identified would be about 430;

  - the cumulative estimated mean infectivity is about 38,000 $BoID_{50}$ in the unprocessed intestines and about 800 $BoID_{50}$ in the mesentery of these animals;

  - the mean of the estimated total length of intestine in these animals is about 280,000 metres.

- As the re-emergence of the disease would be associated with the infection of young animals, the mean contribution of the ileocaecal plate and the caecum to the total amount of infectivity associated with intestine and mesentery would be prominent (about 99%).

- In an infected animal that would enter the food and feed chain in the EU27, the average level of infectivity per metre of ileocaecal plate (used to estimate the potential maximum level of exposure for an individual consumer) would be about 4.7 $BoID_{50}$/m. In comparison with 2012, this value would represent about a 3 fold increase.

Final remark

- Whatever the scenario, the removal of the last 4 metres of the small intestine and of the caecum from the food and feed chain would result on average in a reduction exceeding 90% of the total infectivity associated with intestine and mesentery in BSE infected cattle up to 36 months of age.

RECOMMENDATIONS

- The TSEi model relies on a number of assumptions. If more specific data would become available the model should be updated to obtain refined outputs.
- If data related to L- and H- type BSE become available, the TSEi model should be used to provide quantitative estimates of the exposure to these TSE agents.
- If a modification of the SRM measures would be envisaged, a potential re-emergence of BSE in cattle should also be considered by the risk manager.
- The TSEi model would be useful to provide quantitative estimates of the infectivity in tissues:
  - other than intestine and mesentery of BSE infected cattle;
  - of TSE infected small ruminants.

DOCUMENTATION PROVIDED TO EFSA

1. Letter (ref. n. Ares(2012)117123 dated 01/02/2012) from the European Commission with a request for a scientific opinion on a quantitative evaluation of BSE risk in bovine intestines and mesentery.

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APPENDICES

Appendix A. Summary table of input parameters and function used in the model on TSE infectivity level in animal tissues

* Uncertain range (U), variable range (V), U V indicates two alternative distributions indicated
t=tissue type, where 1=ileum, 2=duodenum, 3=jejunum, 4=colon, 5=caecum, 6=mesenteric lymph nodes, 7=mesentery nerves, 8= celiac and mesenteric ganglion complex (CMGC).
a=age at slaughter (months)
m=months post infection

Parameter	Symbol	Value	Unit	U/V*	Reference
Surveillance					
Random age at slaughter of an infected animal that has by-passed testing	$Age_{slaughter}$	$Discrete\left(\{a\},\left\{\frac{N\_infected(a)}{\sum_{a=0}^{a=21} N\_infected(a)}\right\}\right)$	months	V	
Random number of infected animals that have by-passed testing by age interval	$N\_infected(a)$	$N\_infHS(a) + N\_infES(a)$	head		Function
Number of infected animals in HS that have by-passed testing by age interval	$N\_infHS(a)$	$Pert(HS_L, HS_{ml}, HS_U)$ where uncertainty considered, HS_M for variability	head	U V	Output from C-TSEMM, Adkin et al. (2012)
Number of infected animals in ES that have by-passed testing by age interval	$N\_infES(a)$	$Pert(ES_L, ES_{ml}, ES_U)$ where uncertainty considered, ES_M for variability	head	U V	Output from C-TSEMM, Adkin et al. (2012)
Random age at infection of infected animal	$Age_{infection}$	$Discrete(\{exa\},\{P_{exposure(exa)}\})$	months	V	Arnold and Wilesmith (2004)
Random months post infection of infected animals that has by-passed controls	$Age_{postinf}$	$Age_{slaughter} - Age_{infection}$	months		Function
Abattoir					
Random animal length of small intestine	$Length(1+2+3,a)$	*Uniform*($Length_L(1+2+3,a)$, $Length_U(1+2+3,a)$)	m	V	
Minimum and maximum length of small intestine by age at slaughter	$Length_L$ $(1+2+3,a)$ $Length_U$ $(1+2+3,a)$	*a=0-6: 28, 35* *a=6-12: 32, 36* *a=12-18: 34, 39* *a=18-24: 38, 44* *a>24: 40, 56*	m	V	Carlens (1928)

Parameter	Symbol	Value	Unit	U/V*	Reference
Length of ileum by age at slaughter	$Length(1,a)$	*a=0-6: 0.5* *a=6-12: 0.63* *a=12-18: 0.75* *a=18-24: 0.88* *a>24: 1.0*	m	V	ENSCA, pers. Comm. 2012
Length of duodenum by age at slaughter	$Length(2,a)$	*a=0-6: 0.9* *a=6-12: 0.98* *a=12-18: 1.05* *a=18-24: 1.13* *a>24: 1.2*	m	V	Nickel et al. (1987)
Length of ileocaecal plate in ileum and jejunum	$Length(PP1+PP3i)$	$Uniform{\sim}Length_{l(PP1+PP3i)}, Length_{u(PP1+PP3i)}$	m	V	
Minimum length of ileocaecal plate in ileum and jejunum	$Length_{l(PP1+PP3i)}$	*a=0-6: 2.24* *a=6-12: 2.07* *a=12-18: 2.35* *a=18-24: 0.35* *a>24:0.35*	m	V	Carlens (1928)
Maximum length of ileocaecal plate in ileum and jejunum	$Length_{u(PP1+PP3i)}$	*a=0-6: 3.85* *a=6-12: 3.9* *a=12-18: 4.06* *a=18-24: 2.56* *a>24:2.56*	m	V	Carlens (1928)
Length of jejunum by age at slaughter	$Length(3,a)$	$Length(1+2+3,a) - Length(1,a) - Length(2,a)$	m		Function
Length of caecum	$Length(4,a)$	*a=0-6: 0.5* *a=6-12: 0.5625* *a=12-18:0.625* *a=18-24:0.6875* *a>24:0.75*	m	V	Sisson and Grossman (1953); Nickel et al. (1987)
Length of colon	$Length(5,a)$	*a=0-6: 6* *a=6-12: 7* *a=12-18:8* *a=18-24:9* *a>* $Uniform{\sim}10,12$ 90% correlated to small intestine length	m	V	Sisson and Grossman (1953); Nickel et al. (1987)
Random animal weight of ileocaecal plate	*Weight(PP1+PP3i,a)*	$Pert(Weight_{l(PP1+PP3i,a)}, Weight_{ml(PP1+PP3i,a)}, Weight_{u(PP1+PP3i,a)})$	g	V	

Parameter	Symbol	Value	Unit	U/V*	Reference
Minimum, maximum and most likely ileocaecal plate weight	$Weight_{l(PP1+PP3i,a)}$ $Weight_{ml(PP1+PP3i,a)}$ $Weight_{u(PP1+PP3i,a)}$	*a=0-6: 274.9, 374.8, 439.8* *a=6-12: 276, 385.8, 497* *a=12-18: 252, 346.8, 461* *a=18-24: 30.2, 189.4, 472.3* *a=24-60: 0, 0.9, 4* *a=60-120: 0, 1.1, 3* *a=>120: 0, 0.2, 1* 90% correlated to ileocaecal plate length and Peyer's patch weight	g	V	Modified from Figure 1 and 2, Carlens (1928)
Random weight of Peyers patches in duodenum and jejunum	$Weight(PP2 + PP3ii, a)$	$Pert(Weight_{l(PP2+PP3ii,a)}, Weight_{ml(PP2+PP3ii,a)}, Weight_{u(PP4,a)})$	g	V	
Minimum, maximum and most likely weight of Peyers patches in duodenum and jejunum	$Weight_{l(PP2+PP3ii,a)}$ $Weight_{ml(PP2+PP3ii,a)}$ $Weight_{u(PP2+PP3ii,a)}$	*a=0-6: 69, 104.8, 142* *a=6-12: 102, 124.2, 206* *a=12-18: 160, 191.0, 252* *a=18-24: 155, 184.3, 242* *a=24-60: 111, 159.0, 207* *a=60-120: 66, 111.3, 149* *a=>120: 40, 66.1, 96*	g	V	Modified from Figure 1 and 2, Carlens (1928)
Proportion of ileocaecal plate in ileum	$P(PP1)$	$\frac{Length(1,1)}{Length(PP1 + PP3i, 1)}$	P		Function
Proportion of Peyer's Patches in duodenum	$P(PP2, a)$	$\frac{Length(2,a)}{Length(2,a) + Length(3,a)}$	P		Function
Weight of ileocaecal plate in jejunum	$Weight(PP3i, a)$	$\big(1 - P(PP1)\big) * Weight(PP1 + PP3i, a)$	PP g		Function
Weight of ileocaecal plate in ileum	$Weight(PP1, a)$	$P(PP1) * Weight(PP1 + PP3i, a)$	PP g		Function
Weight of Peyer's patches in duodenum	$Weight(PP2, a)$	$P(PP2, a) * Weight(PP2 + PP3ii, a)$	PP g		Function
Weight of Peyer's patches in jejunum	$Weight(PP3ii, a)$	$\big(1 - P(PP2, a)\big) * Weight(PP2 + PP3ii, a)$	PP g		Function
Surface area of lymphoid tissue in caecum	$Area(PP4, a)$	$Uniform(60,80)$	cm^2	V	Carlens (1928)
Radius of ileum	$Radius(1)$	$Uniform(3.2,5.2)$	cm	V	ENSCA, pers. Comm. 2012

Parameter	Symbol	Value	Unit	U/V*	Reference
Weight of mesenteric lymph nodes	$Weight(6,a)$	*a=0-6:* $Pert\sim(55.5,70.9,101.5)$ *a=>6* $Pert\sim(69.8,163.4,283.0)$	g	V	Jänicke (1911)
Random weight of mesentery tissue (nerves)	$Weight(7,a)$	$Pert\sim(Weight_{l(7,a)}, Weight_{ml(7,a)}, Weight_{u(7,a)})$	g	V	Assumption
Minimum, maximum and most likely weight of mesenteric nerves	$Weight_{l(7,a)}$ $Weight_{ml(7,a)}$ $Weight_{u(7,a)}$	*a=0-6: 50, 87.5, 200* *a=6-12: 62.5, 103.1,275* *a=12-18: 75, 118.8, 350* *a=18-24: 87.5, 134.4, 425* *a=24-60: 100, 150, 500*	g	V	Assumption
Random weight of celiac and mesenteric ganglion complex (CMGC)	$Weight(8,a)$	$Pert\sim(Weight_{l(8,a)}, Weight_{ml(8,a)}, Weight_{u(8,a)})$	g	V	Dyce (1958) adapted by assumptions
Minimum, maximum and most likely weight of CMGC	$Weight_{l(8,a)}$ $Weight_{ml(8,a)}$ $Weight_{u(8,a)}$	*a=0-6: 12.5, 22.0, 50* *a=6-12: 15.6, 27.4, 62.5* *a=12-18: 18.8, 32.9, 75* *a=18-24: 21.9, 38.4, 87.5* *a=24-60: 25.0, 43.9, 100*	g	V	Assumption
Processing					
Reduction in infectivity due to processing into casing	$P_{processng}(t)$ for *t*=2, 3, 4, 5	$Uniform(0,0.5)$	%	V	EFSA (2007); Wijnker et al. (2008)
	t=1, 6, 7, 8	0			Assumption
Infectivity					
Infectivity titre of ileocaecal plate in ileum	$Titre(1,m)$	$Normal(0.37, 0.81)$	Mouse i.c. i.p. ID_{50}/g	V	Arnold, 2013 adapted from Hoffmann et al. (2011)
Lower titre of Peyer's patches in jejunum relative to ileum	$Titre_{lower}$	$Pert(2.5th, 3.072, 3.7377, 97.5th, 4.384)$ where uncertainty concerned, 3.735 for variability	Mouse ic.ip ID_{50}/g	U V	Arnold, 2013 adapted from Hoffmann et al. (2011)
Infectivity titre of mesenteric lymph nodes	$Titre(6,m)$	$Uniform(0, 10^{-6.7})$ where uncertainty considered, -7.68 for variability	RIII Mouse ic.ip ID_{50}/g	U V	Buschmann and Groschup (2005) adapted by Arnold, 2013

Parameter	Symbol	Value	Unit	U/V*	Reference
Infectivity titre of mesenteric nerves	$Titre(7, m)$	$(m * Age_{postinf}) + c$ to a maximum of -0.013 Where for uncertainty: $m = Pert(2.5th, 0.20, 0.224, 97.5th, 0.25)$ $c = Pert(2.5th, -9.22, -8.40, 97.5th, -7.47)$ Where variability only: $m = 0.224$ $c = -8.4$	RIII Mouse ic.ip ID_{50}/g	U V	Kaatz et al. (2012) adapted by Arnold, 2013
Infectivity titre of CMGC	$Titre(8, m)$	$(m * Age_{postinf}) + c$ to a maximum of -0.01 Where for uncertainty: $m = Pert(2.5th, 0.20, 0.224, 97.5th, 0.25)$ $c = Pert(2.5th, -8.53, -7.70, 97.5th, -6.82)$ Where variability only: $m = 0.224$ $c = -7.7$	RIII Mouse ic.ip ID_{50}/g	U V	Kaatz et al. (2012) adapted by Arnold, 2013
Conversion of cattle oral ID_{50} to equivalent RIII mouse log_{10} i.c./i.p. ID_{50}	BO_{unit}	$Pert(2.5th, 2, 2.7, 97.5th\ 3.4)$ where uncertainty considered, 2.7 for variability	-	U V	Wells et al. (2007)

Appendix B. Individual bovine intestine and mesentery tissue type results

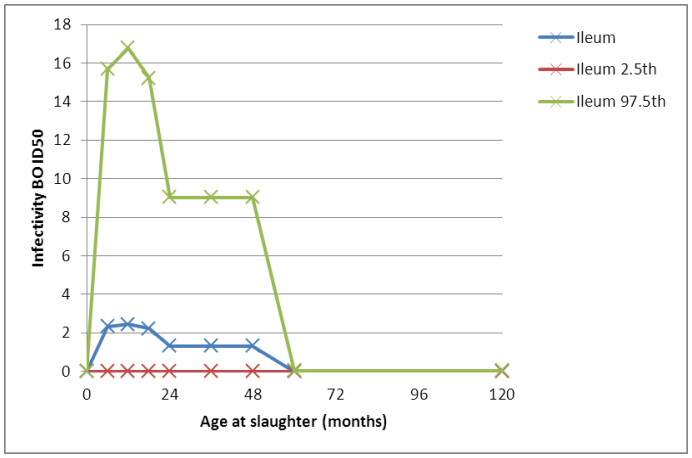

Figure 24: Graph of mean, 2.5th and 97.5th estimates of infectivity in the ileum ($BoID_{50}$ per infected animal) by age at slaughter

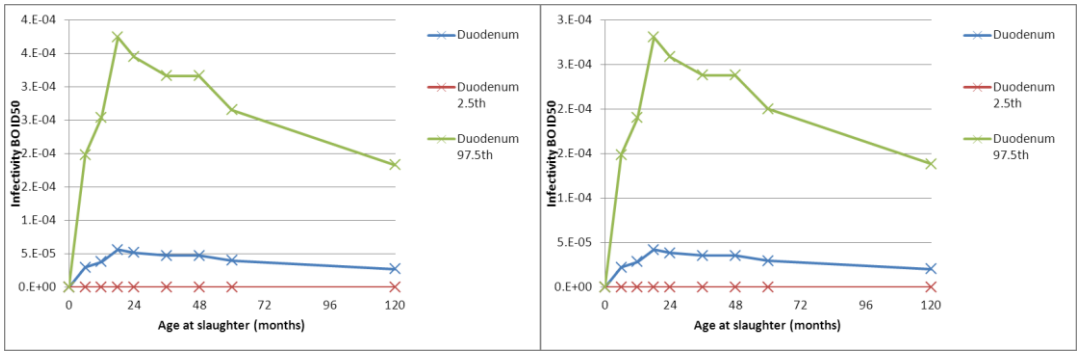

Figure 25: Graph of mean, 2.5th and 97.5th estimates of infectivity in the duodenum before processing LHS, after processing RHS ($BoID_{50}$ per infected animal) by age at slaughter

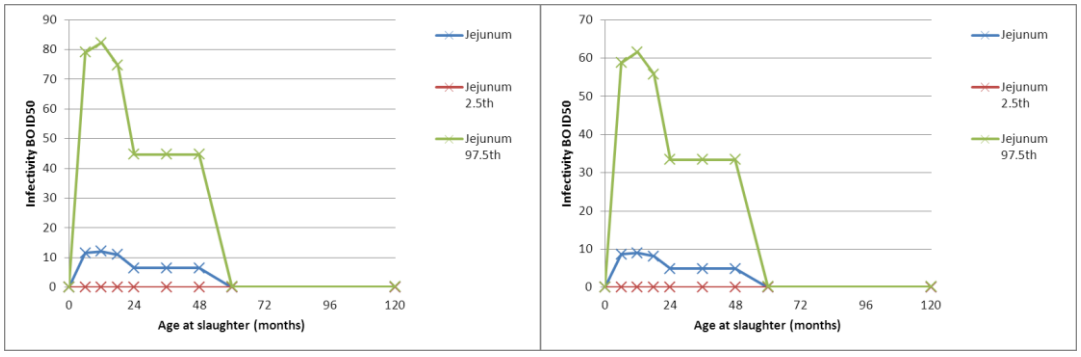

Figure 26: Graph of mean, 2.5th and 97.5th estimates of infectivity in the jejunum before processing LHS, after processing RHS ($BoID_{50}$ per infected animal) by age at slaughter

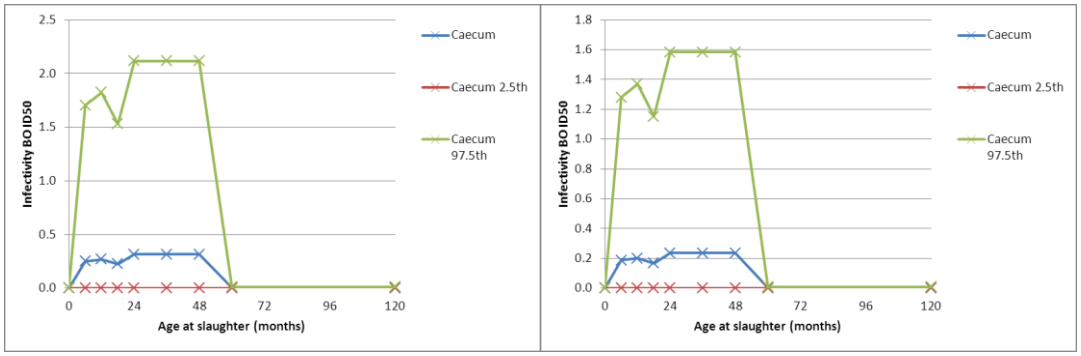

Figure 27: Graph of mean, 2.5th and 97.5th estimates of infectivity in the caecum before processing LHS, after processing RHS ($BoID_{50}$ per infected animal) by age at slaughter

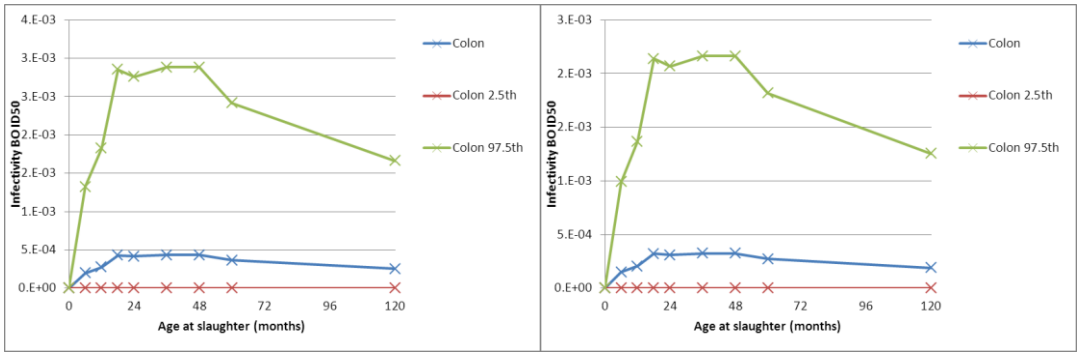

Figure 28: Graph of mean, 2.5th and 97.5th estimates of infectivity in the colon before processing LHS, after processing RHS ($BoID_{50}$ per infected animal) by age at slaughter

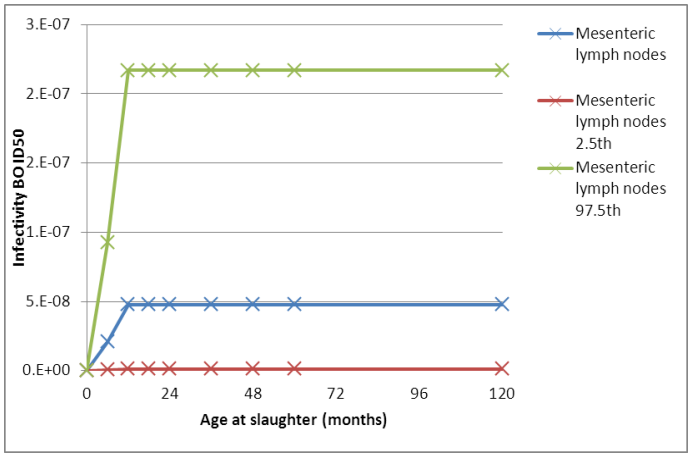

Figure 29: Graph of mean, 2.5th and 97.5th estimates of infectivity in the mesenteric lymph nodes ($BoID_{50}$ per infected animal) by age at slaughter

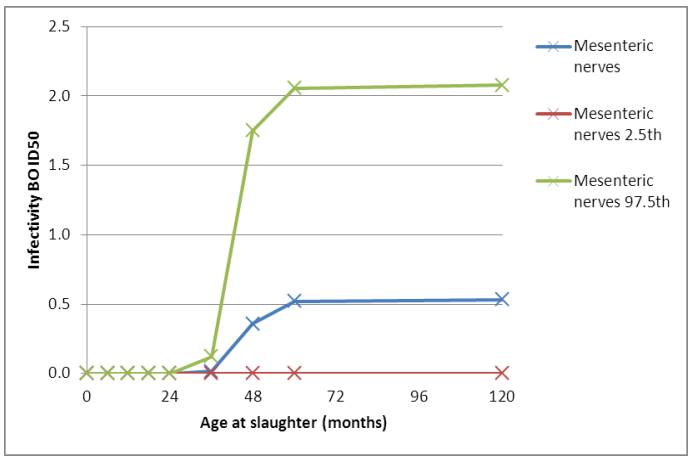

Figure 30: Graph of mean, 2.5th and 97.5th estimates of infectivity in the mesenteric nerves ($BoID_{50}$ per infected animal) by age at slaughter

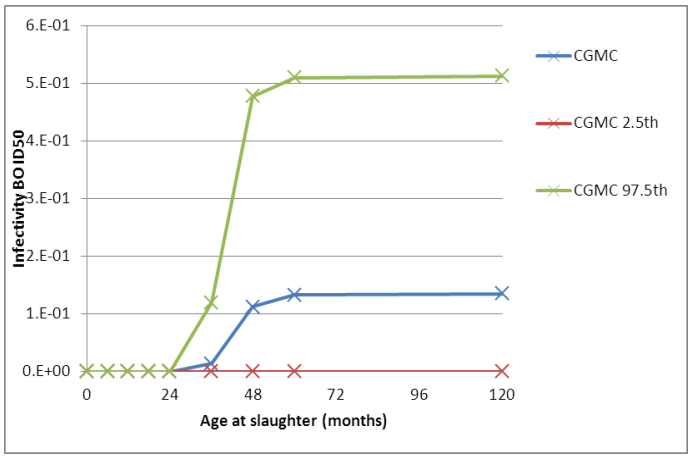

Figure 31: Graph of mean, 2.5th and 97.5th estimates of infectivity in the mesenteric CMGC ($BoID_{50}$ per infected animal) by age at slaughter

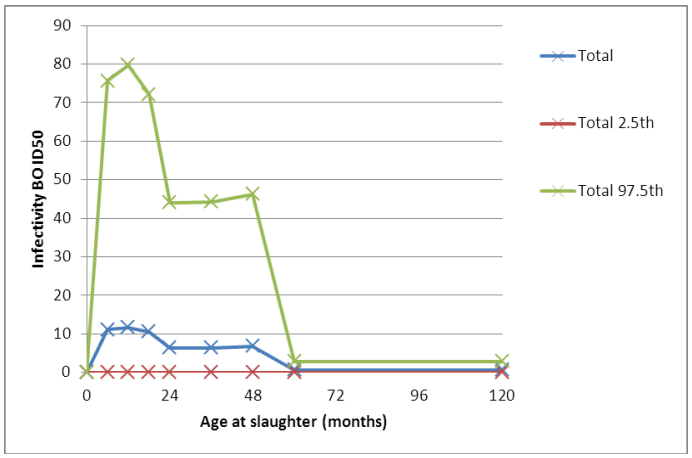

Figure 32: Graph of mean, 2.5th and 97.5th estimates of infectivity in total bovine intestines and mesenteries ($BoID_{50}$ per infected animal) by age at slaughter

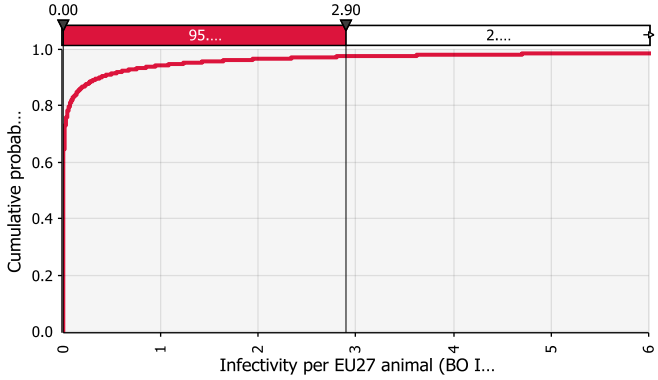

Figure 33: Cumulative probability function describing the amount of infectivity in ileum ($BoID_{50}$) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

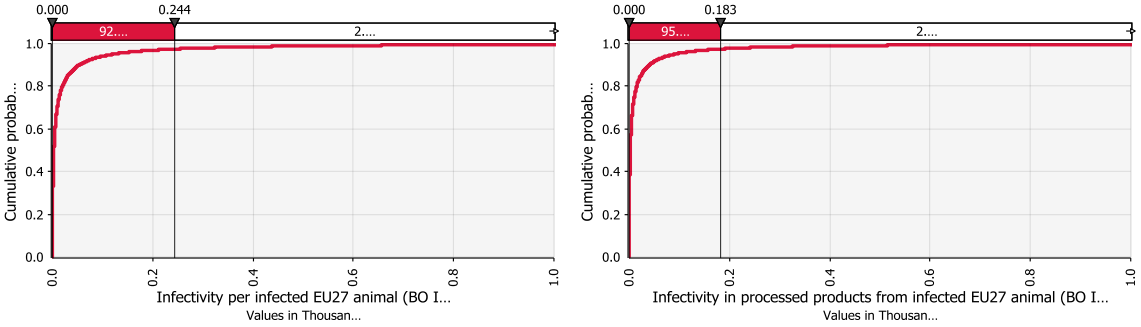

Figure 34: Cumulative probability function describing the total amount of infectivity in duodenum before processing RHS, after processing LHS ($BoID_{50}$) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

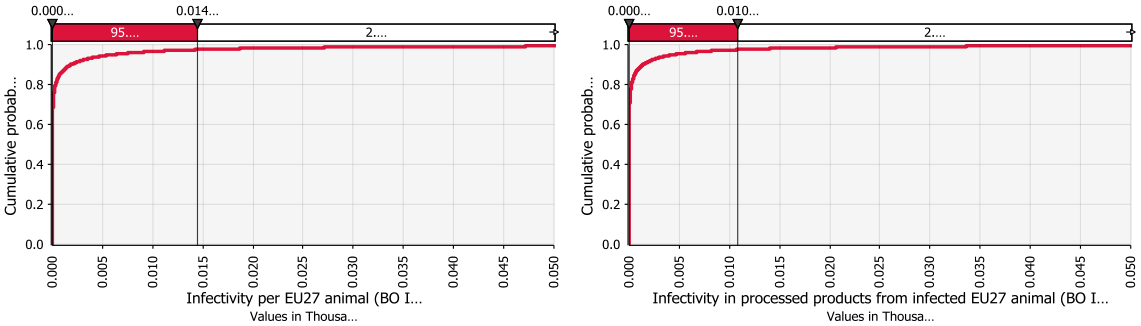

Figure 35: Cumulative probability function describing the total amount of infectivity in jejunum before processing RHS, after processing LHS ($BoID_{50}$) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

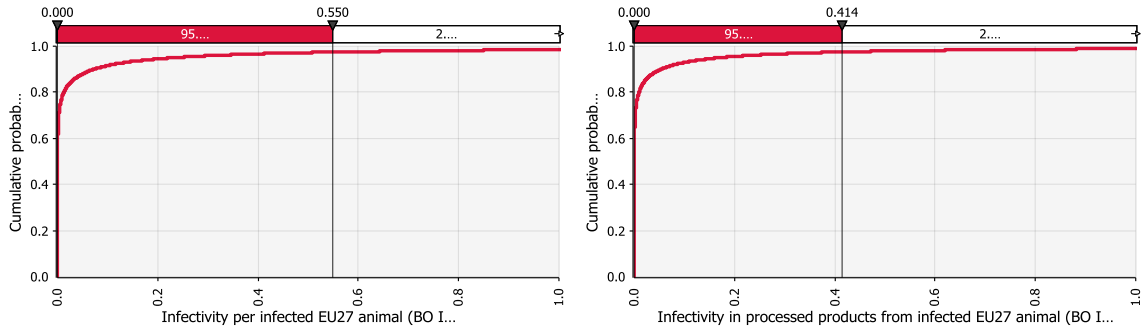

Figure 36: Cumulative probability function describing the total amount of infectivity in caecum before processing RHS, after processing LHS ($BoID_{50}$) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

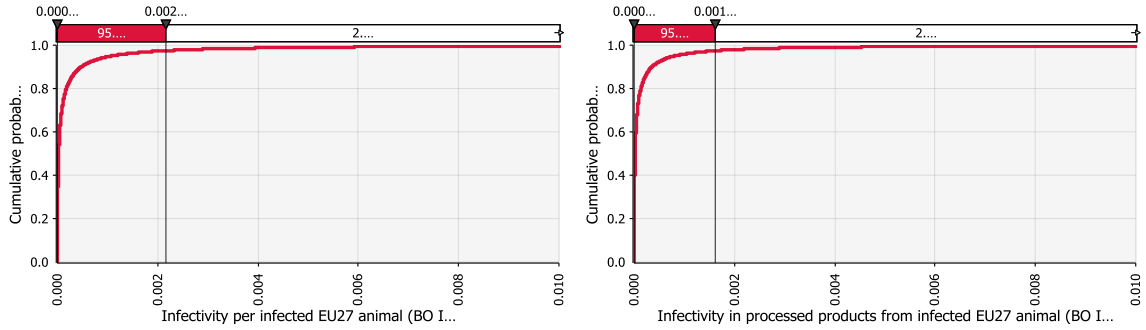

Figure 37: Cumulative probability function describing the total amount of infectivity in colon before processing RHS, after processing LHS ($BoID_{50}$) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

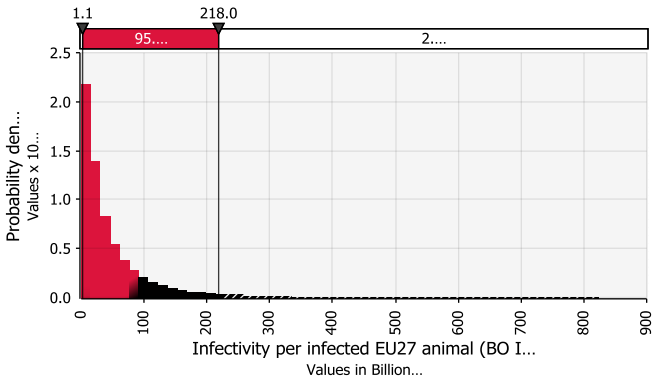

Figure 38: Probability density function describing the total amount of infectivity in mesentery lymph nodes ($BoID_{50}$) in a slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

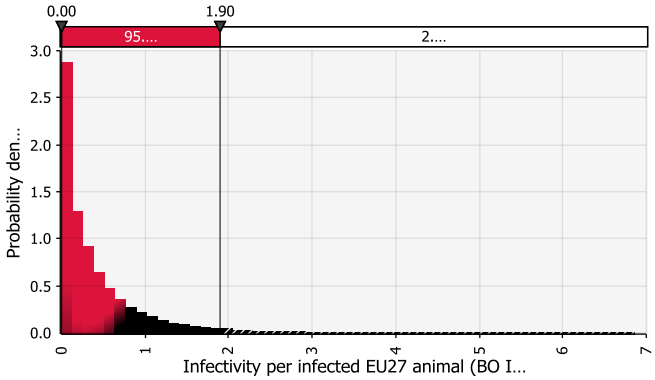

Figure 39: Probability density function describing the total amount of infectivity in mesentery nerves ($BoID_{50}$) in a slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

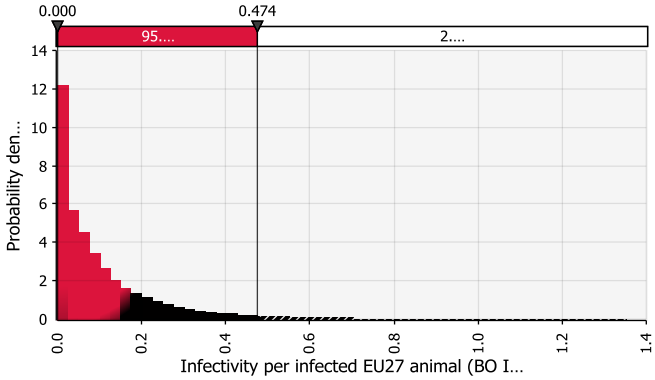

Figure 40: Probability density function describing the total amount of infectivity in mesentery CMGC ($BoID_{50}$) in a slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

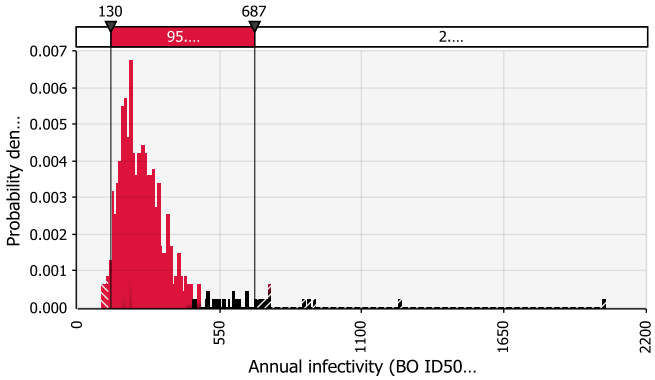

Figure 41: Cumulative probability function describing the total amount of infectivity in ileum per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

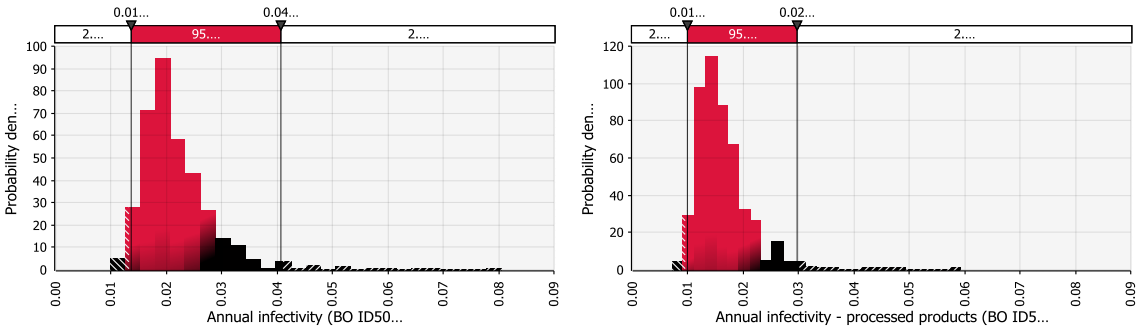

Figure 42: Probability density function describing the total amount of infectivity from duodenum before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

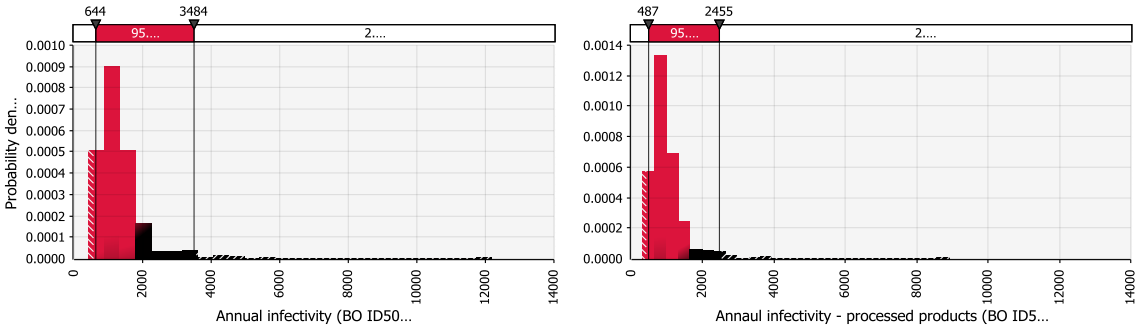

Figure 43: Cumulative probability function describing the total amount of infectivity in jejunum before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

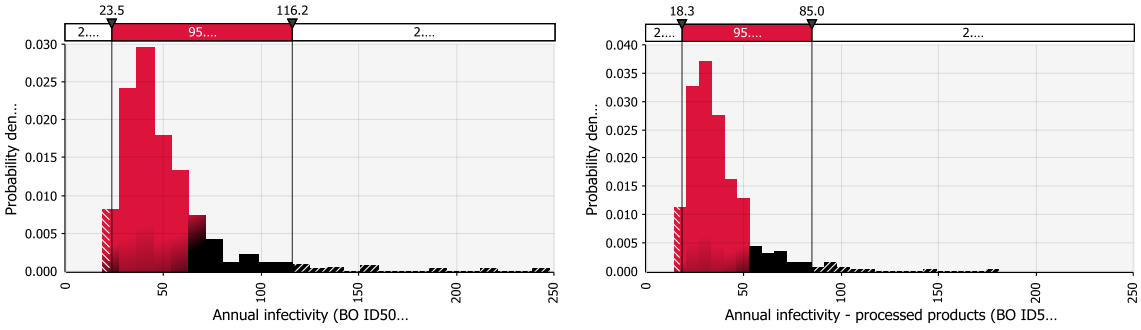

Figure 44: Cumulative probability function describing the total amount of infectivity in caecum before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

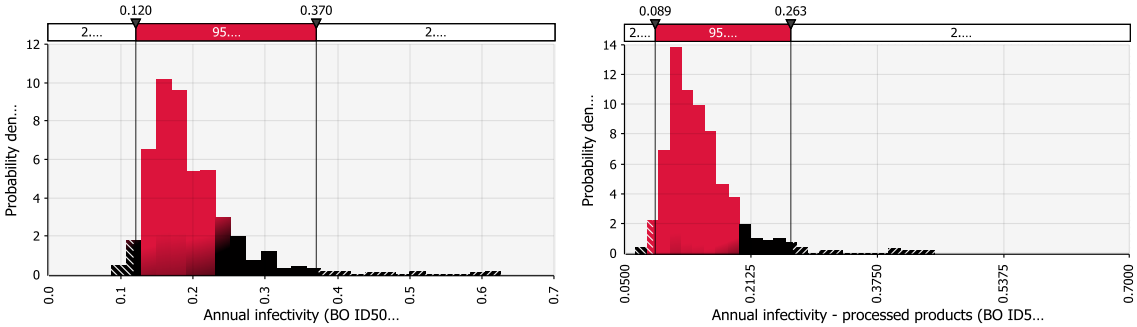

Figure 45: Probability density function describing the total amount of infectivity in colon before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

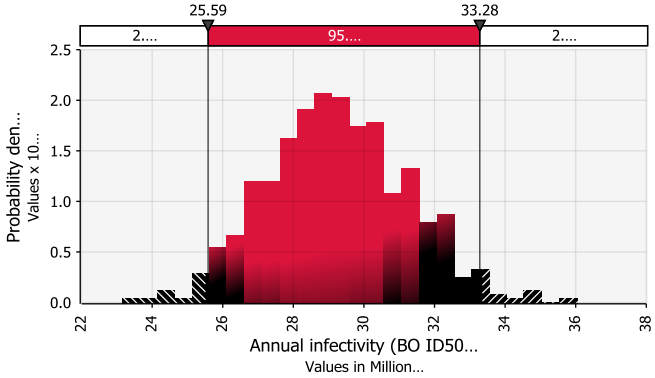

Figure 46: Probability density function describing the total amount of infectivity in mesentery lymph nodes per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

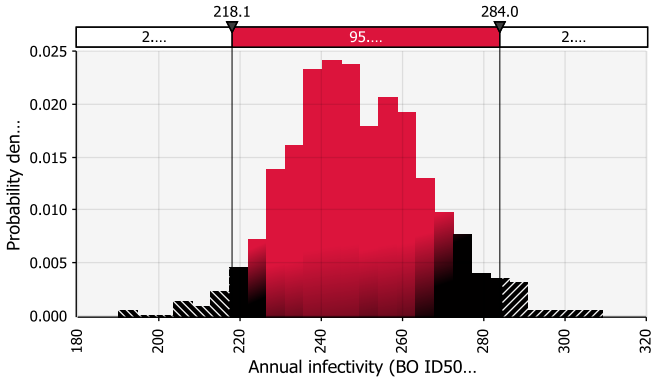

Figure 47: Probability density function describing the total amount of infectivity in mesentery nerves per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

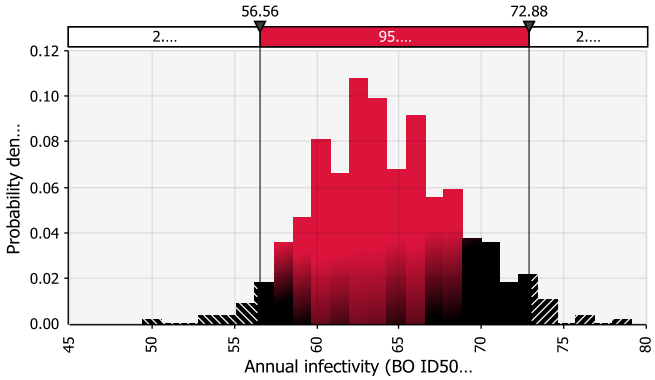

Figure 48: Probability density function describing the total amount of infectivity in mesentery CMGC per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)