Estimating the impact on food and edible materials of changing scrapie control measures: the Scrapie Control Model

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Highlights (85 characters)

- Specified risk materials controls changing in Europe for small ruminants
- A risk assessment developed to estimate the impact for one country
- Limiting to brain and spinal cord would cause little impact in edible products
- Reducing to brain only would increase the impact to food chain to a higher degree
- Recommend other countries with different health demographics evaluate their risk

ABSTRACT (400 words)

Multiple controls established during the bovine spongiform encephalopathy (BSE) epidemic were not solely applied to BSE in cattle, but were implemented for scrapie in sheep and goats due to concerns over the occurrence of BSE in sheep. In the absence of BSE in sheep being observed, control measures for prion diseases are now being evaluated to ensure they remain proportionate to risk. This risk assessment, aims to estimate, by use of stochastic simulation, the impact of reducing controls for Specified Risk Materials (SRM) from sheep at abattoir. Three scenarios have been included: 1) current list of SRM; 2) brain and spinal cord of adult sheep; and 3) the brain of adult sheep.

Results indicate the total amount of infectivity passing through British abattoirs is highest for atypical scrapie with nearly 3,500,000 Ovine Oral (OO) ID_{50} per year. The majority of this infectivity enters Category 1 waste for incineration, with only 13,000 OO ID_{50} per year within edible products. Under Scenario 2, an additional 4,000 OO ID_{50} per year would be classified as edible products from the lifting of restrictions on the distal ileum of adult sheep. However, if SRM removal was limited to brain, an additional 110,000 OO ID_{50} per year would be permitted into edible products with the lifting of restrictions on the spinal cord of adult sheep.

For classical scrapie, there is a mean estimate of infectivity of 30,000 OO ID_{50} per year at abattoir. This is lower than for atypical scrapie due to the lower occurrence of this disease in Great Britain. However, more infectivity is destined to reach the food chain as the disease is peripherally distributed in the carcase. The highest contributor to the

total amount of infectivity consumed per year is the intestines (duodenum and jejunum). If SRM removal is limited to the brain and spinal cord of sheep over 12 months of age, there is an approximate mean increase from 19,000 to 21,000 OO ID_{50} per year diverted to edible products. If the SRM list is restricted to brain only, this increases to over 23,000 OO ID_{50} per year.

For the potential of sheep-BSE, there is a very low estimate of 29 OO ID_{50} per year in total from carcases entering abattoir, due to the potential very rare occurrence of this disease. Given changes in SRM regulations there is a change of an additional 4 OO ID_{50} per year being diverted to edible products.

Keywords: QRA, abattoir, prion protein, scrapie, specified risk materials

INTRODUCTION

In view of the steady decline in observed cases in European countries for Bovine Spongiform Encephalopathy (BSE) in cattle, European wide regulations have been amended to ensure that controls at the animal level and food chain are proportionate to the current level of risk. Such discussions are not limited to control measures focused on the prion protein in cattle, but also classical scrapie and atypical scrapie in sheep and goats. This is due to the escalation of controls on prions occurring in small ruminants in the wake of the BSE in cattle epidemic in the 1990s. Concern was high that detected scrapie in sheep may have been hiding the occurrence of BSE in small-ruminants which was justified subsequently after confirmation of naturally occurring BSE in goats (Eloit *et al.,* 2005; Spiropoulos *et al.,* 2011). In view of this concern, specified risk materials

(SRM) (Table 1) have been removed from small ruminant carcases at abattoir and categorised as the highest risk tissues defined as Category 1 materials. Such waste is incinerated to reduce any risk associated with use in the food chain or in the production of other protein materials such as Category 3 materials, which may be used in products such as pet food and soil improvers.

For classical scrapie, both active and passive surveillance are vital in achieving the goal of eradicating the disease. Active surveillance began in January 2002 as a result of Regulation (EC) No. 999/2001 (EC, 2001) and the recommendation of the Spongiform Encephalopathy Advisory Committee (SEAC) to estimate the prevalence of sheep and goat scrapie in the British flocks. The programme includes surveys on the slaughtered population and the fallen stock on farm. In addition to this, passive surveillance is also conducted in parallel where suspect cases are reported and tested.

The European Union (EU) requirement for testing sheep over 18 months of age for the United Kingdom (UK) is 20,000 in total with a baseline of 50% from sheep slaughtered for human consumption at abattoirs and 50% from fallen stock. However, the UK takes advantage of a derogation of the EU TSE Regulation to replace up to 50% of their requirement for sheep tested for human consumption with the same number of sheep fallen stock. On this basis, the UK tests at least 5,000 sheep over 18 months of age slaughtered for human consumption and the remaining 15,000 samples obtained from the fallen stock survey over 18 months to meet the overall requirement of 20,000. The EU requirement for testing fallen goats over 18 months of age is set at 500 for the UK. Table 2 displays the results of UK surveillance from 2005 to 2016.

In the last 5 years new outbreaks of classical scrapie have not been confirmed in GB. The surveillance has been further enhanced by the introduction of the Compulsory Scrapie Flock Scheme (CSFS), which aims to closely monitor scrapie-affected holdings through surveillance of fallen stock and slaughtered animals. The holdings where scrapie has been confirmed are placed under movement restrictions, which lasts for two years following the detection of the last case of classical scrapie and follows the full implementation of the relevant controls, as appropriate, during which animals aged over 18 months of age that die (fallen stock) or are killed for human consumption must be TSE tested. Should this monitoring option fail to be successful, the holding will be reassessed to where other options such as killing and destruction of all sheep or goats in the holding or compulsory culling of all sheep genetically susceptible to classical scrapie will be implemented as laid down in Annex VII of the EU TSE regulations. An additional control for classical scrapie was called "The National Scrapie Plan" or NSP, introduced in July 2001 and ending in 2012, which had the effect of reducing the most susceptible genotypes within the national sheep population.

Atypical scrapie was first identified in Norway in 2003. Previous studies have not found any risk factors associated with an infectious origin suggesting that atypical scrapie in sheep is a spontaneous disease (Fediaevsky *et al.,* 2009). However, oral transmission of atypical scrapie has been experimentally demonstrated (Simmons *et al.,* 2011) suggesting this prion disease could be transmitted by feed and environmental pathways.

BSE in small ruminants had been experimentally produced by the oral route (Foster *et al.,* 1993) and it was known that BSE-infected bovine material in meat and bone meal feed was fed to small ruminants as protein supplements in the 1980s and 1990s.

Therefore it was not a significant surprise when two naturally occurring cases of classical BSE were found in goats, one in France, and one in Scotland. The Scottish case of BSE in a goat was originally tested and confirmed as a scrapie case in 1990. Following the confirmation of BSE in a goat in France in 2002 (Eloit *et al.,* 2005), the UK carried out discriminatory immunohistochemistry on the scrapie positive goat samples. This case was found to be positive for BSE in 2005 (Jeffrey *et al.* 2006). Tissue samples from this case were further characterised by mouse bioassay and the outcomes reported in 2009 as BSE (Spiropoulos *et al*. 2011).

With only two naturally occurring cases, it has been difficult to estimate an approximate prevalence of the occurrence of BSE in small ruminants. Kao and colleagues (2002) and Ferguson *et al*. (2002) used data from a small sample of brains (156 and 180, respectively) that were being studied by bioassay to estimate that approximately 2% of scrapie cases could be masking sheep-BSE at the 95% confidence limit. The 95% confidence limit is often quoted, rather than the mean, as selection bias and other complexities in the data make this the most appropriate estimate of the data available (Gravenor *et al.,* 2003). Other risk assessments have utilized different maximum and minimum proportions of scrapie that are assumed to be sheep-BSE cases. Ferguson *et al*. (2002) utilized 0.5% to 2%, while Det Norske Veritas (DNV) (2001) used much larger intervals by considering arbitrary scenarios where the value ranged from a minimum of 0.01% to a maximum of 10%, with a "medium" value of 0.1%. In 2004, the Veterinary Laboratories Agency (VLA) reported to the Food Standards Agency (FSA) on the outcome of a 2-year project to screen brains of scrapie suspects using the method of Stack *et al.* (2002). Gubbins (2004) has used these data to estimate the maximum proportion of sheep TSE cases that could be BSE under different assumptions about the sample size. Based on the assumption that the cases tested

represent a random sample, the upper 95% confidence interval is that 0.14% of cases could be BSE positive. Finally in 2007, after the discovery of the two natural cases of small ruminant BSE, the European Food Safety Authority (EFSA) published an Opinion which calculated that, depending on the statistical model and the sub-set of input surveillance data, there was 95% confidence that in the UK there were less than 2-4 sheep-BSE cases per 10,000 healthy-slaughter sheep (EFSA 2007).

The BSE control model was a risk assessment developed in the UK to monitor the estimated amount of BSE infectivity from cattle entering the food chain each year based on current or theoretical surveillance and SRM control scenarios (Adkin *et al.,* 2010). This work was previously developed under the TSE research portfolio in 2004, and has been subsequently updated and maintained under the surveillance contract, with annual results for GB provided to assist policy makers at Defra and FSA when considering the UK response to European proposals to change BSE legislation.

Over the last few years a number of scrapie related projects have yielded data on the estimated occurrence and titres of scrapie infectivity in different tissue types for classical and atypical scrapie. Additional statistical work has used these data to parameterise certain variables of use in risk assessment. Given the interest at a European level for the deregulation of scrapie SRM controls in line with BSE control deregulation, a quantitative assessment on the infectivity arising from scrapie affected livestock at a national level would assist current policy considerations in this area. Therefore a risk assessment, termed the Scrapie Control Model, has been developed. Within the UK market, sheep meat dominants the proportion of meat consumed from small ruminants. In 2016, a provisional estimate of 289,590 tonnes of sheep meat and only 340 tonnes of goat meat were slaughtered for human consumption (Eurostat,

2017). Therefore, combined with a higher uncertainty regarding prion disease in goats, the risk assessment has been developed and parameterised only for sheep.

This paper describes the quantification of the total amount of infectivity present in an infected sheep and lamb carcase for the prion diseases sheep-BSE, sheep classical scrapie and sheep atypical scrapie. In addition, the annual amounts of infectivity that subsequently enters the food chain, Category 1 materials and that are lost to the floor and diverted to drains are estimated for three SRM removal scenarios shown in Table 3.

METHODS

Estimating the amount of infectivity was conducted by development of a probabilistic model with random variables and uncertain parameters described by appropriate probability distributions. The model was implemented in @Risk (© Palisade) Version 6, an add-on package within Microsoft Excel (© Microsoft). The results presented follow the standard form of the arithmetic mean and the 5^{th} and 95^{th} percentile values. Accordingly the latter represent the range of values for which we are 90% certain that the true value lies between. The variables and parameters are described in each of the subsequent sections, with a summary of the values provided in Table 4.

Model overview

The risk assessment is divided into four components – Surveillance, Abattoir, Infectivity and the Annual extension. Parameters from the first three components are used to estimate the amount of infectivity per infected animal that has by-passed testing controls and entered the slaughterhouse for food production. These components are run

194 in a separate simulation (Model 1). The Annual extension uses the results from Model
195 1 to estimate the annual amount of infectivity consumed nationally (Model 2). A
196 summary diagram of the model framework outlining the links between the components
197 in Model 1 and Model 2 is provided in Figure 1.
198
199 **Surveillance component**
200 The surveillance component estimates the number of infected adult sheep and lambs fit
201 for human consumption, in the last 12 months of the incubation period, that enter
202 abattoirs annually. The estimated number of infected animals at abattoir ($N_{a,i}$), per
203 animal group $a \in \{S, l\}$ and disease $i \in \{bse, sc, at\}$ is given by Equation (1):
204 $$N_{a,i} \sim Poisson(P\_infected_i * N\_animals_a) \quad (1)$$
205 where *bse* refers to sheep-BSE, *sc* to classical scrapie and *at* to atypical scrapie; *S* refers
206 to sheep over 1 year of age and *l* denotes lambs (sheep less than 1 year of age). A
207 Poisson distribution has been used to approximate the binomial distribution due to a
208 large number of animals and small probability of infection. Sheep were divided into
209 these two age groups because SRM controls are different for the two groups. For each
210 animal group and disease, $P\_infected_i$ denotes the probability that an individual animal
211 is infected (i.e. prevalence of infection), and $N\_animals_a$ the annual number of animals.
212
213 The mean infection prevalence and 2.5th and 975.5th percentiles for classical scrapie at
214 abattoir has been estimated previously (Ortiz-Pelaez and Arnold, 2008; Arnold and
215 Ortiz-Pelaez, 2014). The prevalence has been updated by calculation of the trend 2005-
216 2017, derived from a confidence interval from the uncertainty in the trend parameter.
217 This estimate includes sampling error but relies on the assumption that there has been
218 a constant declining trend through the years 2005-2017 which cannot be verified in the

absence of any observed cases (Arnold, pers. comm. 2017). These estimated values are represented in the risk assessment by a Betapert distribution. This calculation of prevalence is not stratified by population genotype.

There are little data for atypical scrapie cases. In the absence of further information, the Abattoir Survey was used to estimate the prevalence of atypical scrapie. A number of key assumptions were applied when using these data: (1) the incubation period of atypical scrapie was estimated from atypical passive surveillance and fallen stock positives, assuming fallen stock had reached clinical onset and died of scrapie (atypical scrapie is known to have a longer incubation period than classical scrapie); (2) the survivability of sheep infected with atypical scrapie is the same as for classical scrapie; and (3) the sensitivity of the post-mortem test for atypical scrapie is the same as for classical scrapie. The total number of lambs and sheep entering the abattoir, *N_animals a,,* is recorded by Defra statistics (Defra, 2015).

As detailed in the introduction, prevalence values for sheep-BSE as a proportion of the estimated classical scrapie prevalence vary. From the most recent Opinion, the estimates from EFSA have been used in the risk assessment in the following equation:

$$P\_infected_{bse} = P\_infected_{sc} * \theta_{sc} \qquad (2)$$

where θ_{sc} is the proportion of scrapie that is assumed to be sheep-BSE. In a survey of 2483 scrapie cases, the differential test for sheep-BSE was applied with 0 results. (Stack et al., 2002; SEAC, 2006). Results were 0 in sample size of 2483 cases, with the uncertainty associated with the probability of occurrence described in the assessment by a beta distribution with non-informative prior.

Infectivity component

The infectivity component estimates the amount of infectivity by tissue type, in infected adult sheep and lambs in the last 12 months of the incubation period.

Classical scrapie

There are a number of tissues that have been identified as carrying significant levels of classical scrapie infectivity in sheep because of peripheral spread. The titre of infectivity $Infectivity_{a,sc,t}$, (Ovine Oral (OO) ID_{50} per gram) was estimated by the following equation (values in Table 4):

$$Infectivity_{a,sc,t} = P\_infectivity_{a,sc,t} * \frac{10^{Max_{sc,t}}}{10^{OOunit}} \quad (3)$$

where $Max_{sc,t}$ denotes the maximum titre of infectivity (log_{10} Mouse i.c. ID_{50} per gram) for tissue type $t \in \{1 \text{ to } 14\}$. For tissues t = 1 to 9 estimates of infectivity were taken from Kimberlin and Wilesmith, (1994) with a mean value expressed as log_{10} Mouse i.c. ID_{50} per gram and standard error. The uncertainty about the maximum was described by a normal distribution (Table 4). Other tissues have been found to harbour infection or abnormal prion protein has been detected. Low levels of infectivity of the stomach, heart, and kidney (t = 10, 11 and 12) were described by point values (Kimberlin and Wilesmith 1994). In the absence of information regarding the infectivity titre of the duodenum and jejunum (t = 13), it was assumed that the level of infectivity in these tissues is the same as in the ileum (t = 6). This is a worst case assumption since the ileum is classified as SRM for sheep of all ages whereas the duodenum and jejunum are not. Evidence of classical scrapie infectivity has been detected in the blood of sheep at low titres (Kimberlin and Wilesmith 1994) and Andreoletti has investigated the transmission of scrapie through blood transmission with the use of transgenic mice tg338 (Andreoletti *et al.,* 2012). The minimum infectious dose for brain homogenate

was titrated by end point dilution in tg338 mice, with a titre of $10^{6.6}$ ID_{50} IC/g (Andreoletti *et al.,* 2011). The lowest dose resulting in transmission was observed in sheep (1 infection out of 2 challenged individuals) that were transfused with blood containing 10^3 ID_{50} IC in tg338 mice (Andreoletti *et al.,* 2012). This equates to an approximate 4,000 fold reduction for blood as compared to brain homogenate. When applied in the risk assessment, this equates to blood containing the same infectivity as estimated for stomach and liver tissue. Low levels of infectivity have also been observed in the pituitary gland, cerebrospinal fluid and adrenal gland (Kimberlin and Wilesmith 1994). These tissues are respectively paired with the brain, spinal cord and kidney for ease of assessing the weight. Other tissues containing low amounts of infectivity are the PNS (Kimberlin and Wilesmith 1994; Hadlow *et al.*1982), tongue (Hadlow *et al.,*1982), bone marrow and supramammary lymph node (OIE 2009), and psoas muscles (Andreoletti *et al.,* 2011). Given that the titre of infectivity in these tissues is considered very low, when compared to other infectious tissues which are being considered, these tissues were not quantitatively assessed.

$P\_infectivity_{a,sc,t}$ refers to the proportion of infectivity accumulated when an animal is slaughtered. The majority of clinical cases of classical scrapie appear in sheep between 2 and 5 years of age (Kimberlin and Wilesmith 1994). During the progression of the disease, infectivity accumulates in different tissues at different rates. It is important to note that the vast majority of sheep are slaughtered before the age of 7 in the UK, are clinically in good health, and therefore, the disease may not be fully developed at slaughter. In this risk assessment, two age groups were considered, lambs under one year old and sheep over 1 year old. The percentage increase in infectivity at different ages was adjusted accordingly for lambs under one year of age (Kimberlin and

Wilesmith 1994), except for the lymph nodes and intestine (duodenum, jejunum and ileum) where the percentage of infectivity was estimated as 40% (Simmons, pers. comm. 2009). For sheep over the age of one year, the percentage of infectivity in all tissues was estimated to lie between 70 and 100% described by a uniform distribution (Simmons, pers. comm. 2009).

A conversion factor, *OOunit*, was used to obtain approximations of the titres in units of Ovine Oral ID_{50} per gram. Titres of infection as calculated by intracerebral and intragastric routes of exposure (expressed as sample mean ± standard error of $\log_{10}$ ID_{50} per 30 mg of mouse brain) are given in Kimberlin and Wilesmith, (1994). Using these data, the intracerebral route titre was described by a normal distribution with mean 7.03 and variance 0.0169, and the intragastric route titre is described by a normal distribution with mean 2.03 and variance 0.0361. Since the values of these distributions are in units of $\log_{10}$, an estimate for *OOunit* is obtained by subtracting the intragastric route titre from the intracerebral route titre which results in a normal distribution with mean 5 and variance 0.053.

Atypical scrapie

The titre of infectivity (OO ID_{50} per gram) for tissues from animals infected with atypical scrapie, $Infectivity_{a,at,t}$, was estimated by the following equation with values shown in Table 4:

$$Infectivity_{a,at,t} = P\_infectivity_{a,at,t} * \frac{10^{Max_{at,t}}}{10^{OOunit}} \quad (4)$$

$Max_{at,t}$ denotes the maximum titre of infectivity ($\log_{10}$ Mouse i.c. ID_{50} per gram) by tissue type *t*. The number of potentially infectious tissues for atypical scrapie has been found to be much more restricted than for classical scrapie. Previous studies suggest that high levels of infection are limited to the central nervous system (CNS) and lymph

318 nodes as detected by the presence of PrP^{sc}. However the use of more sensitive transgenic
319 mice bioassay has been able to detect some cases of tissue infectivity present in the
320 distal ileum in orally infected atypical cases (Simmons *et al.,* 2011), brachial nerve,
321 sciatic nerve and eye (external ocular muscle) from experimentally infected atypical
322 cases via the intracerebral inoculation route (Andreoletti *et al.,* 2011). In this risk
323 assessment, it is assumed that the CNS (brain and spinal cord), lymph nodes, and distal
324 ileum can become infectious for sheep infected with atypical scrapie.
325
326 The titre of infectivity within atypical scrapie tissues has not been less widely reported
327 in the literature and work in this area are ongoing. Andreoletti and colleagues reported
328 on the results from 9 cases of atypical scrapie and 4 cases of classical scrapie for brain
329 material shown in Table 5, together with estimates of infectivity cited in Kimberlin and
330 Wilesmith (1994). The atypical infectivity titres shown in Table 4, are higher than for
331 classical scrapie using the same mouse model (tg338) with a minimum percentage value
332 of 100%, mean 105% and maximum of 132% of the value of infectivity estimated in
333 the brain of a classical case. This may be due to the transgenic mouse model having a
334 higher predilection for atypical proteins over classical, variation between animals in
335 such a small sample size, or may represent a higher concentration of the prion protein
336 in the brains of infected individuals. In this risk assessment, the worst case is assumed
337 that brain material from atypical cases accumulate a higher percentage infectivity than
338 classical scrapie described with uncertainty described using a pert distribution.
339
340 To parameterise spinal cord, there are only one value found from experimentation of
341 $10^{2.9}$ Titre ID_{50} IC/g tg338 from a case where the brain sample measured $10^{5.8}$ Titre ID_{50}

IC/g. Due to only one sample, a worst case assumption is used that the spinal cord has the same titre as classical scrapie.

Limited infection has been demonstrated to be present in the peripheral tissues at low levels detectable by transgenic bioassay but not by PrP^{sc} detection (Andreoletti *et al.,* 2011). The titre of infectivity has been estimated to be between 5 and 6 logs less than in CNS tissues (Simmons, per. comm., 2009; Andreoletti *et al.,* 2011). This has been used in the risk assessment for lymph nodes and distal ileum, described by a uniform distribution.

Based on only 8 clinical cases, the incubation period of atypical scrapie is at least 2 to 3 times longer than for classical scrapie (Simmons, per. comm., 2009). Therefore, the distribution of infectivity with age has been adjusted: for lambs under one year old the percentages are thought to be negligible and represented in the assessment as 0.1% of the maximum clinical titre (Simmons, pers. comm. 2009). This is an important assumption which is further explored in the sensitivity analysis. For sheep over the age of one year, the percentage of infectivity in all of the tissues at the time of slaughter, $P\_infectivity_{S,at,t}$, is estimated to lie between 40 and 80%.

BSE in sheep

Experimental infection of classical BSE in sheep has been well documented (Andreoletti *et al*., 2006; Bellworthy *et al.,* 2005; Jeffrey *et al.,* 2001, 2015; Foster *et al.,* 1993, 2001 a, b; Houston *et al*., 2015; Tan *et al*., 2012; McGovern *et al*., 2015, 2016; van Keulen *et al*., 2008; Hunter *et al*., 2012). The sheep PRNP genotype has been found to influence both localisation of PrP^{d} deposition and incubation rate. Experimental BSE infection has the shortest incubation period in sheep with the

ARQ/ARQ PRNP genotype (Foster *et al*., 2001b; Houston *et al*., 2003; van Keulen *et al*., 2008; Hunter *et al*., 2012; Tan *et al*., 2012) although the incubation period has also been found to be influenced by the genotype at codon 141 of the PRNP gene (Hunter *et al*., 2012; Tan *et al.,* 2012), with LF heterozygotes having a longer mean incubation period than homozygotes of either type; the shortest incubation period was observed in LL141 sheep (Tan *et al.*, 2012). Resistance in sheep to BSE challenge has also been found to be associated with the TARQ allele M112T PRNP variant (Saunders *et al*., 2009; McGovern *et al*., 2015). Houston *et al*., (2015) found that out of eight clinically affected BSE-challenged ARQ/ARQ sheep examined, all had PrP^d deposits in the spleen, mesenteric lymph node, and tonsil. Sheep of other PRNP genotypes showed more restricted distribution of PrP^d in lymphoid tissues and there was no evidence of PrP^d deposition in lymphoid tissues from BSE-infected sheep in the VRQ/ARR, ARQ/ARR or ARR/ARR groups apart from one sheep of ARQ/ARR genotype which was positive in spleen (Houston *et al.,* 2015). In a similar study, neither PrP^d nor infectivity was detected in any tissues of BSE-dosed ARQ/ARR or ARR/ARR sheep (McGovern *et al*., 2015).

Breed of sheep may also influence their susceptibility to BSE challenge. Oral infection of Romney and Suffolk sheep with BSE-infected bovine brain material demonstrated that the brain, spinal cord, spleen, tonsil, ileum, and proximal colon could all be infected by BSE in infected sheep (Jeffrey *et al*., 2001). More recent studies on BSE infection of Romney and Suffolk sheep of the ARQ/ARQ or ARQ/ARR PRNP genotypes found that whilst all clinical BSE cases showed PrP^d accumulation in the brain, lymphoreticular system (LRS) involvement within Romney recipients was significantly lower than for Suffolk sheep (McGovern *et al*., 2016). Differences between the two

breeds were noted in terms of involvement of LRS and enteric nervous system (ENS) tissues, with Romney sheep showing a more delayed and less consistent PrP^d accumulation than Suffolk sheep in such tissues (McGovern *et al*., 2015).

Time course studies of PrP^d deposition following oral inoculation with BSE brain homogenate have found similar results of progression. Van Keulen *et al*., (2008) found initial accumulation of PrP^d in the tonsil and the ileal Peyer's patches then in all gut-associated lymphoid tissues, lymph nodes and the spleen before spreading from the ENS to the medulla oblongata and the spinal cord. More recently, McGovern *et al.,* (2015) found that in MARQ/MARQ sheep of Romney and Suffolk breeds, initial PrP^d accumulation was identified in LRS tissues (retropharyngeal, prescapular, distal jejunal and prefemoral lymph nodes, palatine tonsil, spleen and jejunal and ileal Peyer's patches) followed by the CNS and ENS (jejunum and ileum) and finally in the autonomic nervous system and peripheral nervous system and other organs associated with infection for classical scrapie. Accumulations of PrP^d have also been found in the liver of 100% of sheep challenged with BSE, compared to 89% of sheep naturally infected with scrapie, at both clinical and preclinical stages of the disease (Everest *et al*., 2011).

The bovine atypical L-BSE strain of BSE can be intracerebrally transmitted to sheep of several genotypes, with the exception of ARR/ARR animals, but differs from challenge with classical BSE in that PrP accumulation is predominantly confined to the nervous system. In seventeen out of eighteen positive challenged animals, no immunoreactivity was detected in the liver, spleen, lymph nodes, distal ileum or rectoanal mucosa-associated lymphoid tissue (Simmons *et al.,* 2016). For classical BSE challenge,

417 Bellworthy *et al.* (2005) concluded that despite differences in the experimental
418 protocols, it is safe to say that the spread of BSE infectivity through the sheep carcass
419 reflects that reported by Hadlow *et al.* (1982) for naturally occurring scrapie. At the
420 current time, such experimental data has not been linked to titre. Therefore, with no
421 other information available, it is assumed that all tissues that are infected by scrapie are
422 also infected by sheep-BSE with the same level of infectivity.
423
424 **Abattoir component**
425 The abattoir component estimates the total weight of each tissue type $N\_total_{a,t}$, from
426 adult sheep and lambs, that are removed and (1) classified as food fit for human
427 consumption including Category 3 materials, $N\_food_{a,t}$, (2) classified as Category 1
428 high risk materials for disposal by incineration, $N\_cat1_{a,t}$, and (3) falls to floor or
429 washed down from equipment, $N\_floor_{a,t}$. These amounts were separately estimated
430 for sheep and lambs under three scenarios shown in Table 3. Tissue weights and
431 associated references are presented in Table 4.
432
433 ***Sheep >12 months of age***
434 Blood lost at exsanguination is currently disposed of as Category 1 material through
435 use of a blood tank. It is estimated that 40-60% (Kimberlin and Wilesmith 1994) of the
436 total blood volume of a sheep ($N\_total_{S,14}$ 2,720 g) is collected here. The amount of
437 blood loss due to further processing, that was assumed to fall to the floor $N\_floor_{S,14}$,
438 was estimated to be 10-20% of the original blood content (272-544 g). The remaining
439 weight of blood is assumed to be associated with edible materials produced from the
440 carcase $N\_food_{S,14}$.
441

Under current SRM regulations, the skull including brain and eyes (160 g) and tonsils (3.2g) are classified as SRM for sheep over one year old and are disposed of as Category 1 material, $N\_cat1_{S,1}$. There is a risk of contamination of meat from embolisms and operator transfer of brain tissue as a result of a captive bolt gun (CBG) being used to stun the animal. Coore *et al.,* 2004 found that the frequency of brain tissue embolism was 23% in sheep stunned with a cartridge-activated CBG and 14% in those stunned with a pneumatically activated CBG. The number of sheep slaughtered in England and Wales using a stunning method is approximately 72.8% (FSA, 2017), and is split between CBG and electrical stunning with the later used more frequently (Anil, 2012). There are no data currently available on the proportion of sheep stunned by CBG, but using a pessimistic assumption that 40% of sheep are stunned with a CBG would result in an overall average proportion of 8.3% of carcases (if using cartridge-activated CBG) and 5.0% of carcases (if using pneumatically activated CBG) incurring an embolism event.

In the risk assessment a most likely probability of 10% per carcase (minimum 1%, maximum 15%) is used with a most likely value of 1% brain weight transferred to food/category 3 materials per event (minimum 0.1%, 0.02%). This equates to an average of approximately 0.1 g contamination per carcase. This is lower than that estimated for cattle (Adkin et al., 2010) to take into account the lower likelihood of captive bolt used to stun sheep and less manual handing of the head during processing. Under Scenario 2 and 3, the tonsils are no longer removed as SRM. As a worst case it is assumed that the tonsils are removed together with the tongue and enter edible materials.

467 The spinal cord is removed for adult sheep as SRM under scenarios 1 and 2. However,
468 inspections have shown that infrequently, small pieces of spinal cord may remain post
469 abattoir controls in food or category 3 materials, $N\_food_{S,2}$. From an FSA survey in
470 2007, from 260,833 inspections there were 13 failures (FSA, 2007). This is described
471 in the risk assessment using a beta distribution. Given that there is a failure to correctly
472 remove, between 0.05 and 1% of the spinal cord weight is assumed to remain, described
473 using a uniform distribution. The amount of spinal cord material going to the floor,
474 $N\_floor_{S,2}$, was estimated to be the same proportion by weight as the amount of spinal
475 cord which is lost to the floor for cattle, estimated by the following equation where t =
476 2, spinal cord.

477 $$N\_floor_{S,2} = \left(\frac{N\_floor_{C,2}}{N\_carcase_{C,2}} \right) * N\_carcase_{S,2} \quad (5)$$

478 All remaining spinal cord is assumed to be removed as part of evisceration as disposed
479 of as Category 1 materials, $N\_cat1_{S,2}$. Under Scenario 3, it is assumed that spinal cord
480 is diverted to edible materials.
481
482 Upon evisceration it is assumed that a very small proportion of the spleen (300g) and
483 ileum (200g) falls to the floor, between 0.01 and 0.1%, described using a uniform
484 distribution (assessors assumption based on observation, 2009). This equates to a mean
485 of less than a gram of tissue per carcase. The remainder is assumed to enter either
486 Category 1 materials if classified as SRM or edible materials, in the absence of controls
487 in Scenario 2. It was assumed that all other infectious sheep tissues remaining in the
488 carcase enter edible materials.
489
490 ***Lambs <12 months of age***

The amount of infectious material going to the food chain, floor and Category 1 bin at abattoirs for lambs differs from sheep. This is due to different SRM controls being in place for sheep under 1 year old and lamb tissues weighing less than sheep (lamb tissue weights and references are given in Table 4).

At exsanguination it was estimated that 40-60% of the total amount of blood in a lamb ($N\_total_{L,14}$ 1,700 g) is disposed of as Category 1 material using a blood tank, $N\_cat1_{l,14}$ (Warriss 2000). The amount of blood falling to floor, $N\_floor_{l,14}$, was estimated to be between 170 and 340 g (10-20% of the original blood content).

Unlike sheep aged over one year the skull, including brain, eyes and tonsils, are not classified as SRM and can be disposed of as Category 2 material. However, most abattoirs dispose of the head as Category 1 material in order to reduce collection costs. Infectious tissues associated with the head are the brain and eyes (100 g) and tonsils (2 g). It was estimated that the amount of brain material which is lost to the floor due to handling of the head, $N\_floor_{l,1}$, is between 1 and 2 g (i.e. 1-2% of all brain material) and the amount transferred to meat is proportionally the same as that assessed for sheep. The remaining brain material is disposed of as Category 1 waste, $N\_cat1_{l,1}$. Tonsils are assumed to remain on tongue meat removed for the food chain and Category 3 materials. The spinal cord (40 g) is not classified as SRM for lambs and it is assumed that carcasses are not split to remove the vertebral column. Therefore all spinal cord is assumed to enter the food chain and category 3 materials, $N\_food_{l,2.}$ The spleen (75 g) and the ileum (100 g) which are SRM for sheep of all ages in Scenario 1 were assumed to be removed at evisceration to Category 1 materials with the same proportions going to floor as for sheep. For Scenario 2, both tissues are assumed to enter the food chain

516 or Category 3 materials. All remaining lamb tissues in the carcase enter the food chain
517 or Category 3 materials.
518
519 **Model 1 outputs**
520 As described in Fig. 1, the amount of contaminating tissues from the Abattoir
521 component is multiplied separately, by tissue type, with the titre of infectivity in those
522 tissues (ovine oral ID50/g) from the Infectivity component.
523
524 *Infectivity per carcase entering food chain and Category 3 materials, N_food* $_{a,t}$
525 The amount of infectivity for each disease entering the food chain and Category 3
526 materials (Oral ID_{50} per carcase), is estimated by multiplying the tissue weight by
527 infectivity and summing for each animal group, as shown in equation (6):

528 $$I\_food_{a,i} = \sum_t N\_food_{a,t} * Infectivity_{a,i,t} \quad (6)$$

529 *Infectivity per carcase entering Category 1 materials, N_cat1* $_{a,t}$
530 The amount of infectivity disposed of as Category 1 waste (Ovine Oral ID_{50} per
531 carcase), is estimated by summing the amount of infectivity by tissue type and adding
532 the total material retained by the trap, as shown in equation (7)

533 $$I\_cat1_{a,i} = \left(\sum_t N\_cat1_{a,t} * Infectivity_{a,i,t}\right) + I\_trap_a \quad (7)$$

534 Where $I\_trap_a = \left(\sum_t N\_floor_{a,t} * Infectivity_{a,i,t}\right) * P\_trap$
535 denotes the amount of infectivity (grams) that is retained in the trap and subsequently
536 placed in the Category 1 bins for disposal and $N\_floor_{a,t}$ is the amount in grams of
537 infectious tissue type *t* that falls to the floor per carcase.
538
539 *Infectivity per carcase entering wastewater, N_drains* $_{a,t}$

Material falling to floor or washed from equipment will arrive at the mandatory 6mm trap and, potentially, enter the facility drains. There are two drain areas at abattoirs; one floor area includes the processing stages of stunning, head removal and bleeding and, if required, brain sampling for TSE testing, and flows into the blood tank which is disposed of as Category 1 as shown in Equation 7. Wash down from the floor areas for processing the remainder of the carcase will flow via the trap into wastewater. The amount of infectivity passing through the trap, and thus into the drains, $I\_drain_{a,i}$, (OO ID_{50} per carcase) is estimated for sheep and lambs given by Equation (8)

$$I\_drain_{a,i} = \sum_{t} N\_floor_{a,t} * Infectivity_{a,i,t} * (1 - P\_trap) \quad (8)$$

where the percentage of infectivity retained by the trap is denoted *P_trap*.

Proportion of infectivity retained by trap, P_trap

It is a legal requirement for SRM handling facilities to have a 6 mm trap, with any sludge retained classified as Category 1 material (Saunier 2003). Research conducted by AFSSA attempted to measure the amount of CNS material that was retained at abattoir and the proportion that flowed through the trap (Saunier 2003). However, the experimental protocol used did not enable quantitative estimates. The proportion of material that is retained by the trap, *P_trap,* is based on those estimates available in the literature, between 0.8 and 0.9 (Saunier 2003; Kimberlin and Wilesmith 1994).

Model 2: annual extension

Model 2 estimates the annual amount of infectivity for pathogen *i* entering Category 1 materials, the food chain and drainage by summing the amount of infectivity per infected sheep and lamb consumed as given by Equations (9) and illustrated in Figure 1.

565 $$I\_cat1\_yr_i \sim \sum_{j=1}^{N_{i,S}} I\_cat1_{i,S} + \sum_{j=1}^{N_{i,l}} I\_cat1_{i,l}$$

566 $$I\_food\_yr_i \sim \sum_{j=1}^{N_{i,S}} I\_food_{i,S} + \sum_{j=1}^{N_{i,l}} I\_food_{i,l}$$

567 $$I\_drain\_yr_i \sim \sum_{j=1}^{N_{i,S}} I\_drain_{i,S} + \sum_{j=1}^{N_{i,l}} I\_drain_{i,l} \quad (9)$$

568 A separate model is required to estimate the total annual infectivity due to the fact that
569 each infected animal consumed would have different amounts of infectivity due to
570 uncertainty and variability. The index variable *j* has been used to denote the random
571 value for the number of infected animals per year generated by the distribution
572 presented in Equation 1.

573

574 **RESULTS**

575 Uncertainty is considered in the model and results are represented using mean values
576 followed by 5th and 95th percentiles in parentheses. The model was run for 500,000
577 iterations using Latin Hypercube sampling. It should be emphasised that not all
578 uncertainty has been estimated in the calculations, as not all can be quantified.
579 Therefore the 5th and 95th percentiles describe the amount of quantified uncertainty
580 included in the model.

581

582 **Model 1 - Number of infected carcases at abattoir per year, $N_{a,i}$**

583 Assuming a continuation of the decreasing trend in prevalence between 2005 and 2017,
584 it is estimated that there were on average, 6 (1, 12) and 45 (20, 75) infected sheep and
585 lambs with classical scrapie respectively entering GB abattoirs per year. For atypical
586 scrapie this number increases to an annual total of 475 (220, 742) and 3563 (1658, 5549)

587 sheep and lambs infected with atypical scrapie. For BSE in sheep, it is estimated that a
588 potential 0.002 (0, 0) and 0.02 (0, 0) sheep and lambs were infected per year.
589
590 **Model 1 - Amount of infectivity by exit destination per carcase**
591 Table 6 lists the risk assessment estimated values per carcase by disease for the amount
592 of infectivity by exit destination. By comparing the results under each Scenario, it can
593 be seen that for classical scrapie and sheep-BSE where disease is more peripherally
594 distributed, the change in SRM regulations increases the amount of infectivity entering
595 the food chain and category 3 materials by 220 OO ID_{50} per carcase for sheep and 27
596 OO ID_{50} per carcase for lambs if SRM were restricted to the brain and spinal cord.
597 Under Scenario 3, these values are estimated at 397 OO ID_{50} per carcase for sheep with
598 the same value for lambs where only the brain is restricted. There is a far smaller change
599 for atypical scrapie for Scenario 2 as the tissues that are no longer considered SRM are
600 not thought to be significantly infectious for that disease, measured as a mean difference
601 as 0.01 OO ID_{50} for sheep and 0.002 OO ID_{50} for lambs which is within the convergence
602 limits of the model. Under Scenario 3, the infectious tissue spinal cord is no longer
603 removed for adult sheep which results in an increase difference of 127 OO ID_{50} per
604 carcase destined for the food chain rather than Category 1 waste with no difference
605 found for lambs.
606
607 **Model 1 – Contribution by tissue type per carcase to infectivity entering food**
608 **chain and category 3 materials**
609 Table 7 displays the contribution by tissue type to the total infectivity estimated to enter
610 edible materials. It can be seen regardless of the SRM scenario that for classical scrapie
611 the highest estimated contributor to infectivity is the intestines (assuming the worst case

612 that there is the same titre of infectivity as the ileum). However, if SRM controls are
613 lifted, the spleen and ileum combined subsequently contribute around the 22% level in
614 sheep and 11% in lambs of magnitude. If spinal cord is removed from the list of
615 specified risk materials, then that tissue increased to contribute approximately 15% to
616 the total infectivity per carcase. For atypical scrapie, the lymph nodes dominate the
617 contribution to total infectivity, unless ileum or spinal cord is deregulated from the list
618 of SRM.
619

Model 1 - Sensitivity analysis

A multivariate stepwise regression analysis was used to calculate linear regression or sensitivity values for each value represented by a probability distribution. For all simulations of classical and atypical scrapie in sheep and lambs the results were all strongly affected by the uncertainty associated with the titre conversion factor, *OOunit*. Other parameters affecting the results were the maximum titre of classical scrapie infectivity in the brain, spinal cord, spleen and ileum, and the percentage of infectivity for sheep over one year old.

Model 2 – Annual estimate of infectivity for GB

The second model estimates the annual amount of infectivity for each of the prion diseases. From Table 8 it can be seen that the largest amount of infectivity generated per year is from atypical scrapie with a mean estimate of 3,500,000 OO ID_{50} per year. The majority of this infectivity enters Category 1 materials for incineration for scenario 1 and 2. However, there is a significant difference between Scenario 2 and 3. Under baseline conditions, 0.4% of total infectivity enters edible products which increases to

0.5% under Scenario 2. By restricting SRM to the brain only increases this proportion to 3.5% of total infectivity entering food chain and Category 3 materials.

For classical scrapie, there is less infectivity generated annually, with a mean estimate of 30,000 OO ID_{50} per year. This is lower than for atypical scrapie due to the lower estimated occurrence of this disease in the national flock. However, more infectivity is destined to reach the food chain and category 3 materials under all scenarios as the disease is peripherally distributed in many tissue types that are not classified as SRM. If SRM removal is limited to the brain and spinal cord of sheep > 12 months of age, there is an approximate mean increase of 2,000 OO ID_{50} per year diverted to the food chain rather than being destroyed as Category 1 materials under scenario 2 (brain and spinal cord removed) and a mean of over 4,000 OO ID_{50} per year if this is restricted to the brain only.

For the potential of sheep-BSE still being present in the national flock, there is very low estimate of 29 OO ID_{50} per year generated from the national flock, due to the potential very rare occurrence of this disease. Given changes in SRM regulations there is a change of an additional 4 OO ID_{50} per year being diverted to the food chain and Category 3 materials. Due to convergence limits of the risk assessment, the small change between Scenario 2 and 3 cannot be distinguished in the results.

An important assumption in the assessment, that lambs under 1 year would have negligible accumulation of atypical scrapie infectivity, was tested by increasing the baseline value from 0.1% to 1% of maximal infectivity in a clinical animal. Results were an increase in the total infectivity per carcase from an average value of 2.27 to

22.7 OO ID_{50}/lamb carcase. For absolute total accumulation per year, there was additional 12 fold infectivity entering the food chain and 9 fold entering Category 1 materials. However, a change in this parameter value does not affect the relative results by SRM scenario as the change in the baseline value only affects lambs which are not subject to SRM control amendment.

DISCUSSION

Amongst abattoir controls that are subject to review are those intended to minimise the risk of scrapie and BSE from small ruminants exposure of consumers through food. This risk assessment has included three prion hazards for the national sheep flock of classical scrapie, atypical scrapie and sheep-BSE, when considering the impact of changes in the SRM controls at abattoir to infectivity being diverted to Category 1 waste for incineration, the food chain and Category 3 materials and abattoir wastewater.

The risk assessment, assuming that there has been a continued decline in the prevalence of classical scrapie in the absence of cases, estimated a mean of approximately 30,000 OO ID50 per year, with 65% of total infectivity present in meat products that are permitted in the food chain and Category 3 materials. If SRM removal is limited to the brain and spinal cord of sheep > over 12 months of age, there is an approximate mean increase of 2,000 OO ID_{50} per year diverted to the food chain, rising to over 4,000 OO ID_{50} per year if limited to brain only. This infectivity would be spread across a number of different carcases and tissues within that carcase, with the intestines contributing the highest levels. When viewing the absolute values generated by this risk assessment it is important to remember that the estimates of infectivity are dependent on the sensitivity of the detection method. Results in this risk assessment have been produced from, or

converted to the best approximation of, the infectivity as measured by bioassay in wild type mice due to this detection method having been used historically to test the highest number of samples, across the widest range of tissue types in sheep. Therefore, the key value of the risk assessment is in the relative comparisons for SRM control scenarios and identification of the data gaps.

In terms of the relevant of the risk assessment to other countries, the variables has been parameterized for Britain. Given 1) the total number of sheep and lamb slaughtered annually, and 2) estimated prevalence for the three prion diseases, and 3) assuming similar abattoir practices, estimates for other countries could be quickly generated. Other countries in Europe are significantly affected by classical scrapie and therefore the impact of changing SRM controls for Britain may be different for other EU member states.

From the sensitivity analysis the parameters with the greatest impact on the results are the uncertainty associated with disease characteristics of tissue infectivity and ID_{50} conversion units, which have been found to significantly impact other TSE risk assessments (EFSA 2007, Adkin *et al.,* 2010). Further data are currently being investigated at APHA which could be used to further reduce the uncertainty associated within risk assessment outputs. There are other key assumptions which influence the model results, whose impact has not been quantitatively evaluated but need highlighting when considering the outputs and are presented in the following sections and listed in Table 9.

The most important model assumption, when considering the aim of this work is to estimate the exposure of consumers via the food chain, is the degree of zoonotic potential of sheep prion diseases. BSE in cattle has been demonstrated as the cause of variant Creutzfeldt Jakob disease in humans, but the zoonotic potential of scrapie prions remains unknown. Various mice models genetically engineered to overexpress the human prion protein gene (tgHu) have been developed to investigate the capacity of prions to transmit to humans. For example, Cassard *et al.,* 2014 demonstrated that scrapie prions can be transmitted to such humanized transgenic mice. However, such models are difficult to validate. Epidemiological studies have so far found no links between scrapie and any human prion diseases. Sheep-BSE has been included in the risk assessment due to the links with Cattle-BSE. However, there are little quantitative titre data to parameterise the risk assessment. The worst case assumption is used that sheep-BSE is peripherally distributed using classical scrapie titres as a proxy.The outputs of the risk assessment are presented in units of ovine oral ID_{50} and with the uncertainty associated, no attempt has been made to estimate the species barrier required to cause infection and disease in people.

Assumptions have been made to estimate the current prevalence of disease, with lambs affected to the same level as adult sheep. Great Britain is currently experiencing low levels of classical scrapie identified on farm and a trend analysis has been used to assume a continuing declining trend. One of the control strategies for scrapie was the National Scrapie Plan (NSP), led by APHA, which consisted of a breeding programme to increase the number of sheep that are naturally resistant to classical scrapie. This scheme was terminated in 2012. Given that the scheme may have contributed to the current low and declining occurrence of confirmed scrapie cases (Arnold and Ortiz-

Pelaez, 2014), and that the scheme has terminated, there may be a risk of a future increase in classical scrapie risk due to an increase in susceptible genotypes which would particularly impact the estimated prevalence in lambs.

This risk assessment makes no distinction between prion protein genotypes, which are known to affect susceptibility to scrapie, incubation period, PrP^{sc} tissue distribution and probably also infectivity, with different classical scrapie strains or sheep breeds possibly contributing to these differences. Nearly all studies in sheep with classical scrapie were carried out in sheep with fully susceptible genotypes, usually VRQ/VRQ, where PrP^{sc} distribution is widespread and occurs early in the incubation period based on immunohistochemical examination whereas accumulation of PrP^{sc} in lymphoid tissue may be limited in sheep with other genotypes (Ligios *et al.*, 2005, González, *et al.*, 2006). However, PrP^{sc} detection does not always correlate well with infectivity (González *et al.*, 2012). Sheep with an ARR/ARR genotype are believed to be generally resistant to classical scrapie although some natural cases have been reported (Groschup *et al.*, 2007) and there is some debate whether sheep with this genotype simply act as asymptomatic carriers with an extremely long incubation period. Ultrasensitive *in vitro* methods, such as protein misfolding cyclic amplification which based on one experiment in ARQ/ARQ sheep may correlate well with infectivity (Chianini *et al.*, 2015), are increasing used to study disease susceptibility. One such study produced no significant amplification of PrP^{sc} with brain from an ARR/ARR sheep (Bucalossi *et al.*, 2011), confirming the protective effect of this genotype. Due to these uncertainties, parameters in the assessment were taken from the worst-case scenario of sheep with a genotype fully susceptible to scrapie.

Atypical scrapie has been included as a hazard but little is known about sources of infection and epidemiology. Previous studies have not found any risk factors associated with an infectious origin suggesting that atypical scrapie in sheep is a spontaneous disease (Fediaevsky *et al.,* 2009). However, experimental oral transmission of atypical scrapie has been experimentally demonstrated (Simmons et al., 2011) suggesting this prion disease could be transmitted by feed and environmental pathways to animals if present in sufficient doses. However, at the current time it is viewed that atypical scrapie is likely to be spontaneous in older animals originating in the brain and will continue to occur in GB at a constant, albeit very low level of approximately in 8 of 10,000 healthy slaughtered sheep (Ortiz-Peláez *et al.,* 2016).

It is estimated that there is a lower amount of atypical scrapie infectivity entering the food chain and Category 3 materials than classical scrapie, equalling approximately 13,000 OO ID_{50} per year, despite the higher prevalence of atypical scrapie in the national flock. This is due to the limited distribution of infectivity in the CNS of atypical cases. Peripheral nerves and tissues have been found to be infected by transgenic bioassay, but at extremely low levels when compared to the CNS. There was an estimated small difference in the amount of atypical infectivity entering the food chain or Category 1 materials from a change in SRM controls limiting them to brain and spinal cord. This was estimated in the order of approximately 4,000 OO ID_{50} per year arising from very low levels of infectivity in distal ileum in adult sheep. However, under scenario 3, whereby only brain is classified as SRM, an additional mean estimate of 110,000 OO ID_{50} per year could be permitted into the food chain and Category 3 materials.

The titre of classical scrapie in the intestines (duodenum and jejunum) was not found in the literature. The titre is likely to be heterogeneously distributed depending on concentrations of gut-associated lymphoid tissue. In the absence of data, the titre was assumed to be the same as the ileum, with all three tissues ranked at the same level by the WHO as 'lower infectivity tissues' (WHO, 2010). This assumption and the heavy weight of these tissues, not classed as SRM, result in the tissues contributing the most to the edible fraction of an infected carcase. The estimate is likely to be an over-estimate within the risk assessment, decreasing the absolute infectivity estimates, but would not impact the SRM scenario relative values. The WHO ranked list also lists a number of other 'lower infectivity tissues', which due to lack of data are not included in the risk assessment, but would form part of the edible fraction of the carcase. Inclusion of these tissues, if quantitative values could be gained, would slightly increase the absolute infectivity estimates, potentially balancing the overestimate for the intestines.

Data on goats, which contribute a very small percentage to meat production in Britain, have not been included in this risk assessment. However, goats may represent a more scrapie-susceptible population than sheep because of the lack of national breeding programs to eradicate classical scrapie in this species. There is very limited knowledge about tissue infectivity and PrP^{sc} distribution in classical scrapie-infected goats, which may not be similar to sheep. Indeed, the presence of PrP^{sc} in pre-scapular lymph node tissue only in some classical scrapie-infected goats and the late and inconsistent detection of PrP^{sc} in the enteric nervous system (Gonzalez *et al.*, 2010) may suggest a different pathogenesis to sheep. There is even less information available about atypical scrapie in goats, which has not been found in the UK and is

less frequent than in sheep, with a prevalence of approximately 1 case per 10,000 tests (EFSA BIOHAZ Panel 2014). Whilst goats have not been covered in the assessment, if the number slaughtered for meat were included and using sheep estimated prevalence (atypical scrapie has never been found in UK goats and in 2017 there were no detected cases of goat classical scrapie), there would be a mean additional 0.04 goats and 0.3 kids infected with classical scrapie, 3 goats and 24 kids infected with atypical scrapie, and 1.2 x 10^4 kids with goat-BSE. Given no further information on the difference in the pattern of infectivity in goats as compared to sheep, and the same small-ruminant SRM controls, inclusion of this highly uncertain data for goats in the risk assessment would slightly raise the absolute infectivity estimates, but would not impact the SRM scenario relative values.

In conclusion, this assessment indicates that reducing the SRM controls for small-ruminants at abattoir to the brain and spinal cord (Scenario 2) would cause little impact on the infectivity of prion diseased contaminating edible products. Limiting SRM to only the brain would cause more of a measurable impact, particularly for atypical scrapie increasing the proportion entering the food chain and Category 3 materials from 0.5% to 3.5%.

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Table 1: Designated specified risk material which are removed from sheep and goats by age in accordance with point 1 of Annex V of EC Regulation 999/2001 (as amended). Meat Industry Guide 2017

Age	**Specified Risk material**
All	Spleen and ileum
Over 12 months (or have a permanent incisor erupted)	Skull including the brains and eyes, tonsils, spinal cord

Table 2: UK surveillance for classical and atypical scrapie from 2005 to 2016

	Abattoir			Fallen stock		
Year	Number Tested	Classical	Atypical	Number Tested	Classical	Atypical
2005	11,864	17	16	9,683	29	6
2006	48,971	8	36	21,225	38	13
2007	26,469	6	19	15,214	19	15
2008	10,761	2	5	11,793	6	6
2009	11,255	3	16	10,819	2	9
2010	8,423	1	13	10,460	0	6
2011	7,423	2	11	12,725	3	12
2012	7,009	0	11	13,452	2	18
2013	7,254	0	3	12,842	3	13
2014	7,396	0	4	12,866	0	7
2015	5,488	0	9	14,767	2	6
2016	6,915	0	4	13,534	0	9

Table 3: Designated specified risk material in sheep and goats by age for Scenario 1 (current regulations) and Scenario 2 and 3 (reduced regulations)

Scenario	**Scenario 1**	**Scenario 2**	**Scenario 3**
All ages Over 12 months	All: Spleen and ileum Over 12 months: Skull including the brains and eyes, tonsils, spinal cord	- Over 12 months: Skull including the brains and eyes and spinal cord	- Over 12 months: Skull including the brains and eyes

Table 4: Parameter descriptions and values used within the risk assessment

Parameter	Symbol	Value	Unit	Reference
Surveillance component				
Prevalence of infection at abattoir in the last 12 months of the incubation period	$P\_infected_{Sc}$	Betapert($1.48x10^{-6}$, $3.16x10^{-6}$, $6x10^{-6}$) at the 2.5th, most likely and 97.5th	%	Arnold and Ortiz-Pelaez, 2014 updated by Arnold, pers. comm. 2017
	$P\_infected_{At}$	Betapert($1.1x10^{-4}$, $2.7x10^{-4}$, $4.54x10^{-4}$) at the 2.5th, most likely and 97.5th	%	Arnold and Ortiz-Pelaez, 2014 updated by Arnold, pers. comm. 2017
Proportion of classical scrapie masking sheep-BSE	θsc	Beta(1, 2483+1)		Stack et al., 2002; SEAC, 2006
Number of sheep slaughtered over 1 year old, per year	$N\_animals_{S}$	1,712,000	head	Defra, 2015
Number of lambs slaughtered less than 1 year old, per year	$N\_animals_{l}$	12,845,000	head	Defra, 2015
Infectivity component				
Conversion factor from Mouse i.c. ID_{50}/g units to Ovine Oral ID_{50}/g	*OOunit*	Normal (5,0.053)	-	Kimberlin and Wilesmith 1994
Maximum titre of infectivity of Classical scrapie in tissue *t*	$Max_{sc,t}$	*t = 1*: brain = Normal (5.6,0.04)† *t = 2*: spinal cord = Normal (5.4,0.09)† *t = 3*: lymph nodes = Normal (4.2,0.01)† *t = 4*: spleen = Normal (4.5,0.09)† *t = 5*: tonsils = Normal (4.2,0.16)† *t = 6*: ileum = Normal (4.7, 0.01)† *t = 7*: liver = Normal (2.0,0.01)† *t = 8*: pancreas = Normal (2.1,0.01)† *t = 9*: thymus = Normal (2.2,0.04)† *t = 10*: stomach = 2* *t = 11*: heart = 1* *t = 12*: kidney= 1* *t = 13*: duodenum & jejunum = $Max_{sc,6}$ ◊ *t = 14*: blood = 2 ◊	log_{10} Mouse i.c. ID_{50}/g	† Kimberlin and Wilesmith 1994 * Gale 2002 ◊ Andreoletti et al., 2011; Andreoletti et al., 2012
Percentage of maximum infectivity in tissue *t* at time of death for Classical scrapie in sheep	$P\_infectivity_{S,sc,t}$	Uniform (70%,100%)		Data adapted Gale 2002 and Det Norske Veritas 2002
Percentage of maximum infectivity in tissue *t* at time of	$P\_infectivity_{l,sc,t}$, for *t = 1,2*	0.10%		Data adapted Gale 2002 and Det Norske Veritas 2002

Parameter	Symbol	Value	Unit	Reference
death for Classical scrapie in lambs				
	$P\_infectivity_{l,sc,t}$, for t = *4,5,7,8,9,10, 11,12,14*	10%		Data adapted Gale 2002 and Det Norske Veritas 2002
	$P\_infectivity_{l,sc,t}$, for *t = 3,6,13*	40%		Data adapted Gale 2002 and Det Norske Veritas 2002
Maximum titre of Atypical scrapie infectivity in tissue *t*	$Max_{at,t}$	*t = 1*: brain = Pert(100%, 105%, 132%) $*Max_{sc,1}$ *t = 2*: spinal cord = $Max_{sc,2}$ *t = 3 and 4*: lymph nodes and distal ileum = $Max_{at,1}$ - Uniform (5, 6)	$\log_{10}$ Mouse i.c. ID_{50}/g	Simmons, TSE National Reference Laboratory, APHA, per. comm., 2009; Andreoletti et al., 2011.
Percentage of maximum infectivity in tissue *t* at death for Atypical scrapie in sheep	$P\_infectivity_{S,at,t}$, for *t = 1,2,3,6*	Uniform (40%,80%)		Simmons, TSE National Reference Laboratory, APHA, per. comm., 2009
Percentage of maximum infectivity in tissue *t* at death for Atypical scrapie in lambs	$P\_infectivity_{l,at,t}$, for *t = 1,2,3,6*	0.1%		Simmons, TSE National Reference Laboratory, APHA, per. comm., 2009
Tissue weights				
Tissue weights for lambs	$N\_carcase_{l,t}$	*t = 1*: brain = 100† *t = 2*: spinal cord = 40† *t = 3*: lymph nodes = 38 ◊ *t = 4*: spleen = 75* *t = 5*: tonsils = 2 ◊ *t = 6*: ileum = 100* *t = 7*: liver = 610† *t = 8*: pancreas = 100† *t = 9*: thymus = 50† *t = 10*: stomach = 1,000† *t = 11*: heart = 200† *t = 12*: kidney = 100† *t = 13*: duodenum & jejunum = 930 ◊ *t = 14*: blood = 1,700†	g	†Assessors assumption based on Hart *et al.,* 1997 ◊ Assessors assumption based on Fryer *et al.,* 2007 * DARDNI 2009
Tissue weights for sheep	$N\_carcase_{S,t}$	*t = 1*: brain = Betapert(100,150,200) *t = 2*: spinal cord = Uniform(50*,64)	g	EFSA, 2014

Parameter	Symbol	Value	Unit	Reference
		t = 3: lymph nodes = 60.8 *t = 4*: spleen = 300* *t = 5*: tonsils = 3.2 *t = 6*: ileum = 200* *t = 7*: liver = 976 *t = 8*: pancreas = 160 *t = 9*: thymus = 80 *t = 10*: stomach = 1,600 *t = 11*: heart = 320 *t = 12*: kidney = 160 *t = 13*: duodenum & jejunum = 1,488 *t = 14*: blood = 2,720		Application of 1.6 scale factor Gale 2002 to weight of lamb tissue * DARDNI 2009
Abattoir component				
Amount of blood lost to blood tank at exsanguination for scrapie	$N\_Cat1_{a,14}$	*a = S*: Uniform (0.4,0.6) * 2720 *a = l*: Uniform (0.4,0.6) * 1700	g	Warriss, 2000
Amount of blood to floor from further processing for scrapie	N_floor_{a14}	*a = S*: Uniform (0.1,0.2) * 2720 *a = l*: Uniform (0.1,0.2) * 1700	g	Assessors assumption based on observation (2009); Hart *et al.,* 1997
Probability of brain material contaminating food fit for human consumption	$P\_cont_{a,1}$	Pert(0.01,0.1,0.15)	%	Coore et al., 2004; Anil, 2012, FSA, 2017, Assessors assumption (2017).
Amount of brain material contaminating food fit for human consumption	$N\_cont_{a,1}$	Pert(0.001,0.01,0.02)	% brain wt	Assessors assumption based on observation (2009) and comparison to cattle experimental data summarized in Adkin et al., 2010
Amount of brain to floor for lambs	$N\_floor_{l,1}$	Uniform (0.01,0.02) * 100	g	Assessors assumption; Hart *et al.,* 1997
Amount of spinal cord to floor for scrapie	$N\_floor_{S,2}$	(0.27 / Uniform (200,482)) * 64	g	Assumed to be the same proportion of spinal cord to floor for cattle; Saunier 2003
Probability of failure of spinal cord removal in sheep	$P\_failure_{S,2}$	Beta(13+1, 260833-13+1)	%	UK Sheep (Ewes and Rams) slaughter data - DEFRA slaughter statistics 2007 FSA, inspection data, 2007

Parameter	Symbol	Value	Unit	Reference
Proportion of spinal cord left if failure to remove correctly	$P\_cord_{S,2}$	Uniform(0.05,1)	g	Assessors assumption based on observation (2009) and comparison to cattle experimental data summarized in Adkin et al., 2010
Amount of spleen and ileum to floor	$N\_floor_{a,,t}$	*a=S, t=4*: Uniform (0.0001,0.001) * 300 *a=l, t=4*: Uniform (0.0001,0.001) * 75 *a=S, t=6*: Uniform (0.0001,0.001) * 200 *a=l, t=6*: Uniform (0.0001,0.001) * 100	g	Assessors assumption based on observation (2009); DARDNI 2009
Proportion of infectivity retained by trap				
Proportion of material retained by 6 mm trap	*P_trap*	Uniform (0.8,0.9)	%	Adapted from Saunier 2003; Det Norske Veritas June 1997 and Det Norske Veritas January 1997

Table 5: Estimated infectious titre of brain materials for atypical and classical scrapie from the literature

TSE isolate	Number of samples and case origin	Tissue	Genotype	Titre ID_{50} IC/g	Reference
Atypical scrapie	1 sheep experimental intracerebral	Cerebellum	AFRQ/ARQ	$10^{8.7}$ tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep experimental intracerebral	Cerebral cortex	AHQ/AHQ	$10^{8.3}$ tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep natural	Cerebral cortex	AFRQ/ARQ	$10^{8.7}$ tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep natural	Cortex	ALRQ/ARR	$10^{6.7}$ tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep natural	Cerebral cortex	ARR/ARR	$10^{6.7}$ tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep natural	Cerebral cortex	AFRQ/VRQ	10^{6} tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep natural	Cerebellum	AHQ/AHQ	$10^{6.7}$ tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep natural	Cerebellum	AHQ/AHQ	$10^{5.8}$ tg338	Andreoletti *et al.*, 2011
Atypical scrapie	1 sheep natural	Cerebellum	ARR/ARR	$10^{5.8}$ tg338	Andreoletti *et al.*, 2011
Classical scrapie	1 sheep natural	Posterior brain stem	VRQ/VRQ	$10^{6.8}$ tg338	Andreoletti *et al.*, 2011
Classical scrapie	1 sheep experimental intracerebral	Posterior brain stem	VRQ/VRQ	$10^{6.8}$ tg338	Andreoletti *et al.*, 2011
Classical scrapie	1 sheep experimental oral	Posterior brain stem	VRQ/VRQ	$10^{6.6}$ tg338	Andreoletti *et al.*, 2011
Classical scrapie	1 sheep experimental intracerebral	Posterior brain stem	VRQ/VRQ	$10^{6.6}$ tg338	Andreoletti *et al.*, 2011
Classical scrapie	9 sheep natural	Brain	Not known	$10^{5.6}$ ± 0.2 Wild type	Kimberlin & Wilesmith, 1994
Classical scrapie	3 goats natural	Brain	Not known	$10^{6.5}$ ± 0.2 Wild type	Kimberlin & Wilesmith, 1994

Table 6: Infectivity per infected carcase by exit destination by disease by animal group OO ID_{50} per carcase for three scenarios: 1) current SRM removal, and 2 and 3) reduced SRM removal

		Scenario 1: current SRM	Scenario 2: Brain & spinal cord	Scenario 3: Brain	Sc2-Sc1 Mean	Sc3-Sc1 Mean
Classical scrapie and sheep-BSE						
Sheep	Food chain/Cat 3	763 (246 , 1683)	983 (317 , 2167)	1160 (377 , 2547)	+220	+397
	Category 1	1047 (302 , 2432)	827 (216 , 2008)	651 (153 , 1639)	-220	-397
	Wastewater	0.1 (0 , 0.2)	0.1 (0 , 0.2)	0.1 (0 , 0.2)	0	0
Lamb	Food chain/Cat 3	224 (73 , 490)	251 (82 , 549)	251 (82 , 547)	+27	+27
	Category 1	28 (9 , 61)	1 (0.1 , 2)	1 (0.2 , 2)	-27	-27
	Wastewater	0.01 (0 , 0.02)	0.01 (0 , 0.02)	0.01 (0 , 0.02)	0	0
Atypical scrapie						
Sheep	Food chain/Cat 3	2 (0 , 9)	2 (0 , 9)	127 (20 , 370)	+0.01	+127
	Category 1	2041 (269 , 6555)	2041 (269 , 6551)	1914 (214 , 6409)	-0.01	-127
	Wastewater	0.02 (0 , 0.05)	0.02 (0 , 0.05)	0.02 (0 , 0.05)	0	0
Lamb	Food chain/Cat 3	0.15 (0.02 , 0.4)	0.15 (0.02 , 0.4)	0.15 (0.02 , 0.4)	+0.002	+0.002
	Category 1	2 (0.3 , 7)	2 (0.3 , 7)	2 (0.3 , 7)	-0.002	-0.002
	Wastewater	0.005 (0 , 0.02)	0.005 (0 , 0.02)	0.005 (0 , 0.02)	0	0

Table 7: Percentage contribution by tissue type to infectivity in the food chain and category 3 materials by SRM scenario and disease for a single infected carcase (%)

	Scenario 1: current SRM				Scenario 2: brain & cord				Scenario 3: brain			
	Classical scrapie		Atypical scrapie		Classical scrapie		Atypical scrapie		Classical scrapie		Atypical scrapie	
Tissue type	sheep	lamb	sheep	lamb	sheep	lamb	sheep	lamb	sheep	lamb	sheep	lamb
Brain & pituitary gland	0.1%	0.0%	9.3%	1.4%	0.1%	0.0%	9.3%	1.4%	0.1%	0.0%	1.4%	1.4%
Spinal cord	0.0%	0.1%	0.0%	98.6%	0.0%	0.1%	0.0%	98.6%	14.8%	0.1%	98.5%	98.6%
Lymph nodes	1.3%	1.3%	90.7%	0.0%	1.0%	1.2%	21.1%	0.0%	0.9%	1.2%	0.0%	0.0%
Spleen	0.0%	0.0%	0.0%	0.0%	11.8%	1.4%	0.0%	0.0%	10.0%	1.4%	0.0%	0.0%
Tonsils	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%
Ileum	0.0%	0.0%	0.0%	0.0%	10.3%	9.4%	69.5%	0.0%	8.7%	9.4%	0.0%	0.0%
Liver	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%
Pancreas	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Thymus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Stomach	0.2%	0.1%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%
Heart	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Kidney & adrenal gland	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Intestines (duodenum & jejunum)	98.1%	98.4%	0.0%	0.0%	76.4%	87.7%	0.0%	0.0%	65.1%	87.7%	0.0%	0.0%
Blood	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 8: Infectivity annually by exit destination by disease OO ID_{50} per year for two scenarios: 1) current SRM removal, and 2) SRM restricted to brain & spinal cord, 3) SRM only brain

	Scenario 1: current SRM	Scenario 2: brain & cord	Scenario 3: brain	Sc2-Sc1 Mean	Sc3-Sc1
Classical scrapie					
Food chain/ Category 3	19,320 (7,961 , 34,995)	21,071 (9,011 , 37,037)	23,519 (9,633 , 42,795)	+1,751	+4,200
Category 1 waste	10,266 (2,321 , 26,508)	8515 (642 , 19,871)	6,066 (600 , 17,273)	-1,751	-4,200
Wastewater	1 (0.4 , 3)	1 (0.4 , 3)	1 (0.2 , 3)	0	0
Total per year	29,587	29,587	29,587		
Atypical scrapie					
Food chain/ Category 3	13,079 (6,667 , 29,264)	17,161 (5,262 , 22,023)	122,242 (49,213 , 196,082)	+4,082	+109,163
Category 1 waste	3,504,274 (1,445,152 , 6,366,002)	3,500,191 (1,355,531 , 5,940,187)	3,395,111 (1,116,967 , 5,110,834)	-4,083	-109,163
Wastewater	66 (43 , 126)	67 (36 , 99)	66 (44 , 125)	0	0
Total per year	3,517,419	3,517,419	3,517,419		
Sheep-BSE					
Food chain/ Category 3	23 (0, 0)	27 (0 , 0)	28 (0 , 0)	+4	+4
Category 1 waste	6 (0 , 0)	2 (0 , 0)	2 (0 , 0)	-4	-4
Wastewater	0.001 (0 , 0)	0.001 (0 , 0)	0.001 (0 , 0)	0	0
Total per year	29	29	29		

* difference due to convergence limits of risk assessment

Table 9: Key assumptions and uncertainties not quantified in model results

Model assumption	Data gap	Impact on absolute infectivity estimated	Impact on SRM scenario relative values
There is a zoonotic potential of sheep prion diseases	Level of species barrier which may negate any human clinical disease	Implicitly impacts outcome	Implicitly impacts outcome.
Atypical scrapie is a transmissible disease	Only evidenced in experimental conditions	Implicitly impacts outcome for atypical scrapie	Implicitly impacts outcome for atypical scrapie
Prevalence is continuing to decrease based on previous trend	Changes in breeding programmes may affect trend	Increase, particularly for most recent cohorts - lambs	None
Population is fully susceptible to disease	Population genotype, genotype corresponding infectivity titres	Decrease	None
Intestines have the same infectivity as ileum	Titre data	Decrease	None
Not all 'lower infectivity tissues' included in risk assessment	Titre data	Increase	None
Goats are not included	Prevalence, titre, slaughter practices	Increase	None

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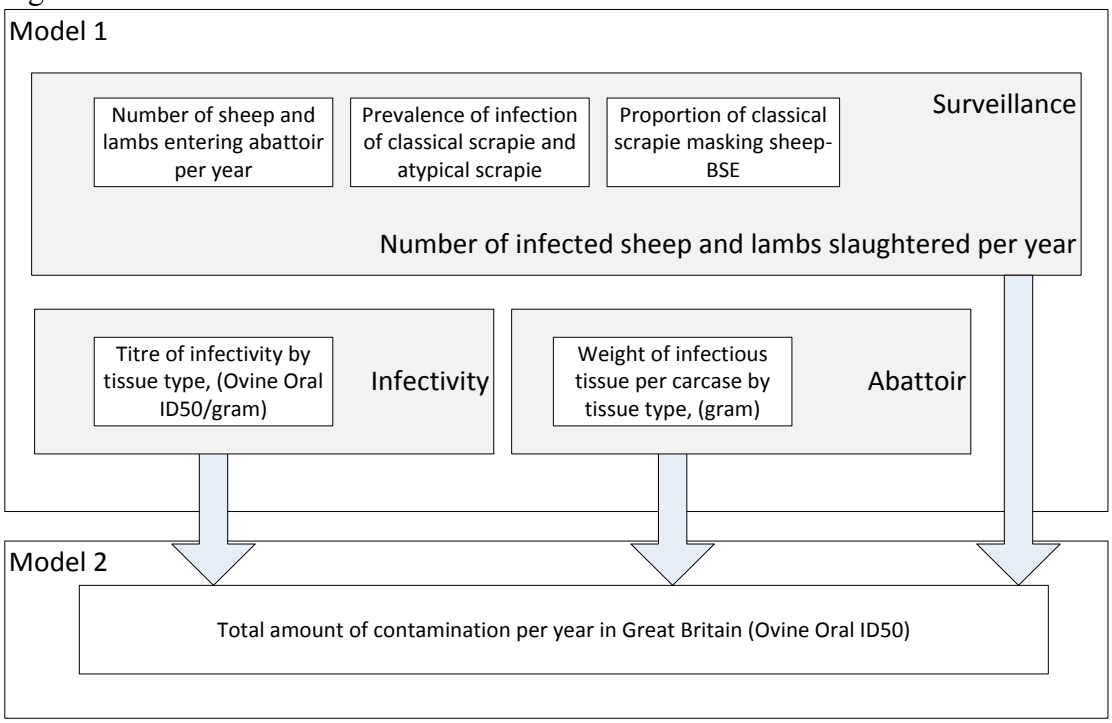
31 Figures



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35 Fig 1: Model framework of the Scrapie Control Model showing the links between

36 Model 1; comprising of the Surveillance, Abattoir and Infectivity components and

37 Model 2 the annual extension component

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