EXTERNAL SCIENTIFIC REPORT

TSE infectivity model (TSEi) in animal tissues: Bovine intestines and mesenteries[1]

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ABSTRACT

A stochastic quantitative risk assessment (QRA) has been developed to (1) compare the level of infectivity of different TSE agents in animal tissues, (2) estimate the impact of amendments to the list/age for the removal of SRM on residual TSE infectivity levels for a single infected animal and at the country level per year, and (3) estimate the impact of certain processing technologies on residual TSE infectivity in selected animal tissues or products. In this report the QRA is focused on bovine intestines and mesentery. The tissue types identified for quantitative modelling are: ileum, duodenum, jejunum, caecum, colon, mesenteric lymph nodes, mesenteric nerves and the celiac and mesenteric ganglion complex (CMGC). Of these tissues processed products include bovine intestines (duodenum, jejunum, caecum, and colon) used to produce sausage casings and the rendering of fats from mesentery tissues. The ileum is not processed for human consumption. This report describes the model approach taken together with the parameterization for each tissue type conceptually divided into five different components: surveillance, abattoir, SRM, processing, and infectivity. Both uncertainty and variability associated with input data have been included separately in the model where estimates are known. A baseline model has been completed using surveillance and demographic data from 2012. Two case studies are also provided, the retrospective analysis of the estimated amount of infectivity in the healthy slaughter and emergency slaughter streams by age at slaughter (2007-2011), and the amount of infectivity accumulating during a theoretical re-emergence of BSE. Results are provided based on the current parameterization and include associated quantifiable uncertainty and variability. When developing the risk assessment a number of assumptions were made which need to be considered when reviewing results.

KEY WORDS

Bovine Spongiform Encephalopathies, BSE, risk assessment, mathematical model, bovine intestines, mesentery tissues

DISCLAIMER

[1] Question No EFSA-Q-2012-00281

Any enquiries related to this output should be addressed to biohaz@efsa.europa.eu. The TSEi model is available upon request to biohaz@efsa.europa.eu.

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SUMMARY

This is the report for project CFT/EFSA/BIOHAZ/2012/02: "Model on the TSE infectivity level in animal tissues". This report presents the model framework and calculations together with the input data required and key assumptions that have been made. Both uncertainty and variability associated with input data were included in the final model results where estimates were known.

The objective of this project is to develop a flexible and transparent model (supported by a user-friendly interface) to assess quantitatively the TSE infectivity level in animal tissues. The first task is focused on developing a risk assessment for bovine intestines and mesentery, more specifically the tissue types identified for quantitative modelling are: ileum, duodenum, jejunum, caecum, colon, mesenteric lymph nodes, mesenteric nerves and the celiac and mesenteric ganglion complex (CMGC). The processing technologies included in this task are those associated with sausage casing (involving duodenum, jejunum, caecum and colon) and mesenteric fat production. The ileum is not processed for human consumption.

The risk assessment has been used to estimate the following defined outputs (mean, 2.5th and 97.5th percentiles within which 95% of the results lie):

For an infected animal:

- Estimated progression of infectivity in an infected animal in bovine intestines and mesenteries over time by age at slaughter, by tissue type (BO ID_{50}), and per length (BO ID_{50} per m)
- Infectivity of processed tissues from an infected animal by age at slaughter, by tissue type (BO ID_{50}), and per length (BO ID_{50} per m)
- Estimated infectivity in an infected animal drawn from the slaughter population of interest (EU27[2]) in bovine intestines and mesenteries, by tissue type (BO ID_{50}), and per length (BO ID_{50} per m)
- Infectivity of processed tissues from an infected animal drawn from the EU27 slaughter population, by tissue type (BO ID_{50}), and per length (BO ID_{50} per m)

For member state or group of member states over one year:

- Estimated total amount of infectivity from infected animals, destined for the EU27 food and feed chain, in bovine intestines and mesenteries at slaughter, by tissue type (BO ID_{50} per year)
- Total infectivity of processed tissues from infected animals, destined for the EU27 food and feed chain, by tissue type (BO ID_{50} per year)
- Total length of infected tissues from infected animals, destined for the EU27 food and feed chain, by tissue type (m per year)

In this report baseline results for the aggregated EU27 in 2012 have been presented, however the risk assessment model contains the input data to generate results for each EU27 member state (results not presented in this report). A sensitivity analysis was performed investigating those uncertain and variable parameters which significantly impact results, and a number of scenarios that were identified during model development and parameterisation have been implemented to investigate the impact on model outputs. Two case studies are provided; (1) the retrospective analysis of the estimated amount of infectivity in the healthy slaughter and emergency slaughter streams by age at slaughter (2007-2011), and (2) the amount of infectivity accumulating during a theoretical re-emergence of BSE.

[2] EU27: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and United Kingdom.

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BACKGROUND AS PROVIDED BY EFSA

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[3] and are considered as the animal tissues potentially containing the highest level of Transmissible Spongiform Encephalopathies (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). So far, the EFSA Scientific Panel on Biological Hazards has issued eight Scientific Opinions that were used by the Risk Manager to define and update the list of SRM. Some examples are reported here below:

- Scientific Opinion on a review of the Bovine Spongiform Encephalopathy (BSE)-related risk in bovine intestines[4].
- Scientific Opinion on BSE/TSE infectivity in small ruminant tissues[5].
- Scientific Opinion on consumption of beef tongue: Human BSE risk associated with exposure to lymphoid tissue in bovine tongue in consideration of new research findings[6].
- Scientific Opinion on the assessment of the age limit in cattle for the removal of certain Specified Risk Materials (SRM)[7].

Taking into consideration the current favourable evolution of the epidemic of BSE in the EU and on the basis of the current scientific knowledge, the strategic paper "TSE Roadmap 2"[8] recently issued by the European Commission foresees the possibility to modify the list/age limit for the removal of SRM without compromising the current level of consumer protection. In the course of recent years the implementation of active TSE surveillance in the EU has allowed the identification of different types of TSEs in ruminants such as Classical BSE, H-type Atypical BSE and L-type Atypical BSE in bovines and Classical scrapie and Atypical scrapie in small ruminants. A recent publication raises the possibility of a new type of TSE in bovine animals being identified[9]. In this context, the European Commission has submitted to EFSA a mandate for a quantitative risk assessment of the BSE risk i) in bovine intestines, both when unprocessed and processed (casings), and ii) in bovine mesentery. It is likely that the Commission may send to EFSA additional mandates on other SRM-related subjects in the future.

3 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.
4 http://www.efsa.europa.eu/en/efsajournal/pub/2104.htm
5 http://www.efsa.europa.eu/en/efsajournal/pub/1875.htm
6 http://www.efsa.europa.eu/en/efsajournal/pub/700.htm
7 http://www.efsa.europa.eu/en/efsajournal/pub/220.htm
8 Available at: http://ec.europa.eu/food/food/biosafety/tse_bse/docs/roadmap_2_en.pdf
9 Seuberlich T, Gsponer M, Drögemüller C, Polak MP, McCutcheon S, Heim D, et al. Novel prion protein in BSE-affected cattle, Switzerland. Emerg Infect Dis. 2012 Jan. http://wwwnc.cdc.gov/eid/pdfs/11-1225-ahead_of_print.pdf

TERMS OF REFERENCE AS PROVIDED BY EFSA

The terms of reference for this work is to provide EFSA with a flexible and transparent model supported by a "user-friendly" interface that would be employed to assess the TSE infectivity level in animal tissues with the aim to provide quantitative answers informing the Risk Manager in the definition/update of the SRM list. This model will support the EFSA BIOHAZ Panel and, where appropriate, the associated *ad hoc* Working Groups in the development of future Opinions related to the review of the SRM list in the EU. A training session to future users of the model must also be provided. The specific objectives of the contract resulting from the present procurement procedure are as follows:

1. To develop a flexible and transparent model to assess and compare:
    - the infectivity level of different TSE agents in animal tissues/organs;
    - the impact of possible amendments to the list/age for removal of SRM on the residual TSE infectivity level both at single animal and at animal population level;
    - the impact of certain processing technologies on the residual TSE infectivity level of some animal tissues/organs or products.

    The model shall employ a "user-friendly" interface that would allow for the input of the various data and parameters needed.

2. To provide results on the application of the model to an initial set of animal tissues/organs and processing technologies. Namely, bovine intestines and mesentery (including mesenteric fat) and their related risk management measures (e.g. their inclusion in or exclusion from the SRM list) and the impact of processing technologies applied to these tissues/organs (e.g. processing of bovine intestines into casings).
3. To provide EFSA with two draft reports and a final report.
4. To develop a user manual.
5. To deliver a training session for EFSA staff and other potential users.
6. To work closely with EFSA and with relevant experts and to participate in up to four physical meetings and four web-conferences with EFSA.
7. To provide assistance during the period after delivery of the final model until the end of the contract where necessary.

This contract was awarded by EFSA to:

Contractor: Animal Health and Veterinary Laboratories Agency, UK

Contract title: "Model on the TSE infectivity level in animal tissues"

Contract number: CFT/EFSA/BIOHAZ/2012/02

INTRODUCTION AND OBJECTIVES

Transmissible Spongiform Encephalopathies (TSEs) are a group of serious diseases that affect the brain and nervous system of man and various animals including cattle, sheep and goats. Regulation in Europe[10] to control the disease in cattle and protect human and animal health currently involves extensive surveillance and removal of Specified Risk Materials (SRM) from the food and animal by-products chain, together with the ban on the use of proteins of animal origin in feed for farmed animals (with certain exemptions). This is complemented by European surveillance in order to monitor the impact of control measures. In view of the continuing steady decline in the number of Bovine Spongiform Encephalopathy (BSE) infected cattle and the lack of emergence of BSE in sheep, it is therefore appropriate to re-evaluate the level of intervention required to achieve acceptable levels of risk reduction in Europe.

The objective of the project is to develop a flexible and transparent model (supported by a user-friendly interface) to assess quantitatively the TSE infectivity level in animal tissues. A quantitative risk assessment (QRA) simulation model has been developed that will permit:

- Comparison of the level of infectivity of different TSE agents in animal tissues/organs
- Estimation of the impacts of possible amendments to the list/age for the removal of SRM on residual TSE infectivity levels both at single animal and member state level over one year
- Estimation of the impact of certain processing technologies on residual TSE infectivity in some animal tissues or products

The TSEi QRA has been developed initially to focus on bovine intestines and mesentery and their related processing technologies, the parameterisation and results of which are contained in this report. The model was written as a transparent and flexible software package in Excel using the add on @Risk (Version 6.2.1 Palisade ™).

[10] 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. Official Journal L 147, 31.5.2001, p. 1-40.

MATERIALS AND METHODS

The overarching modelling framework has been developed to enable users to investigate various SRM regimes and processing techniques in Member States (MSs). Imported products into Europe are not considered. The model framework is provided in Figure 1. The model is divided into five data components: (1) surveillance, (2) abattoir, (3) SRM, (4) processing, and (5) infectivity, which all feed into a central component; the first five components contain the data to characterise a randomly selected infected animal whilst the central component uses this information and scales up to an annual contribution of infectivity into the food and feed chain for a country or country grouping, for example, the EU27.

A user-interface has been developed which permits the user to select the types of required outputs from the risk assessment and other options such as member state data to be used, tissue types and to define specific scenarios to be investigated. The roles and outputs of each of the components are described in more detail in the following sections.

The risk assessment is predominantly a first order variability model and includes estimates, where available, of parameter uncertainty and the natural variability of input parameters through the use of distributions and stochastically modelling individual infected cattle. Via the user interface, stochastic simulations including only variable parameters can be selected to investigate the impact of variability in results and can be compared to those results where both variability and uncertainty have been included.

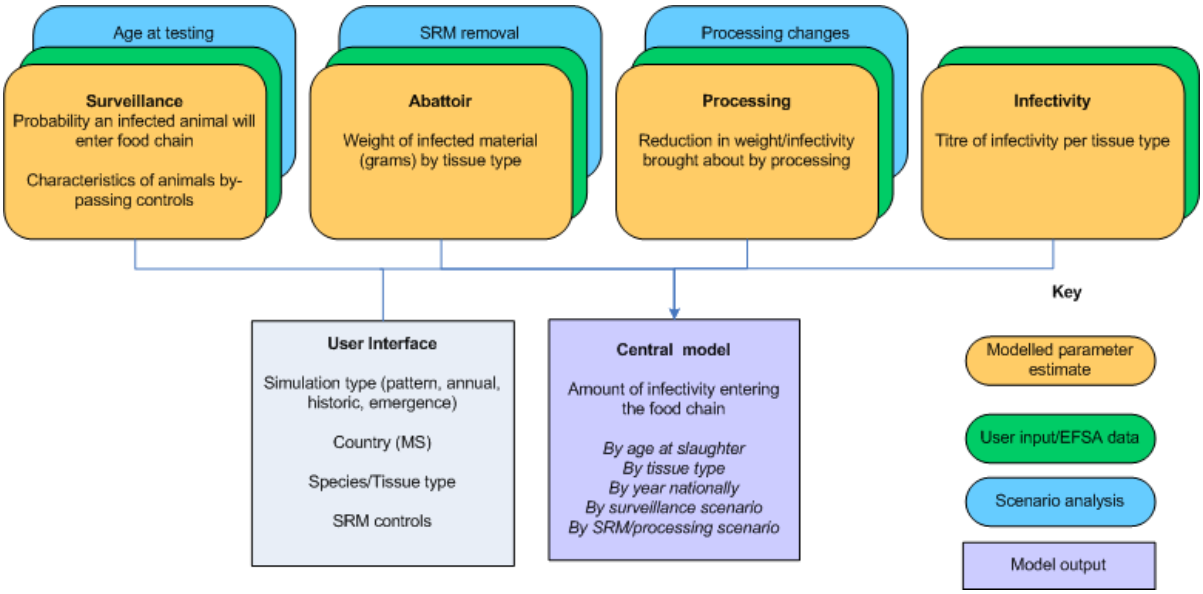

Figure 1: Overarching framework TSE infectivity model (TSEi)

When considering the implementation of the model framework it must be highlighted that the demographic information available for different animal species (cattle, sheep and goats) and scientific knowledge associated with the distribution of infectivity and titre during the incubation period differs for various TSEs. For example, for many EU Member States the age at which cattle are slaughtered is recorded in 12 monthly intervals. However, for sheep, only limited information is recorded. For atypical scrapie and atypical BSE there has been far less experimental data generated to support the modelling of infectivity developing over time when compared to classical BSE in cattle and classical scrapie in sheep. Finally, particularly for TSEs in small ruminants, genetics strongly influence

pathogenesis and thus the probability of infection, the progress of infectivity through peripheral and CNS tissues and final tissue infectivity titres. In conclusion the level of complexity that can be developed within mathematical models for different animal species and TSEs is dependent on the level of data that will be made available as inputs. A demonstration scrapie model has been developed but is not further described in this report.

This report describes the risk assessment approach and specific parameterisation for the development of BSE in infected cattle in the bovine intestines and mesenteries. Infectivity in ileum, duodenum, jejunum and caecum is mainly associated with lymphoid tissue (Peyer's patches, ileocaecal plate and isolated lymphoid follicles) and therefore the QRA model did not consider infectivity present outside those structures for bovine intestines. Any infectivity present in the mesentery is likely to be associated with nerves, autonomic nervous system ganglia and lymph nodes. Fatty deposits are unlikely to contain BSE infectivity. Therefore the tissue types mesenteric lymph nodes, mesenteric nerves and mesentery celiac and mesenteric ganglion complex were included in the QRA.

1. Model overview

The following sections describe the model which is divided into five data components: (1) surveillance, (2) abattoir, (3) SRM, (4) processing, (5) infectivity, and a central component which scales up the estimates based on a single random infected animal to the total infectivity accumulated in one year for a selected country or grouping of countries. The data presented in these sections represents the data as quoted from sources often in the form of a minimum, maximum and mean estimate or percentiles. In some cases these data have been described by distributions with the values used in the model fitted such that each distribution has the same key statistics as that quoted in the literature. Assumptions used in transforming the data, the distributions selected and the specific values used in the model are presented in Appendix A.

2. Surveillance component

The surveillance component estimates the probability that a random infected animal slaughtered for the food and feed chain per year will by-pass testing regimes, and estimates relevant age related or disease characteristics of that animal (for example, age at slaughter, months post infection). In order to estimate these characteristics data are required on the prevalence of infection by age/birth cohort for the exit streams entering the food and feed chain, the number of animals slaughtered, the age at exposure, TSE test sensitivity, and the testing scenario being implemented. Therefore, this component incorporates the demographic information of the slaughter population of interest to the level of definition possible for that animal species and disease information of the likely prevalence within that population and the probability that an infected animal will by-pass controls.

For BSE in cattle, infectivity is estimated for those animals entering the food and feed chain, that is, healthy slaughtered animals, emergency slaughtered animals and those cattle with clinical signs (not BSE) at ante-mortem. A considerable amount of the aforementioned demographic data has recently been gathered and a mathematical model (C-TSEMM) has been developed estimating the trend in prevalence of BSE in European Member States (MSs) (CFT/EFSA/BIOHAZ/2011/02 Adkin et al., 2012, AHVLA). This model estimates the number of BSE infected cattle slaughtered in a year by birth cohort or age at slaughter and of those, the number that tested positive from surveillance data. C-TSEMM can be used to generate results for the most recent year (baseline uses data for 2012), and can also be used to generate the number of infected animals slaughtered in previous years. Finally the model has been extended to include a theoretical emergence scenario where an increasing trend in birth cohort BSE prevalence is assumed and the future number of infected animals slaughtered generated until a point in time where the re-emergence is detected.

C-TSEMM requires annual historical information on the standing population, slaughter/death of animals in each exit stream and of those animals which have been tested, the test results by strain type if available. These data are required for each MS: where individual country estimates are not available an EU average is used. A questionnaire was sent to MSs by EFSA and data gathered up to 2012, to supplement European Commission data up to 2012 and data on standing populations from Eurostat.

2.1. Estimating the random age at slaughter of an infected animal that has by-passed testing, $Age_{slaughter}$

C-TSEMM estimates the number of BSE infected animals that have by-passed testing controls due to (i) not meeting testing criteria, (ii) being insufficiently close to clinical onset to test positive, and, (iii) inadequate test sensitivity, in those streams that may enter the food and feed chain (AHVLA, 2012). Healthy slaughter, emergency slaughter, and animals showing clinical signs (not BSE) at ante-mortem (AM) may enter the food and feed chain subject to certain conditions. The exit stream "clinical signs at AM" does not seem to be uniformly applied in MS data. When considering the definition of the emergency slaughter category there appears little to distinguish between the categories and therefore it has been agreed that the "clinical signs at AM" stream can be merged into the emergency slaughtered stream.

From the 1 March 2013 BSE testing criteria in Europe changed. EU member states (except Bulgaria and Romania) were not required to test healthy slaughtered animals, however, emergency slaughter and clinical signs (not BSE) at AM require testing prior to release into the food and feed chain[11]. C-TSEMM can generate the mean estimate for the number of infected animals in the healthy slaughter stream ($N\_infHS(a)$), emergency slaughter and clinical signs (not BSE) at AM ($N\_infES(a)$) together with 95th confidence intervals, where available, describing uncertainty about the mean, by MS for the EU27. The model can also output the number of animals detected by surveillance for the emergency slaughter and clinical signs (not BSE) at AM, thereby estimating the number of infected animals missed. For the annual estimates in the risk assessment, it is assumed that there is no healthy slaughter testing in the EU27.

The number of infected animals within C-TSEMM can be stratified by strain (classical, unknown, H-type and L-type). It was decided to sum the strain types together for the purposes of the risk assessment.

For bovine intestines and mesenteries, there are significant changes in infectivity levels early in the course of the incubation period. Therefore, it is important to model young animals in 6 monthly increments. The age intervals within C-TSEMM are <24 months, 24-29 months, 30-35 months, and in 12 monthly intervals for those >36 months. In order to model young animals less than 24 months old it is assumed that the number infected can be estimated as the proportion of the total number slaughtered multiplied by the estimated total number infected < 24 months. Data were not available on the number of animals slaughtered between 0 and 24 months. A questionnaire was sent by EFSA and data collected up to 2012 to supplement EFSA data previously gathered. Therefore, the age interval starts 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.

[11] Cattle which require testing before entry into food and feed chain:

- Healthy slaughtered cattle aged over 30 months born in Romania or Bulgaria
- Emergency slaughtered and clinical signs (not BSE) at AM aged over 48 months if born in EU Member States (except Bulgaria and Romania); or aged over 24 months if they were born in Romania or Bulgaria

The age class probability of an infected animal that has by-passed testing at slaughter, $Age_{slaughter}$, can be estimated by the following equation:

$$Age_{slaughter} = Discrete\left(\{a\}, \left\{\frac{N\_infected(a)}{\sum_{a=0}^{a=21} N\_infected(a)}\right\}\right)$$

Where

$$N\_infected(a) = N\_infHS(a) + N\_infES(a)$$

N_infected(a) is the number of infected animals that have by-passed any testing by age interval and is calculated as the addition of those infected animals in the healthy slaughter stream to those in the emergency slaughter and clinical signs (not BSE) at AM streams. The discrete distribution used is a function to describe a variable that can take one of several explicit discrete values with certain probability, for example in the risk assessment, selecting an age at slaughter interval (Vose, 2005).

Assumptions:
(1) In order to model those animals less than 24 months old in 6 monthly time steps, it is assumed that the number infected can be estimated from the proportion of the total slaughtered. For example, the number of animal 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.
(2) Currently there are no data to remove those animals <4 months old where bovine intestines and mesenteries would not be processed for consumption.
(3) In the UK animals are not permitted into the food and feed chain born before August 1996. However, due to the small proportion of the total this represents, all animals have been included in the UK analysis.

The model is set up to enable the user to select the MS or country grouping. Upon selection, the relevant data from C-TSEMM of the number of infected animals entering the food and feed chain and the number of infected young animals (< 24 months old) are automatically inserted into the model.

2.2. Estimating the random age at infection, $Age_{infection}$

For bovine intestines and mesenteries, the infectivity component requires an estimate of the probable age at infection in order to estimate infectivity titre. For other SRM, the age before clinical onset may be a more appropriate measure. Therefore, this component of the model may be further modified as required for other tissue types in future versions.

The probable age at infection, when exposure to BSE occurred and the length of the incubation period has been previously estimated by Arnold and Wilesmith (2004). Resulting probability distributions are used in the model. The distribution assumes that the majority of exposure occurs in the first 6 months of life. The random age at infection, $Age_{infection}$ is estimated by the following equation:

$$Age_{infection} = Discrete(\{exa\}, \{P\_exposure(exa)\})$$

Where *P_exposure(exa)* is the variable probability of infection occurring at each exposure age, *exa*, up to the age at slaughter for that random infected animal.

2.3. Estimating the months post infection at slaughter, $\mathbf{Age_{postinf}}$

The months post infection at point of slaughter for a random infected bovine animal, $Age_{postinf}$ is an output required for the infectivity component. The parameter is estimated by subtracting the age at slaughter ($Age_{slaughter}$) from the age at infection ($Age_{infection}$) for that random infected animal.

3. Abattoir and SRM components

Certain tissues in infected cattle have been identified as containing sufficient infectivity to be deemed Specified Risk Materials (SRM) with required removal and disposal practices from carcases. The SRM component of the risk assessment permits the user to modify SRM controls by tissue type and by age at slaughter to investigate the impact on the amount of infectivity generated by specific subsets of the population. Results in this report were generated for all tissue types, and a subset that could, theoretically, be processed.

For bovine intestines, the duodenum to the rectum is currently classified as SRM together with the mesenteries of all cattle slaughtered. The anatomical tissues distinguished between the duodenum and rectum are the jejunum, ileum, caecum and colon. It is assumed that the amount of infectivity in intestinal tissues can be estimated from the weight of the ileocaecal plate and Peyer's patches, that is, the lymphoid tissue that contains the BSE infectivity rather than the entire tissue weight. Due to a lack of information regarding the rectum this tissue type is not quantitatively assessed in the risk assessment.

For mesentery tissues it is assumed that the amount of infectivity can be estimated from the weight of the mesenteric lymph nodes, nerves and the celiac and mesenteric ganglion complex (CMGC).

Therefore the tissues (t) quantitatively modelled in the risk assessment are ileum (t=1), duodenum (t=2), jejunum (t=3), colon (t=4), caecum (t=5), and mesentery lymph nodes (t=6), mesentery celiac and mesenteric ganglion complex (CMGC) (t=7), and mesentery nerves (t=8).

Several lymphoid tissues types may be continuous through different anatomical tissues as shown in Figure 2. In order to clearly distinguish the location of the lymphoid tissue being described several different codes are used and are summarised as follows:

Ileocaecal plate in ileum	*PP1*
Peyer's patches in duodenum	*PP2*
Ileocaecal plate in jejunum	*PP3i*
Peyer's patches in jejunum	*PP3ii*
Ileocaecal plate in caecum	*PP4*
Lymphoid tissue in colon	*PP5*
Total Ileocaecal plate in small intestine	*PP1+PP3i*
Total Peyer's patches in duodenum and jejunum	*PP2+PP3ii*

For example, the jejunum contains two different types of lymphoid tissue; ileocaecal plate and Peyer's patches. Those areas of the jejunum covered by ileocaecal plate are denoted *3i* and those with Peyer's patches *3ii*. It is assumed that the amount of infectivity in mesentery tissues can be estimated from the entire weight of the mesenteric lymph nodes, nerves and the celiac and mesenteric ganglion complex (CMGC).

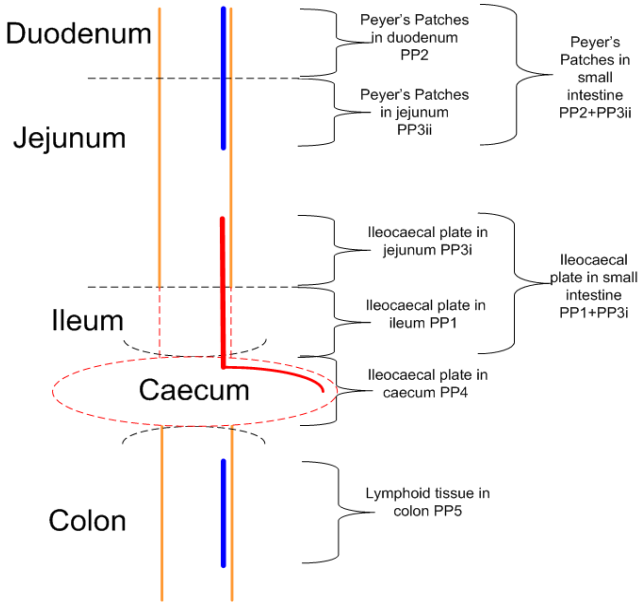

Figure 2: Simplified diagram of bovine intestines indicating location of ileocaecal plate (red line) in jejunum, ileum and caecum, and Peyer's patches PP (blue line) in duodenum, jejunum and colon. Diagram adapted from EFSA, pers. comm. 2012

Therefore, estimates of the weight and distribution of ileocaecal plate, Peyer's patches, mesenteric lymph nodes, mesenteric nerves and the CMGC are required, together with estimates of the length of intestinal tissue types in order to provide infectivity estimates on a per length basis. Such estimates vary according to the age of the animal at slaughter and are included in the model where available.

The age at slaughter estimates within the Surveillance component are <6 months, then in 6 monthly intervals up to 36 months. Therefore, it is assumed that the weight of tissues from infected animals slaughtered <6 months old can be approximated by the 4-6 data age intervals. This is an overestimate as those animals < 4 months old would not be processed into sausage casing. This part of the model could be modified if an estimate for the number of animals aged 0-3 months slaughtered or a proportion is available.

3.1. Length of intestinal tissues

The minimum and maximum length of the entire small intestine (duodenum, jejunum and ileum) in bovine animals at different ages was measured by Carlens (1928) for animals slaughtered at different ages. This variability is described in the model by a uniform distribution between the minimum and maximum estimate.

$$Length(1+2+3,a) \sim Uniform(Length_l(1+2+3,a), Length_u(1+2+3,a))$$

Estimates are available for the variable length of ileum denoted as $Length(1,a)$. The ileum in the risk assessment is anatomically defined as the part of the small intestine marked by the insertion of the plica ileocaecalis, with a length ranging from 0.5 to 1 m (ENSCA, 2012). Estimates for the length of duodenum, $Length(2,a)$, range from 0.9 to 1.2 m depending on the age of the animal (Nickel et al., 1987). 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 these ages in the risk assessment there is a linear increase in length which is seen in the overall length increase of the intestines by age as measured by Carlens (1928).

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

$$Length(3,a) = Length(1+2+3,a) - Length(1,a) - Length(2,a)$$

The length of the caecum and colon in bovine animals, $Length(4,a)$ and $Length(5,a)$, has been estimated to vary depending on the age of the animal (Nickel et al., 1987, Sisson and Grossman, 1953). For caecum the range is between 0.5 and 0.75 m. For 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 these ages there is a linear increase in length which is seen in the overall length increase of the intestines by age as measured by Carlens (1928). For animals aged over 24 months, the colon is assumed to vary between 10 and 12 m (Nickel et al., 1987, Sisson and Grossman, 1953). This variability has been described in the model by a uniform distribution.

Within a randomly selected animal there will be correlation between the lengths of the various tissue types in the intestines, however the degree of correlation is not known. From discussions with the EFSA Working Group a 90% correlation was assumed to be appropriate. In order to represent this dependency, the values selected from the distribution of length for the colon for animals aged over 24 months are 90% correlated to the selected value of the small intestine length. Therefore, for 9 out of every 10 values selected, when the sampling of the small intestine length returns a relatively "high" value, the sampling from the distribution of length for the colon for animals aged over 24 months also returns a relatively high value.

3.2. Weight of ileocaecal plate and Peyer's patches in small intestines

Infectivity in the small intestine is mainly associated with lymphoid tissue: Peyer's patches (PP), ileocaecal plate and isolated lymphoid follicles. Figure 2 shown previously displays a simplified representation of the intestines to indicate where the PP and ileocaecal plate are located within the defined tissue types included in the risk assessment. The total weight of small intestine PP and ileocaecal plate at different ages was measured by Carlens (1928) for cattle slaughtered at different ages, up to but not including the caecum which was investigated separately (refer to section 3.3). In order to model the duodenum, jejunum and ileum separately, the total weight measurements by Carlens are used in this risk assessment and allocated proportionally between the tissue types.

Carlens measured the total weight of ileocaecal plate and PP (*PP1+ PP3i+ PP2 +PP3ii*) in the small intestines and separately the weight of PP minus the ileocaecal plate area (*PP2+PP3ii*). The individual animal data are supplied in the form of two hand drawn graphs of weight against the age of the animal (Carlens, 1928). In order to estimate the individual animal data, the two graphs were loaded into a photo editing tool where the scale was adjusted to match the graph scale, and the x and y co-ordinates recorded for the darkest point of each plus mark denoting an individual animal recorded. This data was checked against the published mean values from the thesis (Carlens, 1928). For each individual animal identified by age, the weight of the ileocaecal plate (*PP1+PP3i*) was estimated from the total weight of ileocaecal plate and PP minus the PP found in duodenum and jejunum (*PP2+PP3ii*) as shown in Figures 3 and 4.

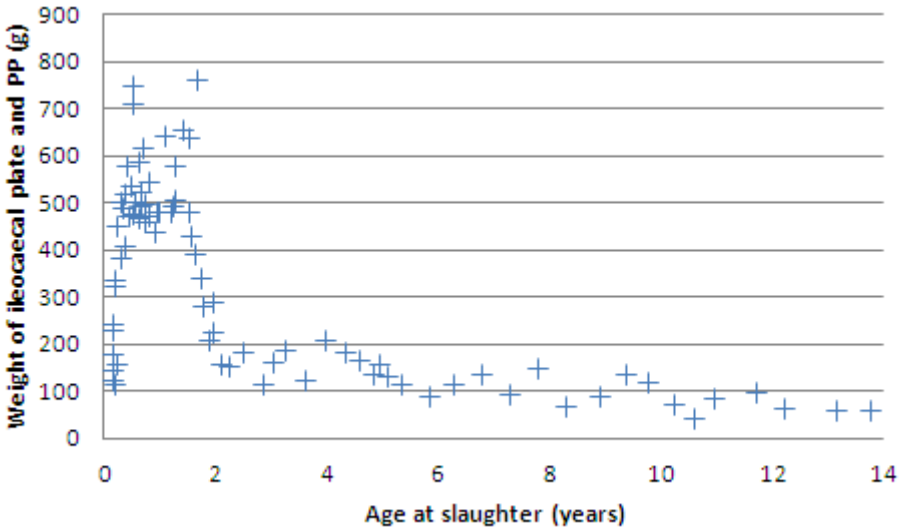

Figure 3: Graph of total weight of ileocaecal plate and Peyer's patches by animal age at slaughter adapted from Carlens (1928)

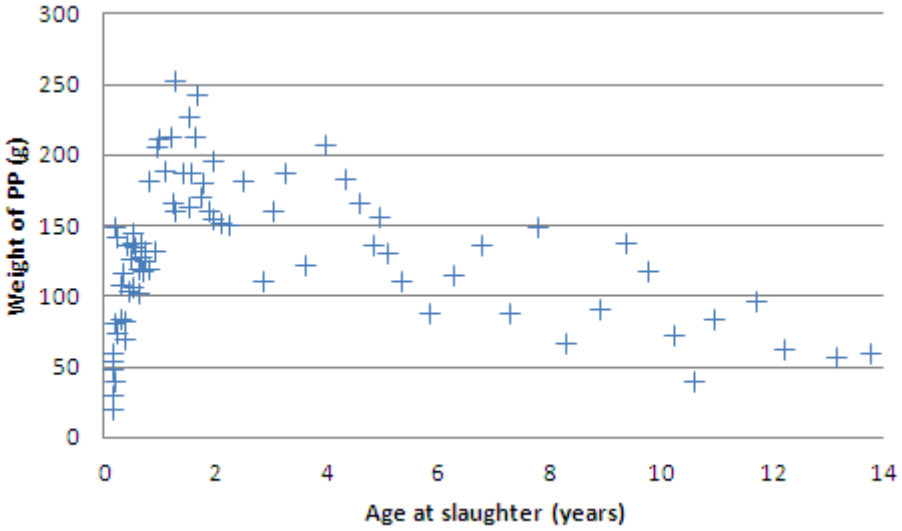

Figure 4: Graph of weight of Peyer's patches by animal age at slaughter adapted from Carlens (1928)

For each age interval, the between animal variability for the total weight of ileocaecal plate in ileum and jejunum, $Weight(PP1 + PP3i, a)$, and PP in duodenum and jejunum, $Weight(PP2 + PP3ii, a)$, is described by the estimated minimum, maximum and a fitted most likely weight by a Pert distribution to yield a distribution with the same mean value as given in Table 1. The fitted most likely values are provided in Appendix A.

Table 1: Weight of ileocaecal plate and Peyer's patches, mean, minimum and maximum values by age at slaughter adapted from Carlens (1928)

	Ileocaecal plate			Peyer's patches		
	Minimum weight (g)	**Mean weight (g)**	**Maximum weight**	**Minimum weight (g)**	**Mean weight (g)**	**Maximum weight**
Age at slaughter (months)						
< 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 there will be correlation between the weight of infectious tissues and the length of those tissues previously selected. However, the degree of correlation is not known. In order to represent this dependency, the values selected from the distribution of the ileocaecal plate weight in small intestines and weight of Peyer's patches in duodenum and jejunum are assumed to be 90% correlated to the selected value of the ileocaecal plate length, based on discussions with the EFSA Working Group (2012). Therefore, for 9 out of every 10 values selected, when the sampling of the ileocaecal plate length returns a relatively "high" value, the sampling from the distribution of the ileocaecal plate weight in small intestines, and weight of Peyer's patches in duodenum and jejunum, also return relatively high values.

3.2.1. Proportion of ileocaecal plate in ileum, $P(PP1)$

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 is assumed that the proportion of the ileocaecal plate located in the ileum, $P(PP1)$, 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). It is assumed that the proportion of ileocaecal plate in ileum and jejunum increases and decreases at the same rate by age at slaughter, such that 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 (EFSA Working Group, 2013). As the ileocaecal plate covers the length of the ileum, it is assumed that the length in the ileocaecal plate in the ileum is equal to the length of the ileum, $Length\,(1, a)$ at age interval 1 (under 6 months).

$$P(PP1) = \frac{Length(1,1)}{Length(PP1 + PP3i, 1)}$$

The total length of the ileocaecal plate, $Length(PP1 + PP3i, a)$, was measured by age at slaughter by Carlens (1928) with minimum and maximum values provided. This variability is described in the model using a uniform distribution.

The total weight of ileocaecal plate is therefore divided between the tissue types as shown in the following equations:

For ileum $\quad Weight(PP1, a) = P(PP1) * Weight(PP1 + PP3i, a)$

For jejunum $\quad Weight(PP3i, a) = \big(1 - P(PP1)\big) * Weight(PP1 + PP3i, a)$

3.2.2. Proportion of Peyer's patches in duodenum, $P(PP2, a)$

The proportion of the total Peyer's patches weight that is located in the duodenum, $P(PP2, a)$, is assumed to be equal to the length of duodenum divided by the total length of duodenum and jejunum by age at slaughter as shown by the following equation:

$$P(PP2, a) = \frac{Length(2, a)}{Length(2, a) + Length(3, a)}$$

The total weight of Peyer's patches is therefore divided between the tissue type as shown in the following equations:

For duodenum $Weight(PP2, a) = P(PP2, a) * Weight(PP2 + PP3ii, a)$
For jejunum $Weight(PP3ii, a) = \big(1 - P(PP2, a)\big) * Weight(PP2 + PP3ii, a)$

3.3. Weight of ileocaecal plate in the caecum

Carlens (1928) investigated the lymphoid tissue present in the caecum. Follicles were seen around the Ostium ileale and are described as a rounded to three-cornered plate. However, an exact size is not mentioned. Additional accumulations of solitary follicles are described nearby the Ostium ileale in the caecal mucosa . These "patches" are distributed in a region of about 60-80 cm^2 (Carlens 1928). In the absence of further data on the occurrence of lymphoid tissue in the caecum, it is assumed 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 ($PP1$), multiplied by the surface area of follicles measured in the caecum, $Area(PP4)$, as shown in the following equation:

$$Weight(PP4, a) = Area(PP4) * \frac{Weight(PP1, a)}{Area(PP1, a)}$$

Where

$$Area(PP1, a) = 2\pi * Radius(1) * Length(PP1, a)$$
$$Length(PP1, a) = Length(PP1 + PP3i, a) * P(PP1)$$

These equations assume that the surface area of the ileocaecal plate in the ileum is tube shaped covering the entire inner surface. Therefore the surface area is equal to 2π multiplied by the radius of the ileum, $Radius(1)$, and length of the ileocaecal plate in the ileum, $Length(PP1, a)$. The area of lymphoid tissue in the caecum has been estimated as 60-80 cm^2 (Carlens 1928), and the radius of the ileum is estimated to vary between 3.2 and 5.2 cm (ENSCA, pers. comm. 2012). The variability associated with the area of lymphoid tissue and ileum radius is described in the model using a uniform distribution.

3.4. Weight of Peyer's patches in the colon

In the absence of data on the weight and occurrence of PP in the colon, it is assumed that the number of follicles in the colon can be approximated by the concentration of Peyer's patches (weight per meter length) that is estimated to be present in the jejunum,$WLength(PP3ii, a)$, multiplied by the length of the colon, $Length(5, a)$. The weight of Peyer's Patches per length of jejunum is estimated by:

$$WLength(PP3ii, a) = \frac{Weight(PP3ii, a)}{Length(3, a)}$$

3.5. Weight of mesentery lymph nodes

There is little quantitative data regarding the weight of mesenteric lymph nodes. The weight, $Weight(6)$, has been measured for 25 calves under 6 months of age and for 33 cattle older than 6 months (excluding two animals of unknown age) (Jänicke, 1911). It is assumed that the entire weight of lymph nodes may be infectious. With no further information, calves under 6 months of age are described in the model using a pert distribution of mean 73.5 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, mesentery lymph nodes from animals older than 6 months are described using a pert distribution of mean 168.1 g, with minimum value of 69.8 g and maximum value of 283.0 g.

3.6. Weight of mesentery nerves

It is assumed that the entire weight of mesentery nerves may be infectious. There are no known quantitative estimates for the weight of mesenteric nerves in bovine intestines. From discussion with pathologists who routinely conduct post-mortems, with consideration for prior estimates regarding peripheral nerves, expert opinion is used to parameterise this tissue type with a mean weight ranging from 100 g for animals less than 6 months old to 200g for those greater 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 the pert distribution and the assumption used for modelling the small intestine, that there is a linear weight gain relationship between the age intervals for those animals less than 24 months old with resulting values shown in Table 2. 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 2. The fitted most likely values are provided in Appendix A.

Table 2: Expert opinion estimates for the weight of mesenteric nerves with minimum, mean and maximum values

	Mesenteric nerves		
	Minimum weight (g)	**Mean weight (g)**	**Maximum weight (g)**
Age at slaughter (months)			
< 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

3.7. Weight of mesentery CMGC

It is assumed that the entire weight of mesentery celiac and mesenteric ganglion complex (CMGC) may be infectious. From a review of the literature, there are no quantitative data on the weight of this ganglion complex in cattle. However, dimensional data are available from a 350 kg pony. The animal dissected possessed two separate ganglion measured as diameter 3.5-4.0 cm (right hand side) and 6.0cm (left hand side), with a height of 1.5 cm (RHS) and 1.2 cm (LHS) (Dyce, 1958). Assuming the volume of a cylinder and that the density of nerves is equal to 1 g/cm^3, permits the estimation of the weight of each ganglion. The combined estimated weight from the pony equals an average of 50.5 g.
From discussions within the EFSA Working Group, expert opinion is used to parameterise this tissue type. 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 risk assessment. 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 the 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 3. The fitted most likely values are provided in Appendix A.

Table 3: Expert opinion estimates for the weight of CMGC with minimum, mean and maximum values

	Mesenteric nerves		
	Minimum weight (g)	**Mean weight (g)**	**Maximum weight (g)**
Age at slaughter (months)			
< 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

4. Processing component

The processing component estimates the reduction, if any, of the weight of infectious materials or infectivity resulting from the processing of tissues prior to entry into the food and feed chain. Specific to the first task for bovine intestines and mesentery, derived edible products include bovine intestines processed into sausage casings and the rendering of fats from mesentery tissues. The ileum is not processed for human consumption and therefore no reduction in the infectious titre is assumed.

4.1. Reduction of infectivity due to processing, $P_{processing}(t)$

Mesentery tissue may be rendered into edible fats. However, in view of the high resistance of prions to inactivation and the maximum temperatures the process achieves at approximately 95°C, the assumption is made that the tallow production method does not have any impact in reducing the infectivity level in the processed material.

The reduction in any infectivity present in bovine intestinal tissues (duodenum, jejunum, colon, and caecum) which may be processed into casings is not known. Wijnker et al., (2008) conducted a histological analysis of bovine small intestines before and after processing into natural sausage casings, producing an estimate of the reduction in weight of lymphoid tissue of 48% using standard cleaning methods. From discussions concerning this work and other studies, it was estimated that the variability in the reduction of infectivity per animal could be described as a minimum of 0 to a maximum of 50% with this variability described in the model using a uniform distribution.

5. 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. Specific to the first task for bovine intestines and mesentery the age post infection is used together with infectivity data for the following tissue types (t), ileum (t=1), duodenum (t=2), jejunum (t=3), colon (t=4), caecum (t=5), mesentery lymph nodes (t=6), mesentery celiac and mesenteric ganglion complex (CMGC) (t=7) and mesentery nerves (t=8).

BSE infectivity has been detected in the intestinal tissues using different methods including mouse bioassay. Positive data for distal ileum have been generated using bovine transgenic mouse models

(Hoffman et al., 2011) and in conventional mouse lines (Wells et al., 2005). In order to compare the results from such studies and potentially combine them to increase the power of the analysis, it is essential to derive the dose response curves (probability of survival and mean incubation period versus dose) for each mouse line used. Given a reference material the relative sensitivities of each mouse line can then be compared.

5.1. Estimation of RIII and TgBov mice relative sensitivities

In order to estimate titres from mouse bioassay data, it is essential to know the dose-response for a standard BSE inoculum in a given mouse line, in terms of both the probability of survival and the incubation period for a given dose (Arnold et al., 2009). Ideally, dose-response curves would be available for each tissue for which the titre is to be estimated, since there can be difference between the dose-response curves of different tissues (Robinson et al., 1990). However, only titrations of brain homogenate were available with which to produce dose-response curves, so it is assumed that the dose-response relationship for brain is applicable to all the tissues for which the titre is estimated in the present study. For RIII mice, data was available from seven separate titrations of BSE brain homogenate in mice. For each study 5 serial 10-fold dilutions were performed, and each mouse was injected with 0.02mL by the intracerebral (i.c.) route and 0.1mL by the intraperitoneal (i.p.) route from a 10% suspension of brain homogenate. A total of 552 mice were available in the combined dataset. The parameters determining the probability of disease versus dose were estimated using logistic regression. The parameters determining the incubation period versus dose were estimated by fitting a normal distribution to the observed incubation periods of each positive mouse, the mean of the distribution assumed to be linearly related to the $\log_{10}$ dose of inoculum, and the standard deviation assumed to be constant across doses.

The parameters determining the dose-response of TgBov XV mice were estimated from the individual mouse data from Buschmann and Groschup (2005) using a similar approach to that for RIII mice i.e. logistic regression for the survival and linear regression for the mean incubation period.

The titre of infectivity in a reference inoculum denoted BP 12/92 was estimated using the approach described in Arnold et al. (2009) in terms of RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g which is presented in Appendix B. In brief, the estimation takes into account the rate of survival and the observed incubation periods at each dose. This method has been updated by using a Bayesian approach, which was more suitable to determine the uncertainty in each parameter, particularly the uncertainty in the dose response parameters. The titre was estimated separately for the titration performed at VLA (now AHVLA) (5 groups of 20 RIII mice) and FLI (10 groups of 5-6 TgBov XV mice) and compared to investigate if the data from the two titrations could be combined. The titre in terms of TgBov mouse i.c. i.p. $\log_{10}$ ID_{50}/g was estimated using logistic regression (unlike the RIII mice there was no data to parameterise the Arnold model except for the titration data from the Buschmann and Groschup study).

The titre of the BP12/92 inoculum was estimated to be 3.45 $\log_{10}$ ID_{50}/g in terms of RIII mouse i.c. i.p. (95% CI: 3.14-3.77). The estimated titre in terms of TgBov XV mice was 8.21 $\log_{10}$ ID50/g i.e. a relative efficiency of 4.76 $\log_{10}$ greater in TgBov XV compared to RIII mice (95% CI: 4.25, 5.21). Figure 2.1 and 2.2 in Appendix B display the estimated dose-response and mean incubation period versus the dose for each mouse line. These data were used to convert titre of infectivity in tissues in terms of RIII mouse i.c. i.p. ID_{50}/g, estimated by bioassay using TgBov XV mice.

5.2. Estimating the infectivity titre in the ileum by months post infection, $Titre(\mathbf{1}, m)$

The titre of infectivity in distal ileum was estimated using the method developed in Arnold et al. (2009) using both the probability of survival (attack rate at each dilution) and the individual mouse incubation periods at each dilution, except that a Bayesian approach was adopted instead of the maximum likelihood approach. This method is able to provide a more accurate estimation of titre than

using attack rate alone, and provides a natural way to deal with mice that have died before the end of the experiment without having to decide debatable inclusion/exclusion criteria.

There was a difference in the volume of inoculum between the titration of BP12/92 in TgBov XV mice (0.02mL i.c. route and 0.1mL i.p) in the Buschmann and Groschup (2005) study and the Hoffman et al (2011) study (0.03mL i.c.) – for the purposes of the estimation it was assumed that the two doses were equivalent. If one were to assume, for example, that the i.p. route made no difference to the inoculum infectivity, i.e. the 0.03mL i.c. inoculum was 1.5 times the equivalent dose of the 0.02mL i.c. route and 0.1mL i.p then this would relate to a reduction in the estimated titres of 0.17 (i.e. the effect is small relative to the uncertainty in the titres in each tissue at each time point). Estimates of titre for the distal ileum were compared with those estimated in Arnold et al. (2009).

The titre of infectivity was highly variable, especially at 12 months post infection (mpi) with a maximum titre of 1.01 and a minimum titre of -2.31 RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g (refer to Table 2.1 in Appendix B). However, given the limited information available for both tissues, there was no clear pattern of any changes in the titre of infectivity with changes in age for either distal ileum or jejunum in those samples that were positive as shown in Figure 5.

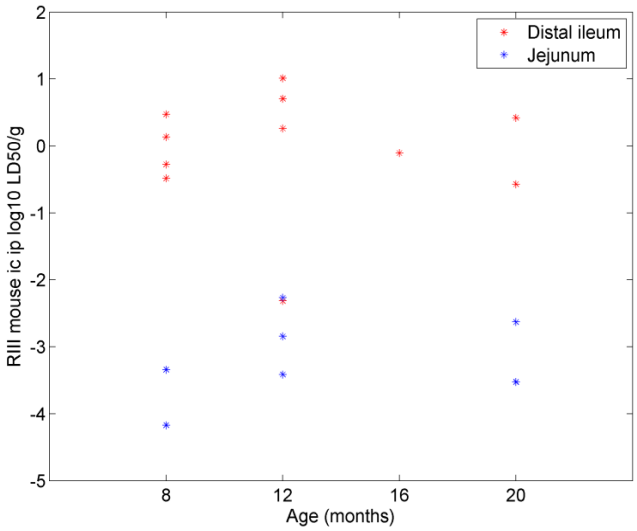

Figure 5: Estimated titre of infectivity in distal ileum and jejunum obtained from TgBov XV mouse bioassay of samples from cattle at 8-20 mpi

Estimates of infectivity in the distal ileum have previously been calculated in Arnold et al (2009) at 6, 10, 14 and 18 mpi using data from the VLA pathogenesis study (estimates are also available for 36, 38 and 40 mpi). The VLA pathogenesis study consisted of pooled samples from four cattle, so to compare an average titre from the German pathogenesis study (samples not pooled), estimates were calculated for pooled animals (the mean of the antilog of each of the titres from the 4 animals at each age range).

Unfortunately comparison is complicated by the differences in ages at sampling between the VLA and German pathogenesis study, but the estimated titres from the VLA pathogenesis study for distal ileum were 1.01, 1.1, 1.59 and 1.58 at 6, 10, 14 and 18 mpi respectively (titres in both studies are in terms of RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g). The estimated titres of a pool of 4 samples for the German data would be 0.11, 0.63, -0.71, -0.14 respectively at 8, 12, 16 and 20 mpi. respectively [0.11= ¼ ($10^{0.47}$+$10^{-0.48}$ +$10^{0.13}$+$10^{-0.28}$)]. The confidence intervals for the estimates at 6 and 10 (VLA) and 8 and 12 (German pathogenesis study) overlap (the lower bound of the credible interval of the Arnold titres at 6 and 10 months, taking into account uncertainty in the dose-response parameters, are -0.65 and -0.55 RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g, i.e. lower than the posterior median estimates obtained for

Hoffman titres at 8 and 12 months), indicating that difference at these ages are not statistically significant. A key difference is that the mean titre declines at 16 and 20 mpi compared to earlier time points in the German pathogenesis study but increases at 14 and 18 mpi in the VLA study compared to earlier time points, leading to a significant divergence between estimates. However, with such a small number of distal ileum samples it remains possible that an individual distal ileum sample had an unusually high titre in the VLA study leading to a relatively high titre of the pool. This is especially possible due to the highly heterogeneous distribution of infectious follicles in the distal ileum (Stack et al., 2011).

Based on available data from the combined analysis of German data from FLI and VLA, there is no discernible pattern to infectivity titres in the distal ileum. Therefore, it is assumed that infectivity in the distal ileum is essentially random with high between animal variability. It is possible to combine the titre data from Arnold et al., (2009) at 6, 10, 14, 18, 38, and 40 months and Hoffman et al., (2011) at 8, 12, 16, and 20 months. A Lilliefors test applied to the 17 individual distal ileum titre estimates from the Arnold and Hoffman studies indicated no significant deviation from a Normal distribution (P=0.37). Therefore the Arnold and Hoffman data were combined by amending the Bayesian model that estimates the titre at each time point for each tissue to a full hierarchical Bayesian model, where the titre at each time point was assumed to arise from a normal distribution with unknown mean and variance. The Bayesian hierarchical model then estimates this mean and variance. This approach, yielded a posterior estimate of mean titre equal to 0.41 RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g with standard deviation of 1.22, yielding a 95% range of between animal variability of -1.98 to 2.8.

The estimate of the distal ileum titres from the Bayesian hierarchic model produced an upper 95% credible interval (2.8) that was much higher than the greatest observed value (2.1) and thus may produce pessimistic estimates. An alternative approach to estimating the titre in distal ileum and its variability is to estimate the titres for each sample using the Bayesian model, and then simply estimate the mean and variance of the resulting 17 estimates. It is a simpler approach than using the Bayesian hierarchical model since it does not account for the uncertainty in the titre at each time point nor any prior information. A normal distribution through the median titres at each time point yields a mean of 0.37, and standard deviation of 0.81 giving a 95% range of -1.2 and 2.1. This is more in line with the maximum value observed but the lower bound is exceeded by one observation.

The risk assessment was run using each of these alternative parameters for the ileum. The infrequent but high values (5% of samples above 2.4 log, 2% of samples greater than 3 log) generated by the full hierarchical Bayesian approach caused a significant impact on the number of iterations required for model convergence, whilst there was no significant impact on key model results (the differences between the mean total infectivity per animal were within the 4% variation in the mean between different simulations). Therefore, the second parameterisation of a mean of 0.37 and standard deviation of 0.81 is used in the risk assessment.

The titre of infectivity estimated for the ileum is based on the infectious lymphoid tissue called the ileocaecal plate within the ileum. Therefore the estimate is used in the risk assessment for estimating the RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g for the ileocaecal plate present in the ileum (*PP1*) and in the jejunum (*PP3i*).

5.3. Estimating the relative titre of infectivity in jejunum, $Titre_{lower}$

The titre of infectivity in Peyer's patches in the jejunum (*PP3ii*) was also estimated using the method developed in Arnold et al. (2009). There was no clear pattern in the likelihood of a jejunum sample being positive in the age range 8-20 mpi with 2/8, 3/8, 0/8 and 2/8 samples being positive for 8, 12, 16 and 20 months p.i. respectively. The titre of infectivity was highly variable, with a 2 log10 difference between the highest and lowest titre (refer to Figure 5 with data supplied in Table 2.2 in Appendix B),

but the titre of infectivity in jejunum was consistently approximately 3 $\log_{10}$ lower than the titre in distal ileum.

Based on the available data there is no discernible pattern to infectivity titres in the Peyer's patches in the jejunum when considered as a separate tissue. 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 RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g, with a 95% uncertainty range of 3.072 to 4.384. This is described in the model using a modified pert distribution.

5.4. Estimating the infectivity titre in duodenum, caecum and colon

There are limited data on the accumulation of abnormal PrP in the duodenum, caecum and colon. Positive results have been recorded for the myenteric plexus of the duodenum (immunohistochemistry only, Kimura and Haritani 2008), lymphoreticular tissue from ileocaecal junction (Hoffman et al., 2011), and colon (Okada et al., 2010).

The Peyer's patches in the duodenum and 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 titre has an equal titre to that estimated for Peyer's patches in the jejunum. For the caecum, it is assumed that any infectivity accumulating will be the same as that estimated for the ileocaecal plate in the ileum. These assumptions can be considered as a worst case scenario and can be revised in the future if new data would become available.

5.5. Estimating the infectivity titre in mesenteric lymph nodes, $\boldsymbol{Titre(6,m)}$

There are few positive results found from testing lymph-reticular structures from infected animals. Negative results have been recorded for spleen, thymus (cervical), tonsil (Wells et al., 2005), submandibular lymph node, retropharyngeal lymph node, bronchial-mediastinal lymph node, hepatic lymph node, mesenteric lymph node, prescapular lymph node and popliteal lymph node in RIII mice (SSC, 2002) and mesenteric lymph node in TgBov mice (Buschmann and Groschup, 2005). However, Franz et al. (2012) detected evidence of BSE agent by Protein Misfolding Cyclic Amplification in mesenteric lymph nodes collected in one orally challenged bovine animal, demonstrating infectivity may accumulate in some animals at low levels.

It is assumed that infectivity in mesenteric lymph nodes ranges from no infectivity to the upper confidence interval established from 0 positive mice out of 12 TgBov XV mice as recorded by Buschmann and Groschup, 2005. This has been estimated as a maximum titre of -6.7 $\log_{10}$ RIII mouse i.c. i.p. ID_{50}/g (Buschmann and Groschup, 2005 as adapted by Arnold, 2012). The uncertainty associated with this range is accounted for using a uniform distribution.

5.6. Estimating the infectivity titre in mesenteric nerves and CMGC, $\boldsymbol{Titre(7,m)}$, $\boldsymbol{Titre(8,m)}$

There are few data on the pattern and titre of prion infectivity in the mesenteric nerves and celiac and mesenteric ganglion complex (CMGC). It is assumed that infectivity could accumulate as early as the ileal Peyer's Patches become positive and persist until clinical onset. Kaatz et al. (2012) reported positive bioassay results in TgBov XV mice inoculated with autonomic nerves (vagus cervical and thoracic nerves) and autonomic ganglia (CMGC and mesenteriale caudale) collected from cattle orally challenged with BSE and killed at various time points months post inoculation. It is assumed that the pattern and titre of infectivity detected in the vagus nerves and ganglia can be used to represent the overall pattern and titre in mesenteric nerves and CMGC.

The individual mouse bioassay data from the study was supplied by the authors and used to estimate the titre at each experimental kill point (16-36 months) in the CMGC and ganglion mesenteriale caudale and the vagus nerve (consisting of cervical and thoracic vagus nerve) with results shown in Figure 6. In both cases there appeared to be a linear increase in the $\log_{10}$ titres as month post infection increased (denoting exponential increase), although there was considerable variability about the line of best fit fitted to the mean titre. It is assumed that infectivity will increase by months post infection until a plateau is reached. There are no data available after 36 months post infection. Therefore it is assumed that the maximum titre reached is equal to the upper 95% credible interval of the titre estimated for the identified clinical animal in the dataset. The upper credible interval of the titre for that animal in the dataset was -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.

For the sciatic, facial and radial nerves measured in Buschmann et al., (2005) the mean titre of infectivity was estimated as -3.1 (-3.6, -2.7) RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g. The titres in the Buschmann paper for the clinical animal are much lower than those from experimental studies, probably due to large differences in dose. Therefore, it is acknowledged that the maximum titres achieved of -0.013 and -0.01 RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g for mesenteric nerves and CGMC are a pessimistic upper limit. The impact on the model results from using the alternative parameters is investigated in the scenario analysis (section 9.1).

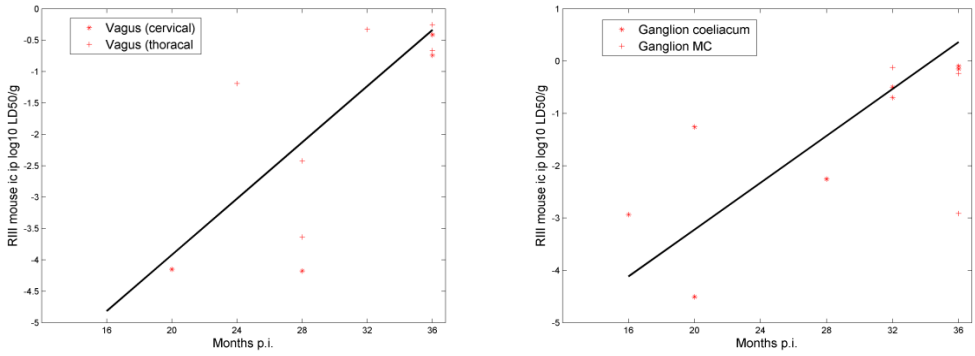

Figure 6: Estimated titres of infectivity in cervical and thoracic vagus nerve and CMGC and ganglion MC, from original data described within Kaatz et al. (2012), and the linear regression line representing the mean titre

The EFSA Working Group considered that the most plausible assumption regarding the rate of growth of CMGC and the vagus nerve was exponential growth, in concert with those of the CNS. Therefore a common rate of increase was estimated for the CMGC and Vagus nerve, using the rate of increase in the CNS reported in Arnold et al. (2009) as a prior for the Bayesian model. The common growth rate of CNS, vagus and CMGC was estimated as 0.224 (95% Credible interval (CrI): 0.20-0.25), with constant terms -8.40 (95% CrI: -9.22, -7.47) for vagus and -7.70 (95% CrI: -8.53, -6.82) for CMGC as shown in Figure 6.

5.7. Estimating the conversion of RIII mouse ic.ip ID_{50}/g to bovine oral infectivity units, BO_{unit}

The majority of infectivity titrations of the BSE agent have been conducted in mice and expressed as mouse intracerebral plus intraperitoneal (ic.ip) ID_{50}/g, whereas for the purposes of this risk assessment infectivity is additionally expressed as bovine oral ID_{50}/g. To convert the titre units, each estimated titre of infectivity assayed in mice, is derived by division with a conversion factor estimated in Wells

et al., 2007 as updated in Konold et al., 2012. Analysis of data from this experimental work estimated a most likely value of $10^{2.7}$ mouse ic.ip ID_{50}/g with 95th uncertainty intervals of 10^{2} to $10^{3.4}$. This uncertainty is accounted for in the model using a pert distribution.

6. Outputs and Central Component

Combining the outputs from the components surveillance, abattoir and processing and infectivity permits estimation of the total infectivity (BO ID_{50}) entering the food and feed chain from a randomly selected infected animal that has by-passed testing controls, together with annual results at the EU27 level, or within a single member state.

At the level of a single infected animal (drawn from the slaughter population of interest), the amount of infectivity at slaughter by tissue type, $Infectivity\_pre(t)$ is equal to:

$$Infectivity_{pre}(t) = Weight(t) * 10^{\left(\frac{Titre(t)}{BOunit}\right)}$$

And after processing by tissue type (for intestinal tissues):

$$Infectivity(t) = Weight(t) * 10^{\left(\frac{Titre(t)}{BOunit}\right)} * (1 - P_{processing}(t))$$

The results on a per length basis for intestinal tissues are given by:

$$InfectivityL_{pre}(t) = \frac{Weight(t) * 10^{\left(\frac{Titre(t)}{BOunit}\right)}}{Length(t)}, \text{ and}$$

$$InfectivityL(t) = \frac{\left(Weight(t) * 10^{\left(\frac{Titre(t)}{BOunit}\right)}\right) * (1 - P_{processing}(t))}{Length(t)}$$

At the EU27 level over one year, the total amount of infectivity resulting from infected animals by tissue type over one year at slaughter (BO ID_{50} per year) is given by

$$Inf_{yr_{pre}}(t) = \sum_{N_{carcases}=1}^{N_{carcases}} Infectivity_{pre}\,(t)$$

And for processed tissues (duodenum, jejunum, caecum and colon):

$$Inf_{yr}(t) = \sum_{N_{carcases}=1}^{N_{carcases}} Infectivity\,(t)$$

Results to investigate the progress of infectivity by tissue type over time are produced by sequentially simulating a random infected animal of selected age at slaughter, that is, assigning a constant value for $Age_{slaughter}$ rather than randomly selecting age from the expected slaughter population. Results are provided for animals aged <6 months, 6 to 12 months, 12 to 18 months, 18 to 24 months, 24 to 36 months, 36 to 48 months, 48 to 60 months, and those animals 60 to 120 months.

7. Results and Discussion

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 meter length, and the infectivity remaining in processed intestinal 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.

The risk assessment has been developed such that only variability in the parameters can be modelled; however, the final results presented in this report represent both variability and uncertainty. An investigation into the impact of uncertainty is provided within section 9 Sensitivity Analysis and Parameter Uncertainty.

7.1. Pattern of infectivity over time in bovine intestines and mesenteries from an infected animal by age at slaughter

7.1.1. Pattern of infectivity in intestines and mesenteries (BO ID_{50}) per infected animal

The progression of BSE infectivity by age at slaughter for each tissue type included in the risk assessment is shown in Figure 7 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 BO ID_{50} per animal and decline to low levels, less than one BO ID_{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.

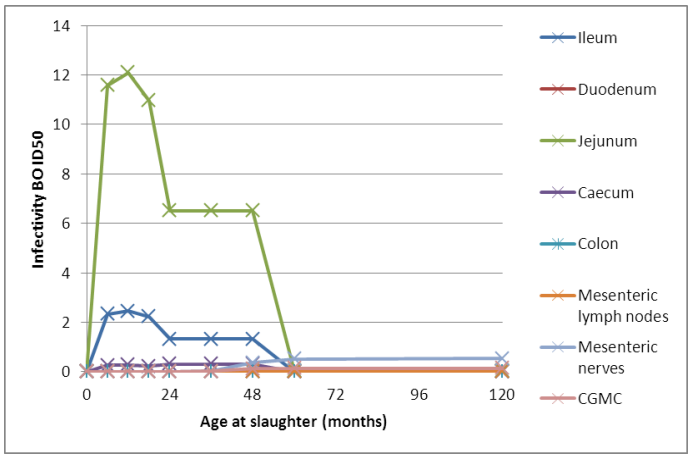

Figure 7: Summary graph of estimated mean infectivity by individual intestine and mesentery tissue type (BO ID_{50} per infected animal) by age at slaughter

All results generated are associated with uncertainty and variability. Refer to Appendix C for each infectivity distribution by tissue type at slaughter with 95% uncertainty and variability range.

Table 5 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 48 months. However, mesenteric nerves dominate the load in older aged animals, 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 5: 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 (BO ID_{50})	**14.16**	**14.81**	**13.41**	**8.15**	**8.17**	**8.62**	**0.70**	**0.72**
Total ileocaecal plate (BO ID_{50})	13.91	14.54	13.18	7.83	7.83	7.83	0.05	0.05
Jejunal ileocaecal plate (BO ID_{50})	11.58	12.09	10.96	6.51	6.51	6.51	0.04	0.04

7.1.2. Pattern of infectivity in intestines by length (BO ID_{50}/m)

For each individual intestinal tissue (ileum, duodenum, jejunum, colon and caecum) and the combined ileocaecal plate (ileum and jejunal plate) the estimated mean, 2.5th and 97.5th percentiles of infectivity per meter length by age at slaughter is provided in Table 6. It can be seen from individual tissues that the ileum has the highest amount of infectivity per meter with a mean estimate of 4.6 BO ID_{50}/m at age 6 months. This reduces to less than one BO ID_{50}/m by age 60 months. The caecum and jejunum also contain significant infectivity per meter, which peaks at 6 months with a mean of 0.51 and 0.39 BO ID_{50}/m respectively. The duodenum and colon exhibit much lower infectivity per meter, 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 BO ID_{50}/m between 24 and 48 months, before declining to approximately 0.03 BO ID_{50}/m.

Table 6: Mean infectivity per length (BO ID_{50}/m) for intestinal tissues by age at slaughter (2.5th and 97.5th percentiles in brackets)

	Infectivity per length (BO ID_{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

7.1.3. Pattern of infectivity for processed products, infectivity load (BO ID_{50}) per infected animal

Results have also been provided for the infectivity of processed products (duodenum, jejunum, colon, caecum and mesentery tissues) as shown in Figure 8. Reductions in infectivity due to processing have been applied to the duodenum, jejunum, caecum, and colon equating to an average 25% reduction in infectivity by tissue. This results in the largest reduction of approximately 3 BO ID_{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 BO ID_{50} by processing. Table 7 presents the mean infectivity of processed tissues by age at slaughter. From the Table it can be seen that for animals greater than 60 months, the processed mesentery tissues contribute the most to a low total infectivity in processed products (0.7 BO ID_{50}). All results generated are associated with uncertainty and variability. Refer to Appendix C for each distribution by tissue type after processing with 95% uncertainty and variability range.

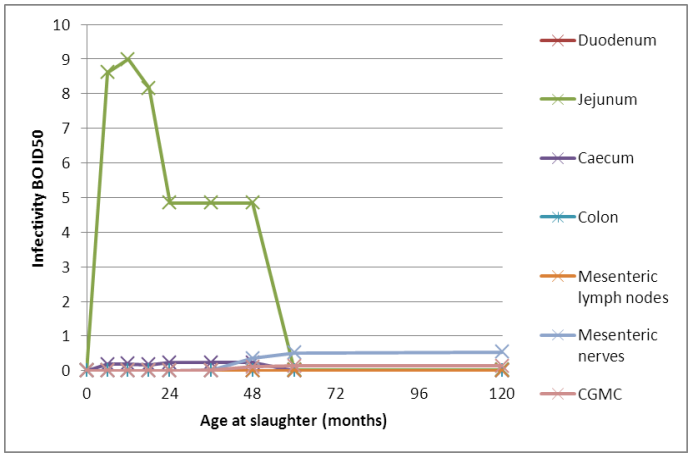

Figure 8: Summary graph of estimated mean infectivity of processed products (BO ID_{50} per infected animal) by age at slaughter

Table 7: 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 (BO ID_{50})	**8.83**	**9.22**	**8.35**	**5.14**	**5.16**	**5.59**	**0.68**	**0.70**
Jejunal ileocaecal plate (BO ID_{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

7.1.4. Pattern of infectivity for processed intestinal products, infectivity per length (BO ID_{50}/m)

For processed intestinal tissue (duodenum, jejunum, colon and caecum) the estimated mean, 2.5th and 97.5th percentiles of infectivity per meter length by age at slaughter after processing is provided in Table 8. The infectivity per meter length for a subset of the jejunum tissue is provided for the jejunal ileocaecal plate. Comparing these results for the individual tissues from those before processing in Table 6, it can be seen that the mean results reflect, on average a 25% reduction in infectivity per meter.

Table 8: Mean infectivity per length (BO ID_{50}/m) for processed intestinal tissues by age at slaughter (2.5th and 97.5th percentiles in brackets)

	Infectivity per length (BO ID_{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

7.2. Amount of infectivity per infected animal (drawn from EU27 slaughter population) by tissue type, BO ID_{50}/animal

7.2.1. Amount of infectivity in intestines and mesenteries (BO ID_{50}) per EU27 infected animal

In the previous sections 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. Therefore, the mean infectivity estimated by tissue type reflects the average infected animal destined for the food and feed chain in 2012. Figure 9 displays the estimated distribution of total baseline infectivity from intestines and mesenteries per infected animal. An estimated mean amount of infectivity of 3 BO ID_{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 BO ID_{50}/animal accounting for uncertainty and variability. The distributions by tissue type before any processing are provided in Appendix C.

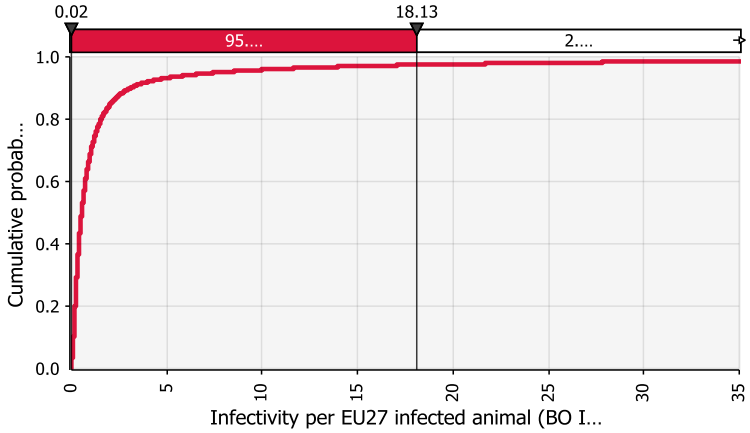

Figure 9: Cumulative probability function describing the baseline total amount of infectivity in bovine intestines and mesenteries at slaughter (BO ID_{50}) for an EU27 infected animal considering uncertainty and variability (95% percentiles indicated by top bar)

It can be seen that the mean total infectivity at slaughter (3 BO ID_{50}/animal) for the average EU27 infected animal equates in Table 5 (also shown in Figure 7) 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.

Figure 10 displays the estimated distribution of infectivity from processed products (duodenum, jejunum, colon, caecum and mesentery tissues) from an infected animal from the EU27 where the duodenum, jejunum, colon and caecum have undergone processing treatment which reduces BSE infectivity levels by, on average, 25%. An estimated mean amount of infectivity of 2.2 BO ID_{50} arises from processed products from a single animal, with 2.5th and 97.5th percentiles this varies 0.01 and 11 BO ID_{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 C.

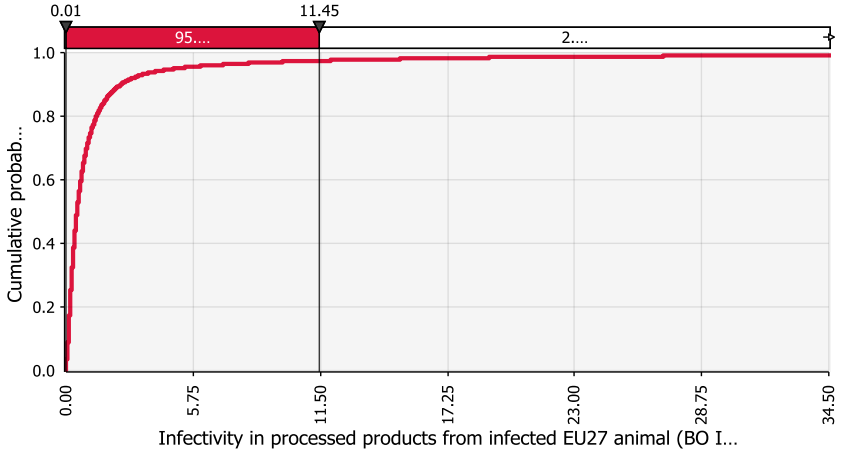

Figure 10: Cumulative probability function describing the baseline total amount of infectivity from processed products (duodenum, jejunum, colon, caecum and mesentery tissues) from an infected animal from the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

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 9. The estimated amount of infectivity for 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 9 that jejunum, ileum and mesenteric nerves contribute significantly to the total of an average EU27 infected slaughtered animal.

Table 9: Baseline mean infectivity (BO ID_{50}) by tissue type in an infected EU27 slaughter animal and post processing (2.5^{th} and 97.5^{th} percentiles in brackets)

	Infectivity (BO ID_{50}) Mean (2.5^{th} and 97.5^{th})	**Mean % contribution**	**Infectivity post processing* (BO ID_{50}) Mean (2.5^{th} and 97.5^{th})**
Tissue type			
Ileum	0.44 ($8x10^{-6}$, 3)	14%	-
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%]	-
Jejunal 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

7.2.2. Amount of infectivity in intestines per EU27 infected animal, infectivity per length (BO ID_{50}/m)

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 meter length for an average EU27 infected slaughter animal is provided in Table 10. Estimated results for processed products are also provided. It can be seen that the infectivity per length are most similar to those animals with a slaughter age older than 48 months.

Table 10: Baseline mean infectivity per length (BO ID_{50}/m) for intestinal tissues within an infected EU27 slaughter animal (2.5th and 97.5th percentiles in brackets)

	Infectivity unprocessed per length (BO ID_{50}/m) Mean (2.5th and 97.5th)	**Infectivity post processing* per length (BO ID_{50}/m) Mean (2.5th and 97.5th)**
Tissue type		
Ileum	0.55 (8×10^{-6}, 4)	-
Duodenum	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})
Jejunum	5.7×10^{-2} (1×10^{-6}, 0.4)	4.2×10^{-2} (7×10^{-7}, 0.3)
Caecum	0.12 (3×10^{-6}, 0.8)	0.1 (2×10^{-6}, 0.6)
Colon	3.1×10^{-5} (3×10^{-8}, 2×10^{-4})	2.3×10^{-5} (2×10^{-8}, 2×10^{-4})
Total ileocaecal plate	1.5 (4×10^{-5} , 10)	-
Jejunal ileocaecal plate	1.5 (4×10^{-5} , 10)	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

7.3. Amount of infectivity per year (EU27 slaughter population) by tissue type, BO ID_{50}/yr

7.3.1. Amount of infectivity in intestines and mesenteries (BO ID_{50}) per year in EU27

Previous sections of results have 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.

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. 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 BO ID_{50}/year is present within bovine intestines and mesenteries in infected animals at slaughter, with 2.5th and 97.5th percentiles this varies between 1,117 and 4,557 BO ID_{50}/year accounting for uncertainty and variability as shown in Figure 11.

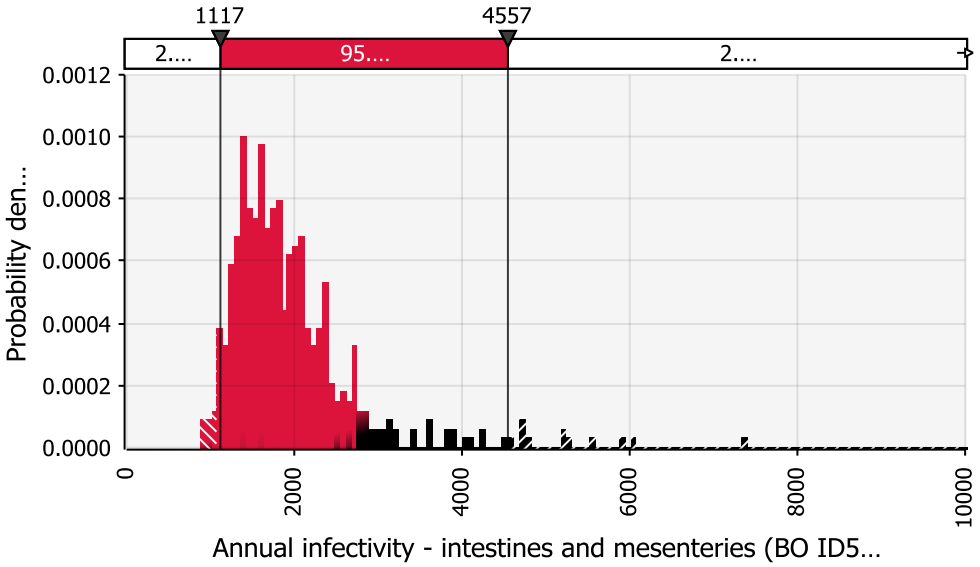

Figure 11: Probability density function describing the baseline total infectivity in bovine intestines and mesenteries in the slaughter stream for the EU27 in one year (BO ID_{50}/year) considering uncertainty and variability (95% percentiles indicated by top bar)

Figure 12 displays the estimated distribution of infectivity from processed products (duodenum, jejunum, colon, caecum and mesentery tissues) per year in the EU27 where all duodenum, jejunum, colon and caecum tissues have undergone processing treatment which reduces BSE infectivity levels. An estimated mean amount of infectivity of 1,362 BO ID_{50} arises from processed products per year in the EU27, with 2.5th and 97.5th percentiles this varies between 796 and 2,791 BO ID_{50}/animal accounting for uncertainty and variability. The distributions by tissue type before any processing are provided in Appendix C. This represents a reduction in the amount of infectivity of, on average, of 352 BO ID_{50} per year, approximately 21% of the unprocessed total (excluding ileum tissues).

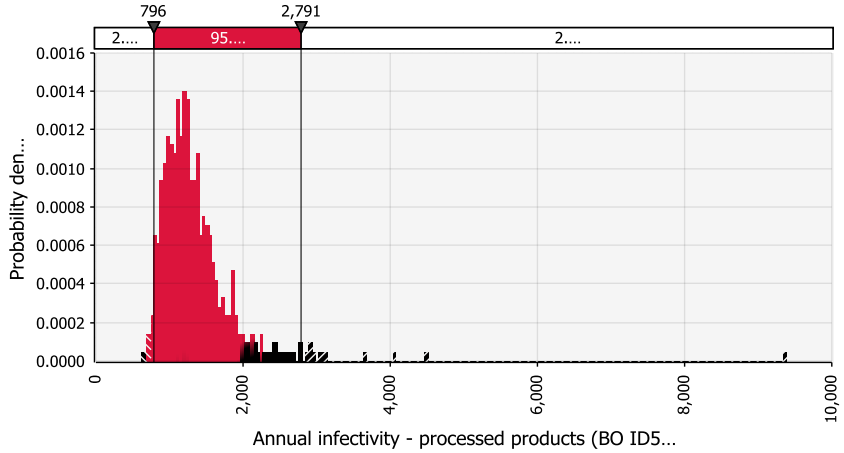

Figure 12: Probability density function describing the total infectivity in processed tissues (duodenum, jejunum, caecum, colon and mesenteries) for the EU27 (BO ID_{50}/year) considering uncertainty and variability (95% percentiles indicated by top bar)

The contribution by tissue to the annual total and results for the subset of the total ileocaecal plate and that present in the jejunum are shown in Table 11 with the estimated mean and 2.5th and 97.5th

percentiles. For slaughtered animals it can be seen that jejunum tissues dominate the estimate, with ileum and mesenteric nerves making a smaller but significant contribution. For processed products jejunum again dominated with mesenteric nerves making a significant contribution (percentage contribution not shown).

Table 11: Baseline mean total infectivity per year for intestinal tissues at slaughter and post processing in the EU27 (2.5^{th} and 97.5^{th} percentiles in brackets)

	Infectivity per year (BO ID_{50}/yr) Mean (2.5^{th} and 97.5^{th})	**Mean percentage contribution**	**Infectivity post processing* (BO ID_{50}) Mean (2.5^{th} and 97.5^{th})**
Tissue type			
Ileum	271 (130, 687)	14%	-
Duodenum	0.02 (0.01, 0.04)	0%	0.02 (0.01, 0.03)
Jejunum	1,350 (644, 3484)	68%	1006 (487, 2455)
Caecum	50 (24, 116)	3%	37 (18, 85)
Colon	0.2 (0.12, 0.4)	0%	0.15 (0.09, 0.3)
Mesenteric lymph nodes	3×10^{-5} (2.6×10^{-5}, 3.3×10^{-5})	0%	3×10^{-5} (2.6×10^{-5}, 3.3×10^{-5})
Mesentery nerves	249 (218, 284)	13%	249 (218, 284)
CMGC	64 (57, 73)	3%	64 (57, 73)
Total per year	**1,985 (1117, 4557)**		**1,362 (796, 2791)**
Total ileocaecal plate	1,620 (767, 4116)	[82%]	-
Jejunum ileocaecal plate	1,349 (643, 3482)	[68%]	1005 (486, 2454)

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

7.3.2. Length of infected intestines (m) per year in EU27

The estimated length of infected intestinal tissue per year in the EU27 varies depending on the age at slaughter of infected animals, the number of infected animals and the tissue type concerned. The estimated total length of intestinal tissues by tissue type from infected animals per year in the EU27 is provided in Table 12. It can be seen that the jejunum accounts for the longest total length with an estimated mean of 27,370 m in one year with 2.5^{th} and 97.5^{th} percentiles that this varies between 25,141 and 29,474 m per year accounting for uncertainty and variability. This is approximately the mean number of infected carcases (613) multiplied by the estimated length of jejunum in animal >60 months of age of 45.8 m.

Table 12: Baseline total length of intestinal tissues from infected animals in the EU27 per year (m/yr) (2.5^{th} and 97.5^{th} percentiles in brackets)

	Total length per year (m/yr) Mean (2.5^{th} and 97.5^{th})
Tissue type	
Ileum	595 (548, 642)
Duodenum	726 (668, 782)
Jejunum	27,370 (25141, 29474)
Caecum	451 (415, 486)
Colon	6,534 (6002, 7044)
Total ileocaecal plate	968 (883, 1056)
Jejunal ileocaecal plate	805 (736, 878)

8. Case Studies

Two case studies have been completed investigating (1) the historical situation of the BSE epidemic in Europe from 2007 to 2011, and (2) a theoretical scenario of a re-emergence of BSE. The results are provided in the following sections.

8.1. Estimated historical levels of BSE infectivity

As described in section 2, the mathematical model (C-TSEMM) has been developed to estimate the trend in prevalence of BSE in European Member States (MSs). Using data on the number of animals slaughtered by age at slaughter, 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 2012 by age at slaughter.

C-TSEMM requires annual historical information on the numbers of cattle slaughtered each retrospective year from 2007 to 2012. 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 2012 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 13. It can be seen that the number of infected animals missed in the EU27 steadily decreases to the baseline year.

Table 13: 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)

HS and ES - Healthy and Emergency slaughter

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 and subset of ileocaecal plate are provided in Table 14.

Table 14: 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 (BO ID_{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 meter length for an average EU27 infected slaughter animal for each historical year is provided in Table 15. Table 16 provides the estimated total length of these tissues with the addition of jejunal ileocaecal plate length for each historical year.

Table 15: Historical mean infectivity per length (BO ID_{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 (BO ID_{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 16: 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)

8.2. Estimated levels of infectivity from a theoretical re-emergence of BSE

A re-emergence scenario has been implemented in the model C-TSEMM extending the exponential trend as estimated by the baseline model into future years (AHVLA, 2012). A user selected percentage increase is applied to the estimated prevalence trend of successive birth cohorts of animals born after 2012. The extended trend has been used to estimate the expected total number of animals infected (by exit stream) and the cases detected each subsequent year. A threshold number of cases required for the prevalence increase to be observed is defined by the user. Additionally, the user is able to specify an 'age window' in which the detected cases must fall. The occurrence of cases originating from the previous outbreak may falsely trigger detection of the re-emergence in the immediate years following 2012. Therefore, detection of the re-emergence does not occur whilst the general trend from the previous outbreak is still decreasing.

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 that feed controls remain in place, it is assumed that any future increase in prevalence of disease is attributable to a novel BSE and, or, transmission/dissemination by an, as yet, unknown mechanism. From discussions, a 10% increase in the BSE prevalence between successive birth cohorts has been used to represent the rate of the re-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. 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.

Simulation of 10,000 datasets of detected cases by age group and year were generated to account for Poisson variability about the expected values and the year of detection calculated for each. The expected (or average) year of detection was defined as the mean over all the simulations. The lower and upper confidence limits for the year of detection were used to generate the 2.5th and 97.5th

percentiles for the number of infected and missed animals thus incorporating the variability between the year of detection within the model outputs.

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 17 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 BO ID_{50} would arise from bovine intestines and mesenteries in infected animals at slaughter, with 2.5th and 97.5th percentiles that this varies between 27,626 and 52,554 BO ID_{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 13.

Table 17: 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) (BO ID_{50})**	**Cumulative total infectivity (processed*) (BO ID_{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)

* Ileum is classed as a tissue not processed for consumption and therefore not included in the analysis

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

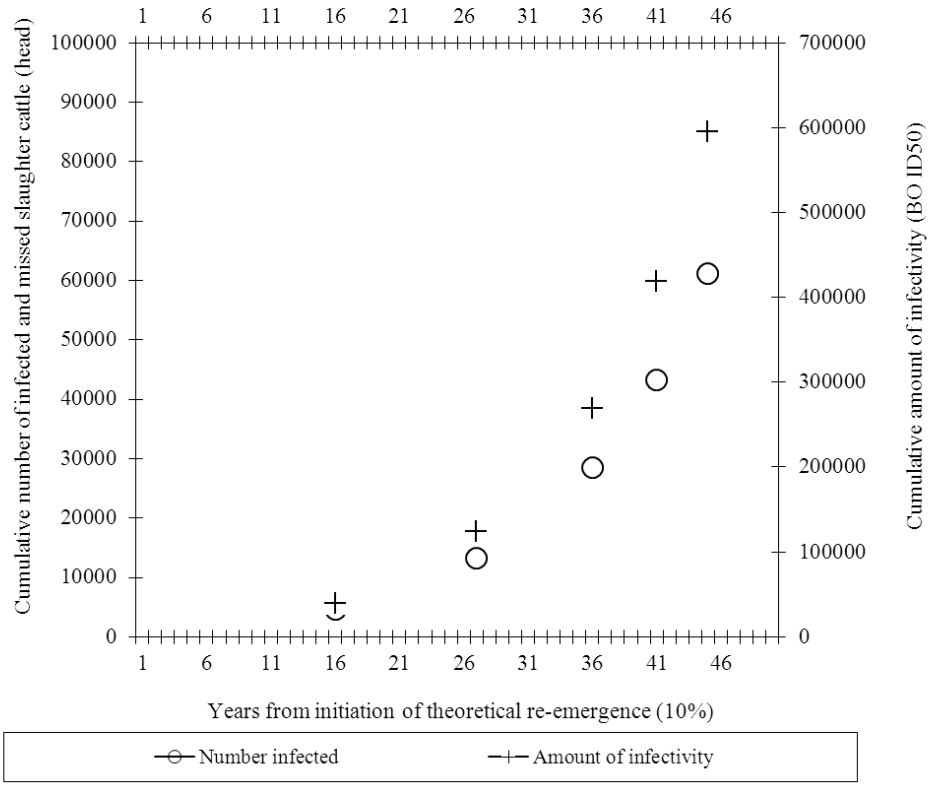

Figure 13: Estimated mean cumulative number of BSE infected and missed cattle slaughtered in EU27 and resulting cumulative infectivity from bovine intestines and mesenteries following a theoretical 10% increase in prevalence by birth cohort from 2012. Points indicate time period of detection of 1 case, 2 cases etc in the age window 48 to 72 month age at slaughter

Table 18 provides the estimated cumulative total infectivity stratified by each tissue type before detection of 1 case and 3 cases within the surveillance window. Table 19 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 re-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 18: 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 (BO ID_{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)	-	43275 (26179, 54844)	-
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)	-	258,008 (154548, 324933)	-
Jejunum ileo 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 19: 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 (BO ID_{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 meter length of an EU27 animal as shown in Table 20 for unprocessed tissues and Table 21 for processed tissues.

Table 20: Re-emergence mean infectivity per length (BO ID_{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 (2.5th and 97.5th percentiles in brackets)

	Unprocessed infectivity per length (BO ID_{50}/m) Mean (2.5th and 97.5th)		
	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 21: Re-emergence mean infectivity per length (BO ID_{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 (BO ID_{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 22.

Table 22: 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) (2.5^{th} and 97.5^{th} percentiles in brackets)

	Total length until detection (m) Mean (2.5^{th} and 97.5^{th})	
	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)

9. Sensitivity Analysis and Parameter Uncertainty

Sensitivity analyses were performed to identify those uncertain and variable parameters, which are quantified in the model, and which significantly impact the final results. To investigate areas where the uncertainty or variability may not be quantified in the model, or where different modelling approaches may be used, scenarios were run and described in the following sections. Finally outputs are provided for a variability only simulation, and with uncertain values fixed at lower and upper bounds to investigate the influence of quantified uncertainty in the results.

9.1. Sensitivity analyses

There are a number of methods available to determine those input variables that contribute the greatest to the output uncertainty or variability. Of the methods proposed by Mokhtari and Frey, 2005 and Saltelli et al., (2000) applicable to simulation data with practical implementation; a rank regression, ANOVA, rank correlation, and sample correlation were performed, together with an @Risk automated step-wise regression with pre-screening of inputs based on their precedence in formulas to the response variable in the model. The regression analyses measured how the main output (total infectivity per EU27 year) varies due to each parameter value selected for that iteration from input distributions. All parameters in the model represented by a range are included in the sensitivity analysis. These analyses were carried out during various stages in the development of the risk assessment, together with a final analysis for the version of the model presented in this report.

Those parameters distributions strongly associated with the final output are shown by method in Table 23. When comparing the results of the different techniques for the final model and results from previous model versions, the ANOVA analysis has given the most reproducible results identifying those variables which during the modelling building process had been previously identified as important. From the estimated sensitivity values that have been calculated, the model was strongly affected by four parameters: (1) variability in the infectivity titre of the ileum, (2) the uncertainty associated with the conversion of log10 RIII i.c. i.p. ID_{50}/g into bovine oral ID_{50}/g, (3) variability in the ileocaecal plate weight in small intestines, and (4) variability in the age at slaughter.

Table 23: Sensitivity analysis ranking of input variables contributing to the output uncertainty and variability

Sensitivity analysis method				
ANOVA	**Rank correlation**	**Rank regression**	**Sample correlation**	**@Risk SMART*+stepwise regression**
Age at slaughter	Infectivity unit	Infectivity unit	Weight of ileocaecal plate	Ileum infectivity
Ileum infectivity	Ileum infectivity	Ileum infectivity	Age at slaughter	Weight of ileocaecal plate
Infectivity unit	Mesenteric nerve weight	Mesenteric nerve weight	Ileum infectivity	Length of ileocaecal plate
Weight of ileocaecal plate	Age at slaughter	Weight of ileocaecal plate	Weight of PP	Weight of PP

*SMART: inclusion only of those parameters proceeding the output (precedence checking)

Variable parameters are those that describe the natural variability in a process and cannot be reduced by collection of further data. Uncertain estimates could be potentially reduced if further information was made available. Of the uncertainty and variability of the parameters identified by the sensitivity analysis as having an impact on the final result, only one is uncertain - the conversion of log10 RIII icip ID_{50}/g into bovine oral ID_{50}/g. The uncertainty associated with this parameter is unlikely to be

reduced as the estimation of this parameter is based on large animal experiments that have taken many years to produce the estimate currently used in the model. Variability with the weight of ileocaecal plate in small intestines and titre of ileocaecal plate in ileum are unlikely to be reduced by further research.

9.2. Scenario analyses

In addition to the sensitivity analysis, a number of scenarios were identified during model development and parameterisation which merited further investigation as to whether an alternative parameterisation would significantly impact model results. This included extrapolation of the experimental tissue measurement data generated by Carlens (1928). Extrapolation of data outside these measurements may lead to implausible tissue values. However, the sample set may not include the true range. To investigate this scenario, the minimum and maximum measurements for small intestine length, ileocaecal plate length, ileocaecal plate weight, surface area of lymphoid tissue in caecum, and total Peyer's patches weight was implemented in the model as the 2.5th and 97.5th percentiles. Results were compared using this alternative method of parameterisation with the estimated infectivity in bovine intestinal tissues by age at slaughter and the EU27 annual infectivity estimate for bovine intestines and mesenteries (results not shown here). The difference between results was insignificant and within the 4% variation possible in the mean estimates between different simulations. Two additional scenarios were: (1) the upper infectivity titre of mesenteric nerves and CMGC and (2) the estimation of lymphoid tissue weight in colon.

9.2.1. Scenario 1: Upper infectivity titre of Mesenteric nerves and CMGC

There are limited data on the maximum titre mesenteric nerves and CMGC may reach given clinical onset of disease. The maximum titre used in the risk assessment represents the upper confidence of data for the only clinical animal as measured by Kaatz et al., 2012, representing the most biologically plausible method of calculation using that dataset. For the sciatic, facial and radial nerves measured in Buschmann et al., (2005) the mean titre of infectivity was estimated as -3.1 (-3.6, -2.7) RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g. The titres in the Buschmann paper for the clinical animal are much lower than those from experimental studies, probably due to large differences in dose. Therefore, it is acknowledged that the maximum titres used in the baseline model of -0.013 and -0.01 RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g for mesenteric nerves and CGMC are a pessimistic upper limit. Results for the EU27 annual infectivity estimate for bovine mesenteric nerves and CMGC were compared using the Buschmann et al., (2005) upper measurement for the sciatic, facial and radial nerves with results shown in Table 24.

Table 24: Estimated mean infectivity of bovine mesentery nerves and CMGC by age at slaughter (BO ID_{50}/infected animal) comparing baseline model and scenario 1

		Infectivity (BO ID_{50}/infected animal)	
	Scenario	**Mesentery nerves**	**CMGC**
Age at slaughter (months)			
6	Baseline	$2.2x10^{-9}$ ($5.6x10^{-1}$, $1.3x10^{-8}$)	$2.5x10^{-9}$ ($7.0x10^{-11}$, $1.5x10^{-8}$)
	Sc1	$2.2x10^{-9}$ ($5.6x10^{-11}$, $1.3x10^{-8}$)	$2.5x10^{-9}$ ($7.0x10^{-11}$, $1.5x10^{-8}$)
12	Baseline	$3.0x10^{-8}$ ($1.0x10^{-10}$, $2.4 x10^{-7}$)	$3.5x10^{-8}$ ($1.2x10^{-10}$, $2.7x10^{-7}$)
	Sc1	$3.0x10^{-8}$ ($1.0x10^{-10}$, $2.4x10^{-7}$)	$3.5x10^{-8}$ ($1.2x10^{-10}$, $2.7x10^{-7}$)
18	Baseline	$7.8x10^{-7}$ ($5.2x10^{-1}$, $6.3x10^{-6}$)	$9.0x10^{-7}$ ($6.4x10^{-10}$, $7.1x10^{-6}$)
	Sc1	$7.8x10^{-7}$ ($5.2x10^{-10}$, $6.3x10^{-6}$)	$9.0x10^{-7}$ ($6.4x10^{-10}$, $7.1x10^{-6}$)
24	Baseline	$2.1x10^{-5}$ ($1.9x10^{-9}$, $1.7x10^{-4}$)	$2.4x10^{-5}$ ($2.4x10^{-9}$, $1.9x10^{-4}$)
	Sc1	$2.1x10^{-5}$ ($1.9x10^{-9}$, $1.7x10^{-4}$)	$2.2x10^{-5}$ ($2.4x10^{-9}$, $1.9x10^{-4}$)
36	Baseline	$1.5x10^{-2}$ ($1.4x10^{-7}$, $1.2x10^{-1}$)	$1.4x10^{-2}$ ($1.7x10^{-7}$, $1.2x10^{-1}$)
	Sc1	$7.5x10^{-4}$ ($1.4x10^{-7}$, $3.6x10^{-3}$)	$2.4x10^{-4}$ ($1.8x10^{-7}$, $9.8x10^{-4}$)
48	Baseline	$3.6x10^{-1}$ ($1.7x10^{-6}$, 1.8)	$1.1x10^{-1}$ ($2.0x10^{-6}$, $4.8x10^{-1}$)
	Sc1	$1.1x10^{-3}$ ($1.8x10^{-6}$, $4.3x10^{-3}$)	$2.8x10^{-4}$ ($1.9x10^{-6}$, $1.1x10^{-3}$)
60	Baseline	$5.2x10^{-1}$ ($6.0x10^{-5}$, 2.1)	$1.3x10^{-1}$ ($7.5x10^{-5}$, $5.1x10^{-1}$)
	Sc1	$1.1x10^{-3}$ ($5.7x10^{-5}$, $4.3x10^{-3}$)	$2.8x10^{-4}$ ($2.1x10^{-5}$, $1.1x10^{-3}$)
120	Baseline	$5.3x10^{-1}$ ($1.9x10^{-4}$, 2.1)	$1.4x10^{-1}$ ($2.3x10^{-4}$, $5.1x10^{-1}$)
	Sc1	$1.1x10^{-3}$ ($7.8x10^{-5}$, $4.3x10^{-3}$)	$2.8x10^{-4}$ ($2.3x10^{-5}$, $1.1x10^{-3}$)

At the individual animal level, it can be seen from Table 24 that there is no change in the amount of infectivity in mesenteric nerves and CMGC within infected animals using the alternative parameterisation until approximately 36 months of age at slaughter. After this point there is an increasing difference between the estimated infectivity between the two parameterisation estimates, particularly evident in infected animals slaughtered when older than 120 months, with an estimated 3 log difference.

Using the alternative parameterisation at the EU27 level results in a mean infectivity estimate of 1,675 BO ID50 per year (792, 4,120) in the slaughter population. This can be compared to the baseline estimate of 1,985 BO ID_{50}/year (1,117, 4,557), indicating that the alternative parameterisation decreases the total amount of infectivity per year by an estimated 16%.

9.2.2. Scenario 2: Estimation of lymphoid tissue weight in colon tissue

There are little data on the weight and occurrence of Peyer's patches (PP) in the colon. In this risk assessment it is assumed that the number of follicles in the colon can be approximated by the concentration of PP (weight per meter length) that is estimated to be present in the jejunum. To investigate the impact of changes in the values used for colon, a 10 fold increase in the weight of PP per meter has been implemented. Results were compared using this alternative method of parameterisation with the estimated infectivity in colon by age at slaughter as shown in Table 25, and the EU27 annual infectivity from infected animals at slaughter.

Table 25: Estimated infectivity of colon by age at slaughter (BO ID50/infected animal) comparing baseline model and scenario 2

	Scenario	Infectivity (BO ID_{50}/infected animal) Colon
Age at slaughter (months)		
6	Baseline	2.0×10^{-4} (2.3×10^{-7}, 1.3×10^{-3})
	Sc2	2.0×10^{-3} (2.3×10^{-6}, 1.3×10^{-2})
12	Baseline	2.7×10^{-4} (3.2×10^{-7}, 1.8×10^{-3})
	Sc2	2.7×10^{-3} (3.2×10^{-6}, 1.8×10^{-2})
18	Baseline	4.3×10^{-4} (5.0×10^{-7}, 2.9×10^{-3})
	Sc2	4.3×10^{-3} (5.0×10^{-6}, 2.9×10^{-2})
24	Baseline	4.1×10^{-4} (4.8×10^{-7}, 2.8×10^{-3})
	Sc2	4.1×10^{-3} (4.8×10^{-6}, 2.8×10^{-2})
36	Baseline	4.3×10^{-4} (5.1×10^{-7}, 2.9×10^{-3})
	Sc2	4.3×10^{-3} (5.1×10^{-6}, 2.9×10^{-2})
48	Baseline	4.3×10^{-4} (5.1×10^{-7}, 2.9×10^{-3})
	Sc2	4.3×10^{-3} (5.1×10^{-6}, 2.9×10^{-2})
60	Baseline	3.6×10^{-4} (4.3×10^{-7}, 2.4×10^{-3})
	Sc2	3.6×10^{-3} (4.2×10^{-6}, 2.4×10^{-2})
120	Baseline	2.5×10^{-4} (2.9×10^{-7}, 1.7×10^{-3})
	Sc2	2.5×10^{-3} (2.9×10^{-6}, 1.7×10^{-2})

At the individual animal level, it can be seen from Table 25 that there is a 10 fold increase in the amount of infectivity in the colon within infected animals using the alternative parameterisation.

The impact of the alternative parameterisation at the EU27 level for infectivity in the slaughter stream per year is insignificant, resulting in a mean infectivity estimate of 1,990 BO ID_{50} per year (1,106, 4,554) in the slaughter population. This can be compared to the baseline estimate of 1,985 BO ID_{50}/year (1,117, 4,557), and within the 4% variation in the mean between different simulations. Therefore, increasing the infectivity of lymphoid tissue in the colon does not significantly influence the final results of the risk assessment.

9.3. Effect of uncertainty on model outputs

The risk assessment is predominantly a first order variability model with six parameters identified as being uncertain: (1) the number of animals infected by age in the baseline year, (2) the lower titre of jejunum Peyer's Patches as compared to ileocaecal plate in the ileum, (3) the infectivity titre of mesenteric lymph nodes, (4) the infectivity titre of mesenteric nerves, (5) the infectivity titre of CMGC, and (6) the conversion factor from RIII mouse ic. ip. ID_{50} units into bovine oral ID_{50} units.

For this specific risk assessment for each of these parameters, there are no variable estimates available about the mean. For (2), (3), (4), (5) and (6) this is due to a lack of experimental data to estimate the between animal variability, with only an estimate of the upper and lower uncertainty available. For (1) the parameter does not require between animal variability.

The final distributed result from the risk assessment has been performed representing both uncertainty and variability. Certain models combining uncertainty and variability have been shown to output erratic results where the uncertainty distribution has a major impact on selected model results (Nauta, 2000). During model development it is important to establish if any of the uncertain parameters significantly impact the final model results as it may be better to present results under 'scenarios' rather than one result covering a range of uncertain values for example.

Each area of uncertainty and others not quantified in the model were separately investigated during model building by fixing values and increasing/decreasing the value within plausible bounds to investigate effect on model results.

Figure 14 displays the estimated mean infectivity per year for the EU27 (BO ID_{50}/year), with Table 26 providing the estimated mean infectivity by age at slaughter (BO ID_{50}/animal) for the following simulations:

(V) variability only model (the six uncertain parameters are not used),
(U+V) uncertainty and variability simulated together (baseline results),
(Umin + V) the variability is simulated and uncertain parameters fixed at 2.5th percentile value, and
(Umax + V) the variability simulated with uncertain parameters fixed at 97.5th percentile value.

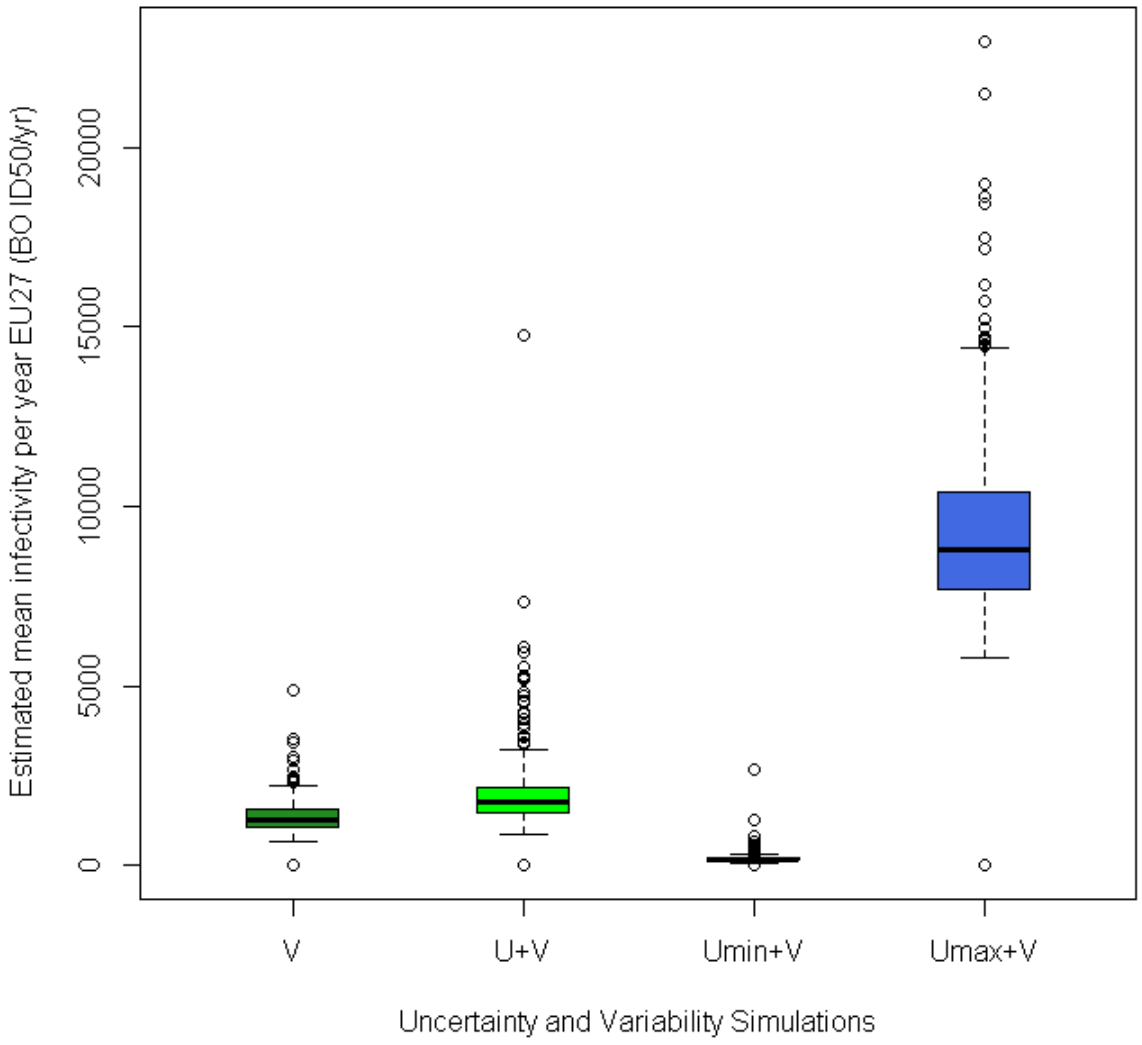

Figure 14: Boxplots of estimated mean infectivity per year in the EU27 simulating variability only, uncertainty and variability (U+V), variability with 2.5th uncertain values (Umin+V), and variability with 97.5th uncertain values (Umax+V)

From Figure 14 it can be seen that the mean result from the variability only model of 1,376 BO ID_{50}/yr (2.5^{th} 819, 97.5^{th} 2,358) can be compared to the result simulating both uncertainty and variability of 1,985 BO ID_{50}/year (2.5^{th} 1,117, 97.5^{th} 4,557) with expected wider percentile range estimated when including uncertainty. The impact of uncertainty being fixed at lower estimated values for each of the six uncertain parameters (2.5^{th} percentile) results in a reduction of the output distribution mean to 191 BO ID_{50}/yr (2.5^{th} 100, 97.5^{th} 407). Fixing the uncertain parameters to the upper estimated values (97.5^{th} percentile) results in an increase in the output distribution mean to 9,293 BO ID_{50}/yr (2.5^{th} 6,202, 97.5^{th} 15,238).

Therefore, for this risk assessment, the inclusion of uncertainty for certain parameters increases the estimated mean by 31% from 1,376 BO ID_{50}/year to 1,985 BO ID_{50}/year. If values for quantified uncertain parameters are fixed at the upper limit of the 97.5^{th} percentile, the estimated mean increases, on average, 7 fold to 9,293 BO ID_{50}/yr when compared to variability only simulations.

Table 26: Total mean infectivity of unprocessed products (BO ID_{50} per infected animal) by age at slaughter (2.5^{th} and 97.5^{th} percentiles in brackets)

	Infectivity (BO ID_{50}/infected animal)			
	Variability only	**Uncertainty and variability (baseline)**	**Uncertainty_min and variability**	**Uncertainty_max and variability**
Age at slaughter (months)				
6	10.0 (0.05, 67.6)	14.2 (0.03, 96.7)	2.0 (0.01, 13.5)	50.1 (0.23, 339.8)
12	10.4 (0.05, 70.7)	14.8 (0.03, 100.9)	2.1 (0.01, 14.1)	52.4 (0.24, 354.9)
18	9.4 (0.04, 64.1)	13.4 (0.03, 91.3)	1.9 (0.01, 12.8)	47.5 (0.21, 321.6)
24	5.8 (0.02, 39.5)	8.1 (0.02, 55.9)	1.2 (0.004, 7.9)	29.3 (0.11, 198.0)
36	5.8 (0.03, 39.5)	8.2 (0.02, 55.9)	1.2 (0.004, 7.9)	30.3 (0.28, 199.0)
48	6.1 (0.16, 39.8)	8.6 (0.08, 56.8)	1.2 (0.01, 7.9)	31.5 (0.16, 4.6)
60	0.5 (0.01, 0.9)	0.7 (0.01, 2.8)	0.1 (0.001, 0.2)	2.5 (0.16, 4.6)
120	0.5 (0.02, 0.9)	0.7 (0.01, 2.8)	0.1 (0.002, 0.2)	2.5 (0.23, 4.6)

From Table 26 it can be seen that the mean results by age at slaughter for the variability only model decrease by, on average, 30% when compared to those simulations including estimates of uncertainty. If values are fixed at the upper limit of the 97.5^{th} percentile (Uncertainty_max), the estimated mean increases, on average, 5 fold when compared to variability only simulations, and 3-4 fold when compared to the baseline results.

In summary the simulation of uncertainty and variability within the risk assessment does not markedly change the key outputs of the risk assessment when compared to the inclusion of only variable parameters, resulting in the same order of magnitude of mean estimates.

10. Conclusions

This report describes the model framework developed to compare the pattern of BSE infectivity in selected animal tissues (bovine intestines and mesenteries) for an individual infected animal. The model also estimates infectivity at the member state, or group of member states level by age at slaughter over one year, results provided in this report for the EU27, and includes the impact of certain processing technologies (sausage casing processing) on residual BSE infectivity. Case studies are provided estimating the annual infectivity historically and in the situation of a future re-emergence of disease. Results, where appropriate, are stratified by age at slaughter, infectivity by meter length (for bovine intestinal tissues), tissue type, and pre and post processing.

There are a number of key assumptions in order to develop the risk assessment:

Assumptions associated with C-TSEMM data used

Three key assumptions are associated with the estimated number of infected animals that have bypassed test controls in the healthy slaughter stream and emergency slaughter stream in the EU27 as estimated by C-TSEMM:

- The exponential distribution can be used to describe the declining trend in BSE prevalence in the EU27. While other distributions could be fitted, analysis of alternative distributions has indicated that an exponential decay of prevalence over time is appropriate for the majority of European data.

- For MSs with no, or very few, BSE cases post 2001 an alternative estimate of prevalence is required. This has been estimated for those MSs based on the average prevalence of the group of MSs with BSE cases under which they were placed in the previous EFSA Opinion (EU17 or the EU8 group). This results in an overestimate of prevalence when using the individual member state data (results not shown here), and therefore number of infected animals for countries with no recorded cases as they are assumed to be a merged epidemiological unit with countries where cases are observed.

- For the re-emergence case study, it is 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 observed between 2002 and 2011. From discussions, a 10% increase in the BSE prevalence between successive birth cohorts has been used to represent the rate of the re-emergence starting in the baseline year 2012.

Key risk assessment assumptions

- Infectivity in ileum, duodenum, jejunum and caecum is associated to lymphoid formation (Peyer's patches and isolated lymphoid follicles) and therefore the QRA model did not consider infectivity present outside those structures for bovine intestines. Much of the infectivity data gathered has been based on experimentally dosed animals. In the absence of other data, it is assumed that the pattern and magnitude of infectivity is broadly applicable to natural field cases.

- Any infectivity present in the mesentery is likely to be associated with nerves, autonomic nervous system ganglia and lymph nodes. Fatty deposits are unlikely to contain BSE infectivity. Therefore the tissue types mesenteric lymph nodes, mesenteric nerves and mesentery celiac and mesenteric ganglion complex were included in the QRA.

- To use the age dependent data available for young animals (<24 months of age) it is assumed that (1) the number of animals infected by 6 monthly interval can be estimated as the proportion of the total number slaughtered multiplied by the estimated total number infected < 24 months, (2) that the weight of tissues from infected animals slaughtered <6 months old can be approximated by the 4-6 data age intervals.

- In the absence of data on the occurrence and infectivity of lymphoid tissue in the caecum, it is assumed that the weight of follicles can be approximated by the weight per surface area present in the ileum and infectivity titre estimated for the ileum. In the absence on the data on the occurrence and infectivity of lymphoid tissue in the colon, it is assumed that the weight of follicles can be approximated by the concentration (weight/length) of Peyer's patches present in the jejunum and infectivity titre estimated for the Peyer's patches in the jejunum. These assumptions can be considered as a worst case scenario and can be revised in the future if new data would become available.

- The proportion of the ileocaecal plate located in the ileum and the remainder in the jejunum can be estimated by dividing the length of ileocaecal plate in the ileum by the total length of the ileocaecal plate (in jejunum and ileum) at a fixed point from that measured in animals slaughtered under 6 months of age.

- There are no known quantitative estimates for the weight of mesenteric nerves in bovine intestines. From discussion with pathologists who routinely conduct post-mortems, with consideration for prior estimates regarding peripheral nerves, expert opinion is used to parameterise this tissue type.

- There are no quantitative data on the weight of the mesentery celiac and mesenteric ganglion complex (CMGC) in cattle. Expert opinion, together with dimensional data measured in a pony has been used to parameterise this tissue with the assumption that such data can be used as a proxy for bovine animals.

- It is assumed that the variability in the reduction of infectivity in bovine intestinal tissues due to the processing of sausage casings could be described as a minimum of 0 to a maximum of 50%.

- Based on available data from the combined analysis of German data from FLI and from VLA in the UK, there is no discernible pattern to infectivity titres in the distal ileum. Therefore, it is assumed that infectivity in the distal ileum is essentially random with high between and within animal variability

Results

Results from the QRA are presented at the individual infected animal level in terms of age at slaughter and drawn from the EU27 merged into one epidemiological area, and at an annual level for the EU27 slaughter population with the year 2012 representing the baseline year. Two case studies have been performed investigating historical levels of infectivity and infectivity that may arise as a result of a theoretical re-emergence.

For an infected animal by age at slaughter:

- At different slaughter ages, different tissue types contribute the most to the total infectivity load per infected animal and several distinctive patterns of infectivity are evident. These

patterns are the result of changes in the weight of infectious tissue 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 BO ID_{50} per animal and decline to low levels, less than one BO ID_{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 estimated mean total of less than 1 BO ID_{50} per animal.

- 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 viewed.

- For processed intestinal tissues (duodenum, jejunum, caecum, colon), an average reduction of 25% has been applied. This results in the largest reduction of approximately 3 BO ID_{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 BO ID_{50} by processing.

For an infected EU27 randomly slaughtered baseline animal

- An estimated mean amount of infectivity of 3 BO ID_{50} at slaughter arises from bovine intestines and mesenteries in a single infected animal, with 2.5^{th} and 97.5^{th} percentiles this varies between 0.02 and 18 BO ID_{50}.

- The 'average' infected EU27 animal is older than 48 months with different infectivity levels in bovine intestines and mesenteries. There are older infected animals as a result of the multiplication of cohort prevalence and the number infected from those cohorts surviving and subsequently being slaughtered.

- The tissue types which are estimated to contribute significantly to the total of an average EU27 infected slaughtered animal are jejunum, ileum and mesenteric nerves.

- For processed tissues (duodenum, jejunum, caecum, colon and mesentery tissues) an estimated mean amount of infectivity of 2.2 BO ID_{50}/animal after processing remains from a single animal, with 2.5^{th} and 97.5^{th} percentiles this varies between 0.01 and 11 BO ID_{50}/animal accounting for uncertainty and variability.

For the EU27 baseline over one year:

- An average of 613 infected animals are estimated to have by-passed testing and been slaughtered in the EU27 in 2012, ranging from 566 to 661 at the 2.5^{th} and 97.5^{h} percentiles representing uncertainty and variability. From these animals, an estimated mean amount 1,985 of BO ID_{50}/year of infectivity arises from bovine intestines and mesenteries, with 2.5^{th} and 97.5^{th} percentiles this varies between 1,117 and 4,557 BO ID_{50}/year accounting for uncertainty and variability.

- The infectivity present in jejunum tissues (68%) dominate the annual estimate for the EU27 arising from bovine intestines and mesenteries, with ileum (14%), and mesenteric nerves (13%) making a smaller but significant contribution.

- For processed tissues (duodenum, jejunum, caecum, colon and mesentery tissues) an estimated mean amount of infectivity of 1,362 BO ID_{50}/year), with 2.5^{th} and 97.5^{th} percentiles this varies

between 796 and 2,791 BO ID_{50}/year accounting for uncertainty and variability. This represents a reduction in the amount of infectivity of, on average, of 352 BO ID_{50} per year, approximately 21% of the unprocessed total (excluding ileum tissues).

For the EU27 historical levels

- An average of 6,816 infected animals are estimated to have by-passed testing and been slaughtered in the EU27 in 2007, ranging from 6,439 to 7,214 at the 2.5th and 97.5^{h} percentiles representing uncertainty and variability. From these animals, an estimated mean amount of infectivity arose 22,994 of BO ID_{50}/year from bovine intestines and mesenteries, with 2.5th and 97.5th percentiles this varies between 18,223 and 31,986 BO ID_{50}/year accounting for uncertainty and variability. This amounts to an estimated 11 fold mean reduction in total infectivity from bovine intestines and mesenteries over the 6 years from 2007 to 2012.

- The historical contribution of different tissue types to total infectivity has remained stable between the years 2007 and 2012.

For the EU27 future theoretical re-emergence

- Given a 10% theoretical increase in prevalence by birth cohort across the EU27 from the baseline year 2012, 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).

- After 16 years of the re-emergence with no change in controls, the estimated mean number of infected animals slaughtered are 4,607 (2.5th 3,475, 97.5th 5,908) yielding a cumulative estimated mean of 38,874 BO ID_{50} (2.5th 27,626, 97.5th 52,554) in unprocessed bovine intestines and mesenteries. Assuming all tissues capable of entering the food and feed chain are processed (duodenum, jejunum, caecum, colon and mesentery tissues) results in an estimated 25,199 BO ID_{50} (2.5th 17,932, 97.5th 37,520) arising from these products. Given no further changes in controls, the number of animals infected and missed and the resulting infectivity increases exponentially.

- As the theoretical re-emergence progresses, the contribution of those tissues most infectious in younger animals (less than 60 months) contribute more to the total infectivity per year 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. The change in the average infected age at slaughter also increases the infectivity per meter of intestinal tissues.

There are a considerable number of parameter ranges within the risk assessment with four strongly affecting the model results: (1) variability in the infectivity titre of the ileum, (2) the uncertainty associated with the conversion of log10 RIII i.c. i.p. ID_{50}/g into bovine oral ID_{50}/g, (3) variability in the ileocaecal plate weight in small intestines, and (4) variability in the age at slaughter. Variable parameters are those that describe the natural variability in a process and therefore would not be reduced by collection of further data. For the uncertain conversion of infectivity units, it is unlikely that any further experimental data will be generated to reduce the uncertainty associated.

Several scenarios were conducted during model development to investigate different parameterisations and model approaches. The upper titre of mesenteric nerves and ganglion is uncertain with different parameter estimates available by analysing experimental data from different nerves (all of which have

a relatively small sample size). Lower estimates were derived from sciatic, radial and facial nerves (Buschmann et al., 2005), whereas high estimates can be derived from the upper confidence intervals of cervical and thoracic vagus nerve and CGMC estimated titre (Kaatz et al., 2012). By running a scenario using the lower estimates, produced an estimated decrease of 16% in the total baseline annual infectivity in the EU27. Parameters were also investigated where there are little data and therefore more reliant on expert judgement. For colon infectivity a 10 fold increase in the weight of lymphoid tissue did not significantly influence the final results of the risk assessment.

The developed risk assessment is predominantly a first order variability model with six parameters identified as being uncertain. The simulation of uncertainty and variability within the risk assessment does not markedly change the key outputs of the risk assessment when compared to the inclusion of only variable parameters, resulting in the same order of magnitude of mean estimates.

In conclusion, the model and results presented in this report permit the estimation of infectivity in bovine intestines and mesenteries at the individual animal level and annual level for member state or group of member states (EU27) using available information. Baseline results are provided for 2012, with historical estimates and the situation of a re-emergence provided as case studies. Results are stratified by age at slaughter, tissue type and allow the comparison for processed tissues before and after sausage casing processing.

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APPENDICES

Appendix A. Summary table of input parameters and functions

	Symbol	Value	Unit	U/V*	Reference
Parameter					
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, O. 1928

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	
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, O. 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, O. 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
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	EFSA Working Group, 2012
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	EFSA Working Group, 2012
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, EFSA Working Group, 2012
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	EFSA Working Group, 2012
Processing					
Reduction in infectivity due to processing into casing	$P_{processng}(t)$ for *t*=2, 3, 4, 5	$Uniform(0,0.5)$	%	V	EFSA Working Group, 2012
	t=1, 6, 7, 8	0			EFSA Working Group, 2012
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 Hoffman 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 Hoffman 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
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	Variability. Katz 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	Variability. Katz 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; Konold et al., 2012

* 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

Appendix B. Algorithm to determine titre from mouse bioassay data

Bioassay, using endpoint titration, is regarded to be the most accurate method for the determination of the concentration of infectivity of TSE agents in tissue. Although regarded as less accurate, dose-response relationships have been used as a method for infectivity estimation when end-point titration data are not available. In this analysis, we adopted a method that is similar in principle to incubation period assay but also takes into account the proportion of mice which become clinical, providing a more accurate estimate of titre at lower doses than solely using the mean incubation period. To derive the dose/incubation relationship to be used to convert the incubation periods from the TgBov XV into RIII mouse bioassay into titres of infectivity, data were analysed from titrations in RIII mice of several different pools of brain material (whole brain or brain stem) from clinical field cases of BSE. The method is then as follows.

There are two potential outcomes for each mouse; either it could become clinical at *t* months post-inoculation, with probability $P_c(t)$, or it could die due to causes unrelated to BSE (through intercurrent disease or be sacrificed at the experimental end point), with probability $P_S(t)$. Denoting the titre of inoculum to which the individual mouse was exposed by ω, and denoting the dose-dependent probability of infection and incubation period (IP) by $S(\omega), f(\omega)$ respectively these outcomes are given by:

$$P_C(t)=S(\omega)f(\omega) \quad (1)$$

$$P_S(t)=S(\omega)\int_t^{\infty} f(\omega)dt' + (1-S(\omega)) \quad (2)$$

The log-likelihood of the observed mouse IP data from tissue *i* taken from a bovine at *T* months post exposure is then given by:

$$\sum_{j=1}^{N_{i,T}^{+}} \log(P_c(t_j)) + \sum_{j=1}^{N_{i,T}^{-}} \log(P_S(t_j)) \quad (3)$$

where $N_{i,T}^{+}, N_{i,T}^{-}$ were the number of mice which became clinical and the number of mice which die without showing clinical signs respectively for tissue *i*, and t_j is the IP of the *j*th mouse. Estimates of the titre for each inoculum for each time point are obtained by fitting the model to the mouse bioassay data using Markov Chain Monte Carlo methods in WinBUGS 1.3. Final estimates were obtained from 5,000 iterations of the model (following a burn-in of 5,000 iterations), from which the median and 2.5 and 97.5 percentiles were derived from the posterior distribution. Convergence was assessed by starting the model with three sets of different starting values and checking that this did not affect the final posterior estimates, through the Gelman-Rubin statistic, implemented in WinBUGS 1.3, as well as the inspection of history plots of each parameter and checking auto-correlation.

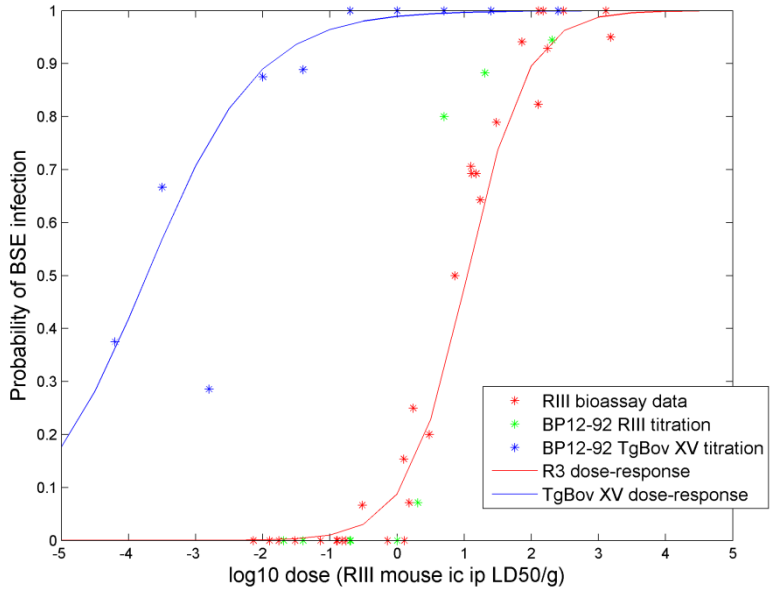

Figure B.1. The estimated probability of BSE infection for RIII and TgBov XV mice versus the dose in terms of RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g. The equivalent TgBov XV ID_{50}/g dose would be 4.76 $\log_{10}$ higher than the RIII dose.

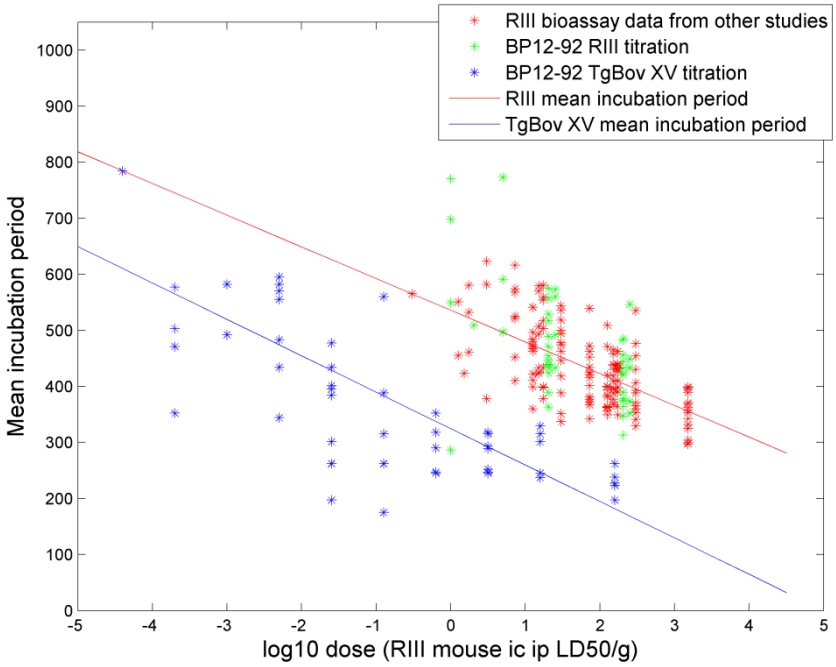

Figure B.2. The estimated mean incubation period of RIII and TgBov XV mice versus the dose in terms of RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g. The equivalent TgBov XV ID_{50}/g dose would be 4.76 $\log_{10}$ higher than the RIII dose.

Table B.1: Estimate of titre of infectivity in distal ileum tissues in terms of RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g, estimated by bioassay using TgBov XV mice (adapted from Hoffman et al., 2011)

	Months post infection	Bayesian estimates		
		Median	Credible intervals	
			2.5%	97.5%
ID				
IT 14 – 96	8	0.47	-0.39	1.3
IT 20 – 96	8	-0.48	-1.34	0.45
IT 39 – 96	8	0.13	-0.81	1.11
IT 55 - 96	8	-0.28	-1.21	0.63
IT 01 – 96	12	0.26	-0.47	1.04
IT 16 - 96	12	-2.31	-3.16	-1.42
IT 57 - 96	12	1.01	0.24	1.79
IT 06 - 96	12	0.7	-0.16	1.53
T 65 - 96	16	-0.11	-0.83	0.66
IT 60 – 96	20	0.42	-0.31	1.19
IT 50 – 96	20	-0.57	-1.38	0.21

Table B.2: Estimate titre of infectivity in jejunum tissues in terms of RIII mouse i.c. i.p. $\log_{10}$ ID_{50}/g, estimated by bioassay using TgBov XV mice (adapted from Hoffman et al., 2011)

	Months post infection	Bayesian estimates		
		Median	Credible intervals	
			2.5%	97.5%
ID				
IT 39 - 95A	8	-4.3	-5.22	-3.4
IT 55 - 95A	8	-3.41	-4.35	-2.32
IT 01 - 95A/B	12	-2.86	-4.01	-1.61
IT 57 - 95B	12	-2.27	-3.55	-0.98
IT 06 - 95B	12	-3.47	-4.58	-2.31
IT 10 - 95B	20	-2.64	-3.77	-1.58
IT 60 - 95A	20	-3.61	-4.71	-2.44

Appendix C. Individual bovine intestine and mesentery tissue type results

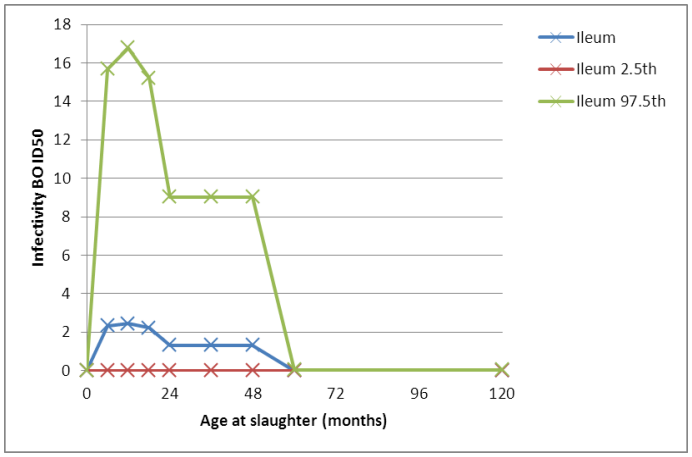

Figure C.1: Graph of mean, 2.5th and 97.5th estimates of infectivity in the ileum (BO ID_{50} per infected animal) by age at slaughter

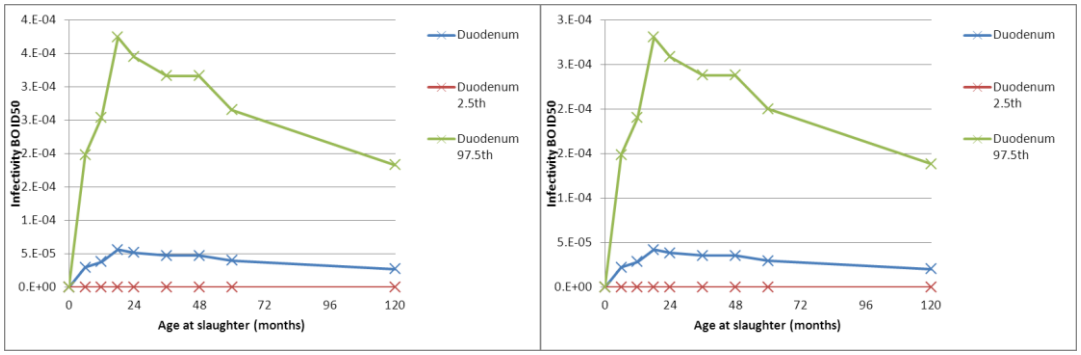

Figure C.2: Graph of mean, 2.5th and 97.5th estimates of infectivity in the duodenum before processing LHS, after processing RHS (BO ID_{50} per infected animal) by age at slaughter

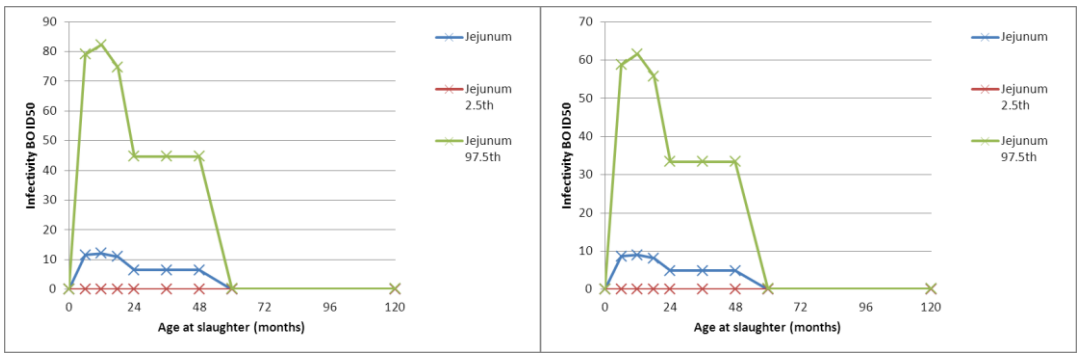

Figure C.3: Graph of mean, 2.5th and 97.5th estimates of infectivity in the jejunum before processing LHS, after processing RHS (BO ID_{50} per infected animal) by age at slaughter

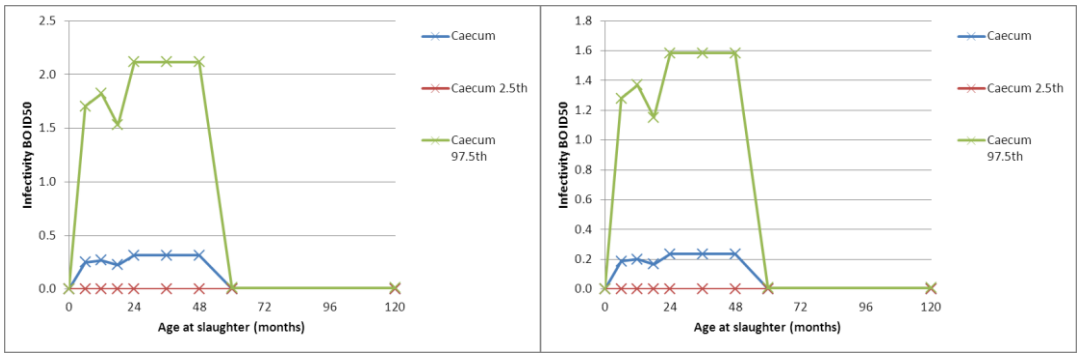

Figure C.4: Graph of mean, 2.5th and 97.5th estimates of infectivity in the caecum before processing LHS, after processing RHS (BO ID_{50} per infected animal) by age at slaughter

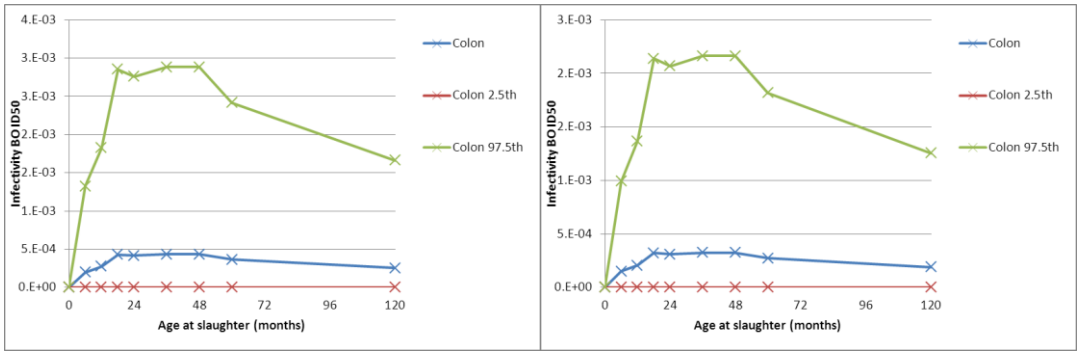

Figure C.5: Graph of mean, 2.5th and 97.5th estimates of infectivity in the colon before processing LHS, after processing RHS (BO ID_{50} per infected animal) by age at slaughter

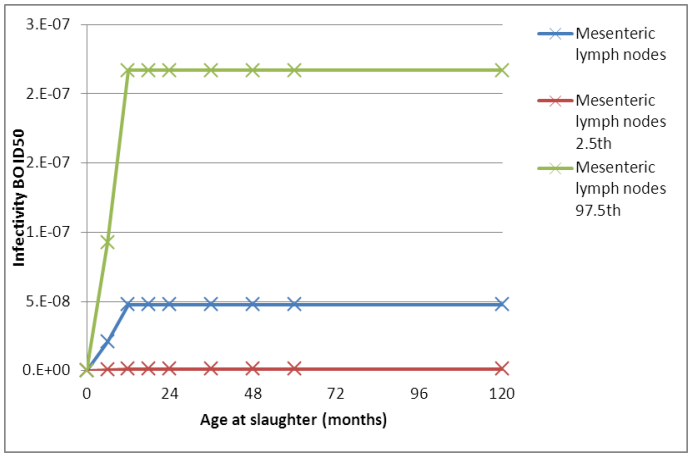

Figure C.6: Graph of mean, 2.5th and 97.5th estimates of infectivity in the mesenteric lymph nodes (BO ID_{50} per infected animal) by age at slaughter

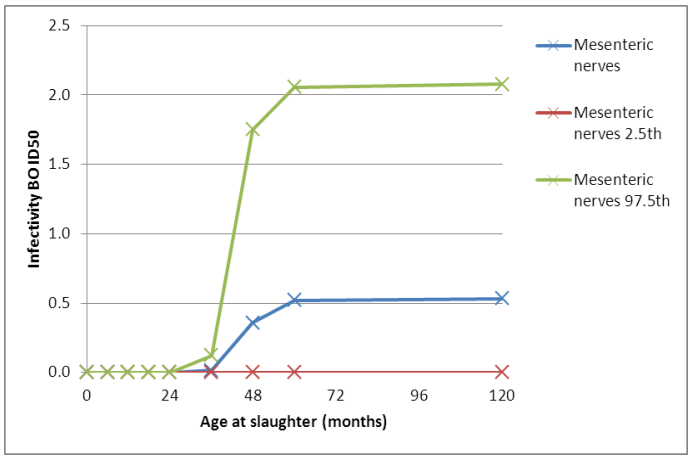

Figure C.7: Graph of mean, 2.5th and 97.5th estimates of infectivity in the mesenteric nerves (BO ID_{50} per infected animal) by age at slaughter

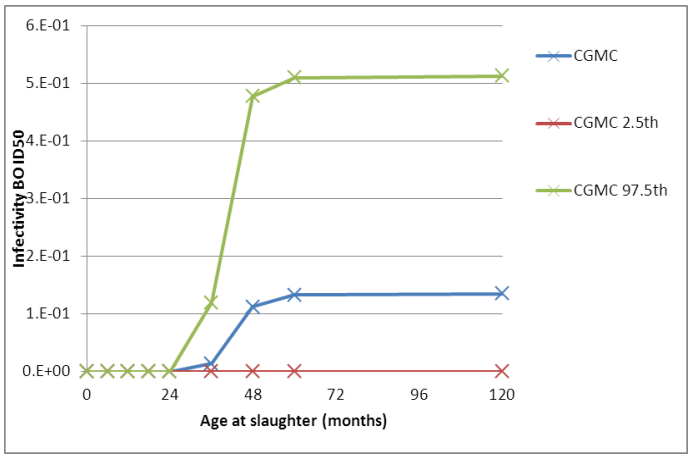

Figure C.8: Graph of mean, 2.5th and 97.5th estimates of infectivity in the mesenteric CMGC (BO ID_{50} per infected animal) by age at slaughter

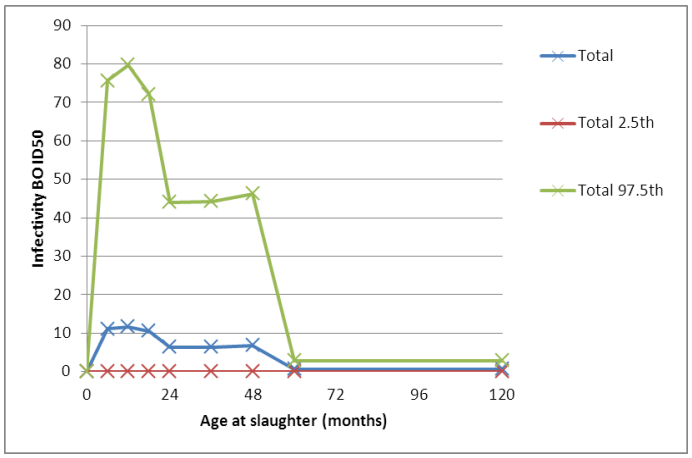

Figure C.10: Graph of mean, 2.5th and 97.5th estimates of infectivity in total bovine intestines and mesenteries (BO ID_{50} per infected animal) by age at slaughter

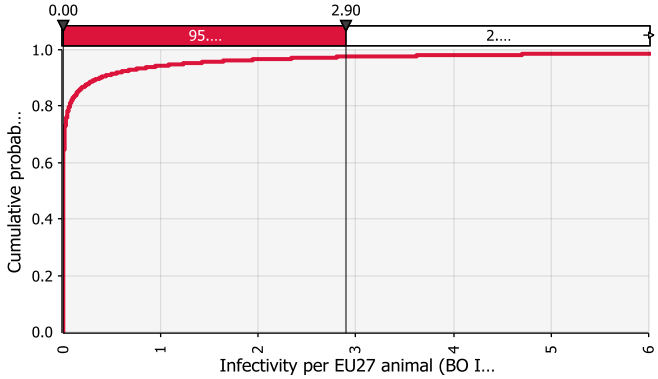

Figure C.11: Cumulative probability function describing the amount of infectivity in ileum (BO ID_{50}) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

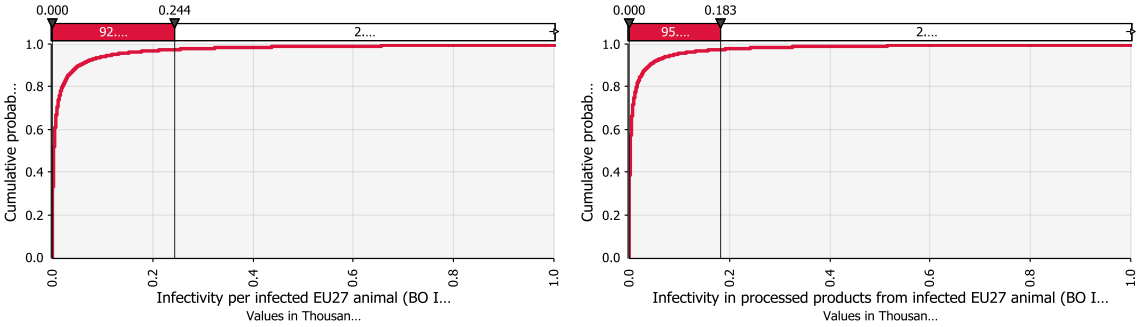

Figure C.12: Cumulative probability function describing the total amount of infectivity in duodenum before processing RHS, after processing LHS (BO ID_{50}) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

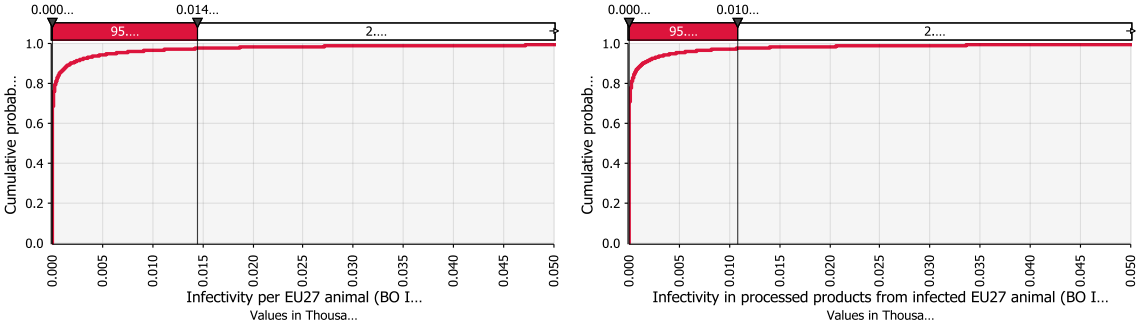

Figure C.13: Cumulative probability function describing the total amount of infectivity in jejunum before processing RHS, after processing LHS (BO ID_{50}) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

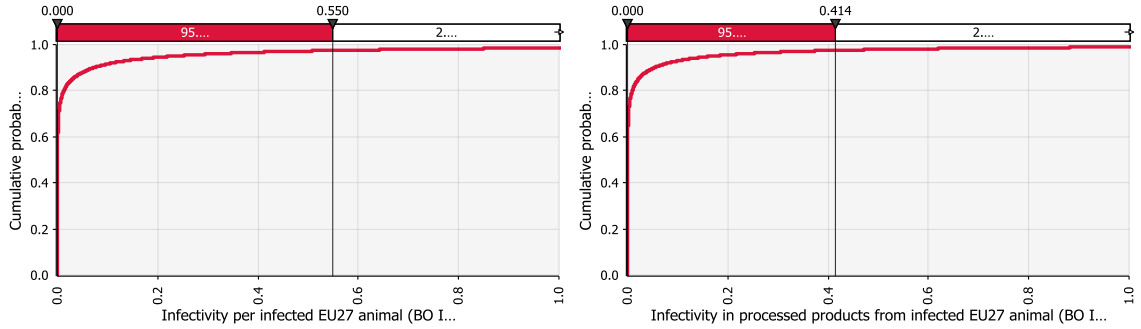

Figure C.14: Cumulative probability function describing the total amount of infectivity in caecum before processing RHS, after processing LHS (BO ID_{50}) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

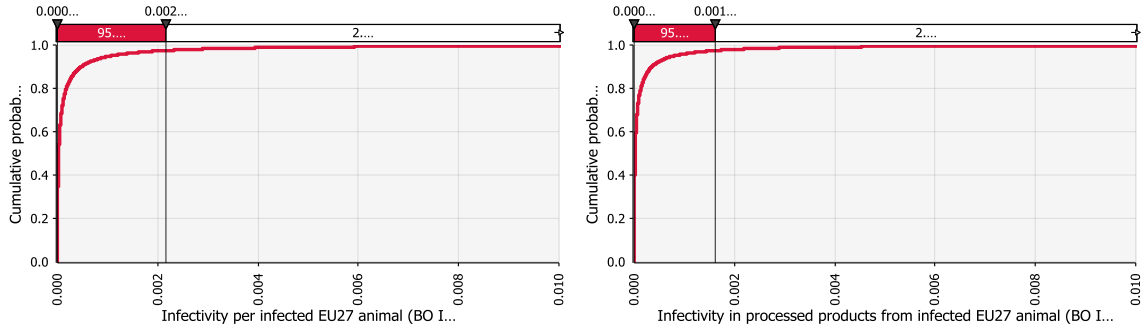

Figure C.15: Cumulative probability function describing the total amount of infectivity in colon before processing RHS, after processing LHS (BO ID_{50}) per infected slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

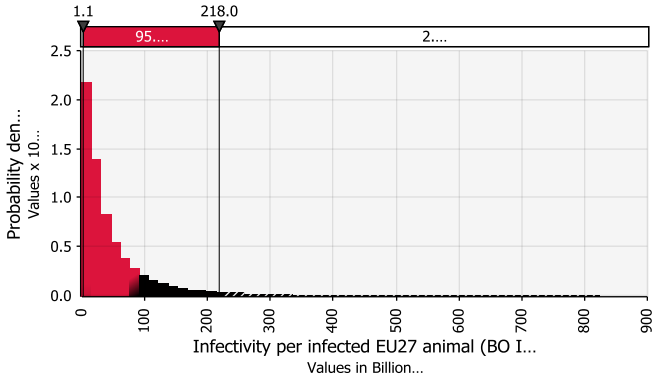

Figure C.16: Probability density function describing the total amount of infectivity in mesentery lymph nodes (BO ID_{50}) in a slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

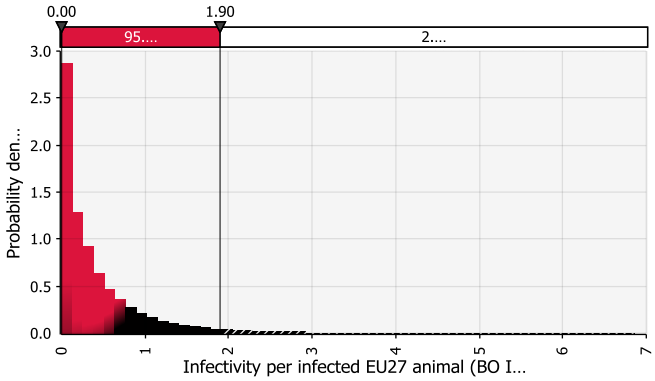

Figure C.17: Probability density function describing the total amount of infectivity in mesentery nerves (BO ID_{50}) in a slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

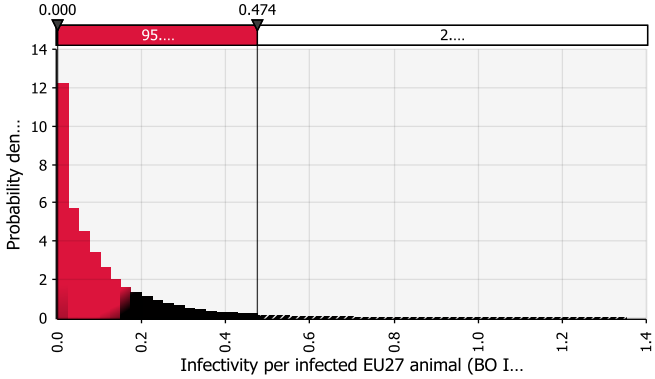

Figure C.18: Probability density function describing the total amount of infectivity in mesentery CMGC (BO ID_{50}) in a slaughter animal in EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

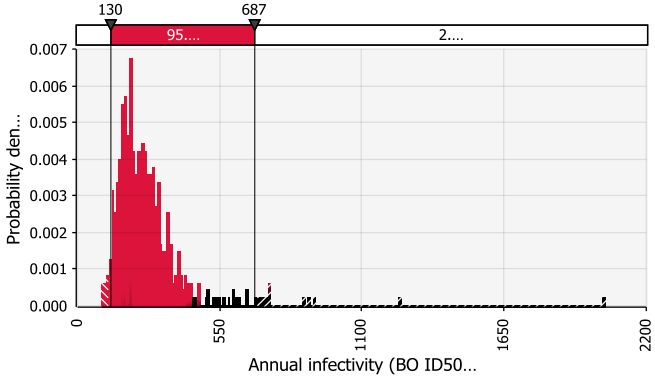

Figure C.19: Cumulative probability function describing the total amount of infectivity in ileum per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

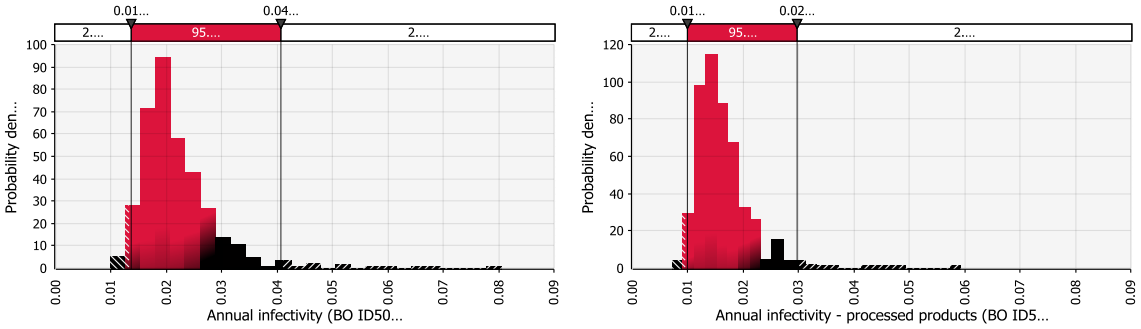

Figure C.20: Probability density function describing the total amount of infectivity from duodenum before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

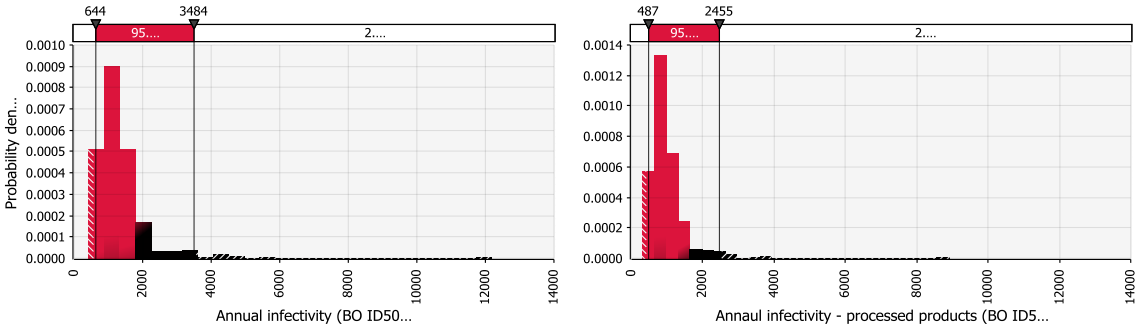

Figure C.21: Cumulative probability function describing the total amount of infectivity in jejunum before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

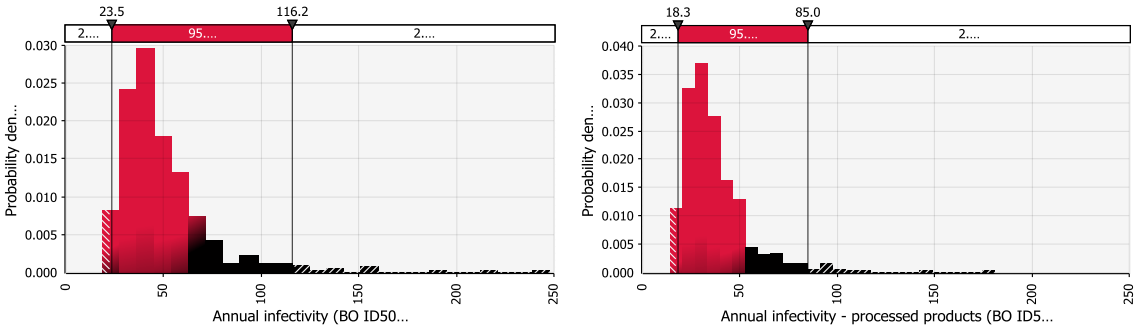

Figure C.22: Cumulative probability function describing the total amount of infectivity in caecum before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

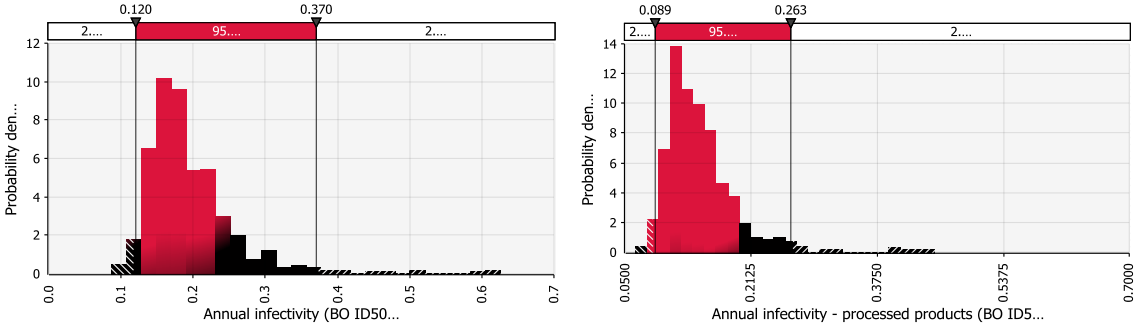

Figure C.23: Probability density function describing the total amount of infectivity in colon before processing RHS, after processing LHS for annual infectivity in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

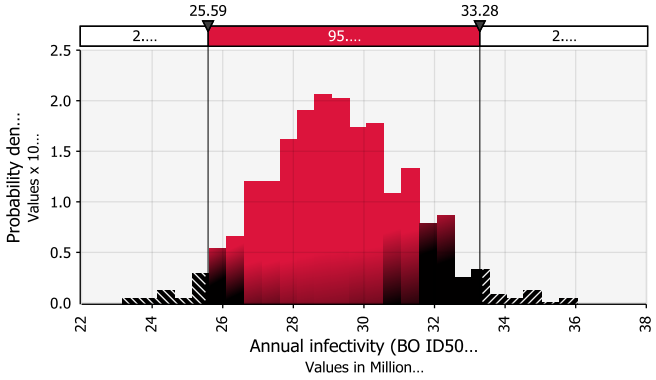

Figure C.24: Probability density function describing the total amount of infectivity in mesentery lymph nodes per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

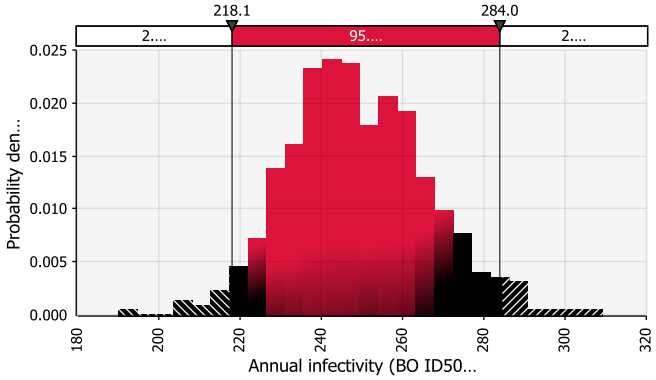

Figure C.25: Probability density function describing the total amount of infectivity in mesentery nerves per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

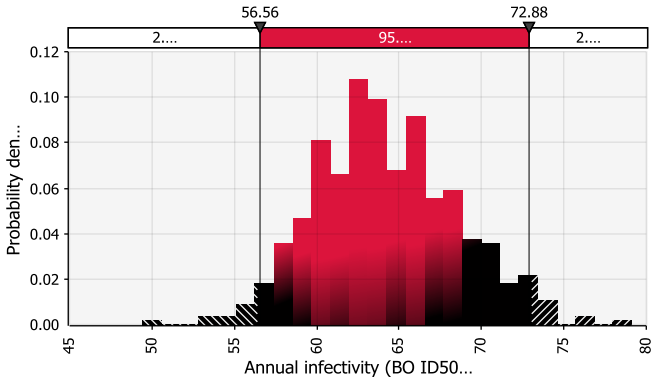

Figure C.26: Probability density function describing the total amount of infectivity in mesentery CMGC per year in the EU27 considering uncertainty and variability (95% percentiles indicated by top bar)

GLOSSARY AND ABBREVIATIONS

BO	Bovine Oral
BSE	Bovine Spongiform Encephalopathy
Cases	Test positive animal that are tested.
CI	Confidence Interval
CMGC	Celiac and mesenteric ganglion complex
CrI	Credible Interval
ES	Emergency slaughtered risk category
HS	Healthy slaughtered risk category
i.c.	Intracerebral inoculation
Infected animals	Total of animals that would test positive, if tested, and those infected that would test negative.
i.p.	Intraperitoneal inoculation
mpi	Months post infection
MS	Member State of the European Community
PP	Peyer's patches
QRA	Quantitative Risk Assessment
Test positive animals	Animals that would test positive if tested.
TSE	Transmissible Spongiform Encephalopathy