Quantitative Microbiological Risk Assessment on Salmonella in Slaughter and Breeder pigs: Final Report

Vigre, Håkan; Coutinho Calado Domingues, Ana Rita; Barfod, Kirsten ; Pedersen, Ulrik Bo; Wingstrand, Anne; Hald, Tine

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Prepared by VLA in consortium with DTU and RIVM

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EFSA *Salmonella* in Pigs QMRA Consortium:

Andrew Hill, Robin Simons, Vick Ramnial, Jane Tennant (nee Tanton), Sarah Denman, Tanya Cheney, Emma Snary (Veterinary Laboratories Agency, UK)

Arno Swart, Eric Evers, Maarten Nauta*, Manon Swanenburg, Frans van Leusden, (RIVM, The Netherlands)

Hakan Vigre, Ana Rita Domingues, Kristen Barfod, Ulrik Bo Pedersen, Anne Wingstrand, Tine Hald (Food-DTU, Denmark).

*Maarten Nauta now works for Food-DTU, Denmark

Executive Summary

Background

Under Article 36 of the European Parliament and Council Regulation (EC) No 178/2002 (EC, 2002), the European Food Safety Authority (EFSA) published a call for a "Quantitative Microbiological Risk Assessment (QMRA) on *Salmonella* in slaughter and breeder pigs".

The aims of the QMRA were to assess:

- **the expected reduction of *Salmonella* cases in humans (or pig meat at retail) by a reduction (e.g. 5- or 10-fold) of *Salmonella* prevalence in slaughter pigs (based on bacteriology or serology at slaughter);**
- **the sources of infection for fattening pigs at farm level;**
- **the reduction of the prevalence in slaughter pigs by the most important potential treatments or control measures at farm level;**
- **the impact of transport, lairage and slaughter processes on contamination of carcasses;**
- **the expected reduction of *Salmonella* cases in humans (or pig meat) by the most important control measures during transport, at lairage or during the slaughter process.**

In order to facilitate the investigation of interventions at different points of the food chain, a farm-to-consumption framework was adopted, so that we could model the prevalence of infection / contamination and the microbial load from the farm to the point of consumption (exposure). The probability of infection, or illness, could then be estimated by applying a dose-response model using the estimated amount of *Salmonella* bacteria ingested at consumption as an input. Numerous QMRAs have been developed or are currently underway for *Salmonella* in pigs within the EU; including QMRAs for the UK (Hill *et al.* 2003; VLA, 2009); Belgium (Delhalle *et al.,* 2009); Denmark (Alban *et al.,* 2002; Hurd *et al.,* 2008), Ireland (Barron *et al.,* 2009) and the Netherlands (van der Gaag *et al.,* 2004). However, EFSA requested a QMRA for *Salmonella* in Pigs that characterised the variability between European Union (EU) Member States (MSs) and, in particular, the inclusion of variability between MSs in their pig farms, slaughterhouses and consumption patterns; this presented numerous challenges. These challenges have been overcome by the development of a generic model with a clearly defined set of parameters that may vary between MSs, the values of which can be easily input for any specific EU MS. To demonstrate the parameterisation and use of the model, four MSs were selected as case studies: MS1, MS2, MS3 and MS4. These MSs were selected by performing a cluster analysis for the EU using criteria relating to pig production and consumption patterns to group the MSs into 'clusters'. Based on which MSs had the most available data, one MS was selected from each cluster. Three product types are included in the QMRA: pork cuts, minced meat and fermented sausage. These products were chosen to represent a range of different production/preparation practices and consumption patterns, which will affect the *Salmonella* levels within these products at consumption and hence the probability of human illness.

Exposure Assessment & Hazard Characterisation

The exposure assessment was split into 4 modules: Farm; Transport & Lairage; Slaughter & Processing and Preparation & Consumption. The output from one module is the input to the

next and so collectively they model the entire farm-to-consumption chain. Efforts were made to take into account the natural variation of *Salmonella* infection and/or contamination in the modelling. This was done by, wherever possible, allowing for stochastic variation of parameter values. Consequently, as much as possible, variability within and between batches of pigs, farms, transport vehicles, slaughterhouses, cutting plants, retail outlets and consumer practices, both within and between MSs, was described.

Within the Farm module, the management of farms within the EU and the associated transmission of *Salmonella* between pigs are mathematically described. The model considers the production of pigs destined for meat production (i.e. slaughter pigs) over a period of 500 days, thereby following batches of pigs from birth to depopulation for slaughter. The consideration of a 500 day time interval allows for the model to capture the extent of the within-farm variation and dynamics over time. Between-farm variation is described by the consideration of different farm management systems, such as size ("large" or "small" farms), type of production (all-in-all-out / continuous), housing (slatted/solid flooring), feed (wet/dry) and sourcing of pigs (breeder–finisher/breeder–weaner/grower–finisher). By allowing for such variation in the farm management structure it is anticipated that a large proportion of EU pig farms can be described by the model. On top of the management model the transmission model describes the infection dynamics of *Salmonella* within and between batches of pigs. The model considers the introduction of *Salmonella* via a number of sources, in particular, sows (infecting piglets), feed and environmental contamination (e.g. rodents etc), as well as the infection of other pigs via new stock (specifically modelled through mixing at the point of weaning). Modelling the farm in such detail produces a complex model, but one which was able to investigate specific farm interventions agreed with the EFSA Working Group and the EC. The primary output of the Farm module is the prevalence of lymph-node positivity and the prevalence/magnitude of pigs actively shedding *Salmonella* within a batch of pigs, at the time of depopulation for slaughter.

The Transport part of the Transport & Lairage module considers the process of transporting finisher pigs to the slaughterhouse (the same framework is also used for the transport of weaners from breeder farms to grower-finisher farms within the Farm module). The number of pigs to be slaughtered by a random slaughterhouse on a particular day, which for both the small and large slaughterhouse models will vary, is determined and batches of slaughter-age pigs are selected at random from the output of the Farm module until enough pigs have been selected. The model then mathematically describes the management of these pigs as well as the infection dynamics of *Salmonella* during transport. The transmission model is similar in structure to the Farm model, except for a few modifications such as the inclusion of increased shedding of *Salmonella* due to stress, and the assumption that cross-contamination between transport pens will not occur over such a short timeframe. Management factors such as transport time and number of pigs per pen in the truck are included in the model as well as the probability of pigs becoming stressed and the possible carry-over of *Salmonella* from previous batches of transported pigs. The Lairage part of the module takes a similar structure to the Transport part, but with the necessary amendments to the management parameters. The outputs of the Transport & Lairage module are: the *Salmonella* infection status per individual pig (positive/negative) at the point of slaughter and the number of *Salmonella* within an infected pig's faeces. At this point we also model the *Salmonella* contamination status per individual pig (positive/negative) at the point of slaughter and introduce an estimate for the external contamination per pig measured in colony-forming units of *Salmonella* (CFU) per cm^2 of skin.

Using the output from the Lairage module, the Slaughter & Processing module predicts the prevalence and number of *Salmonella* present in/on the product at the end of processing. Both large and small slaughterhouses are modelled, where it is assumed that small slaughterhouses use less dedicated machinery and do not have a continuous slaughter line. At each processing stage several processes may increase or decrease the *Salmonella* concentration on the carcass. The model mathematically describes this by considering the immediate effect of the processing stage (e.g. singeing destroys a number of organisms on the carcass) as well as the amount of *Salmonella* (CFUs) moving between the pig and the environment (which may contaminate subsequent pigs) and the environment (from contaminated pigs earlier in the slaughter line) to the pig. Examples of the slaughterhouse environment include the scald tank, knife and polishing machine. Such a highly mechanistic approach allows interventions to be modelled at a very high resolution and to describe between-pig variability during a production day. The output of the slaughterhouse module is the prevalence and level of contamination on the half-carcasses produced for that day.

The cutting plant processes the half carcasses and delivers the food product. This model describes the processing of 10,000 portions of each type of product (pork cuts, minced meat and fermented sausage) produced from half-carcasses, which were randomly sampled from half-carcasses produced per MS, respecting the proportion of production from large and small slaughterhouses. These products are then delivered to retail. The prevalence and level of contamination within each portion are the input for the Preparation & Consumption module.

In the Preparation & Consumption module, the 10,000 portions of each product type are modelled. This module describes the impact of transport, storage and meal preparation on the prevalence and contamination of *Salmonella,* for the three product types. The module includes the possibility of *Salmonella* growth during transport and storage using time and temperature parameters. In relation to meal preparation, both cross-contamination between pork products and salad (for pork cuts and minced meat only) and inadequate cooking (minced meat only) are considered. Routes of cross-contamination modelled include via the chopping board, knife, hands and tap. The final output of the module is the number of *Salmonella* on/in each portion of each product at the point of consumption.

The number of *Salmonella* on each portion of each product is fed into a dose-response model that predicts the probability of illness given consumption of that portion (hazard characterisation). This probability is then used in a binomial trial to predict if that particular serving will result in illness or not. The proportion of illness given 10,000 servings per product type was then calculated and interpreted as the probability of illness. Therefore, the average probability of illness over all 10,000 servings for each MS, for each of the three product types (pork cuts, minced meat and fermented sausage) can be estimated.

The model framework is summarised in Figure 1.

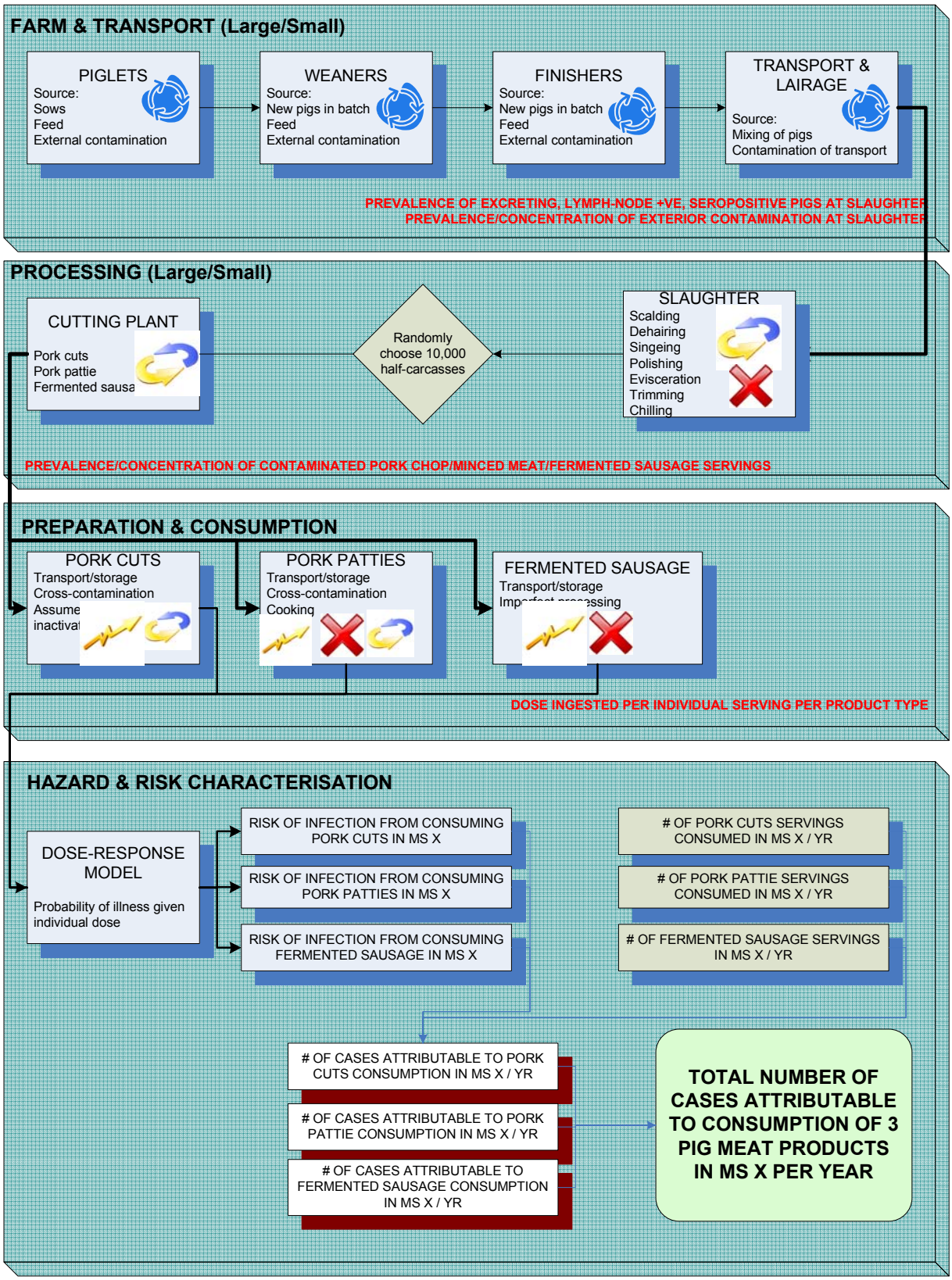

Figure 1 An overview of the modules within the farm-to-consumption QMRA. Icons represent the relevant microbiological processes: all-blue – transmission; blue-yellow arrows – cross-contamination, X - inactivation, bolt – growth.

Risk Characterisation

The results of the QMRA are summarised in Tables 1 & 2. For all four MSs the average probability of illness is between 1 in 100,000 and 1 in 10 million servings across all three product types. MS2 is predicted to have a higher probability of illness. For all of the MSs the product with the highest probability of illness per serving is fermented sausage. The lowest risk per serving is associated with pork cuts (MS1, MS2) and minced meat (MS3, MS4). The total number of cases attributable to the three product types was estimated to be about 1000 (MS1); 25000 (MS2); 1500 (MS3) and 2700 (MS4). It is recognised that the number of predicted cases seem to be an overestimation and this is discussed below. In MS1, MS3 and MS4, the highest number of cases was attributable to pork cuts and in MS2 to minced meat products. The model results suggest that a high prevalence in slaughter pigs will result in a large number of cases, but the complex system involved post-slaughter means that the number of cases is not directly proportional to the slaughter pig prevalence in a country. Although, what is (probably) more important is the total burden of *Salmonella* entering/exiting the slaughterhouse/retail establishment, which is dependent on not only the slaughter pig prevalence but also the contamination level of the slaughtered pig/carcass/end product.

The validity of the model was assessed by comparing predicted results from the model with observed (microbiological/epidemiological) results at two points in the farm-to-consumption pathway (prevalence of lymph-node positive pigs at post-lairage and the prevalence/enumeration of contaminated portions at retail) and also the number of human salmonellosis cases. Like model results, the observed data to which the model is being compared are uncertain due to, for example, restricted test sensitivity, imperfect sampling design, inclusion of imported products in retail surveys and the under-reporting of human cases from epidemiological data.

Table 1: Baseline results from the QMRA: mean probabilities of illness by eating one serving of pork cuts, minced meat or fermented sausage in MS1, MS2, MS3 and MS4.

Member State	$P_{illness}$		
	Pork Cuts	Minced Meat	Fermented Sausage
MS1	7.65×10^{-07}	8.84×10^{-07}	1.87×10^{-06}
MS2	1.86×10^{-05}	2.24×10^{-05}	4.25×10^{-05}
MS3	3.88×10^{-07}	2.32×10^{-07}	5.78×10^{-07}
MS4	2.55×10^{-06}	2.58×10^{-07}	4.29×10^{-06}

Table 2: Number of cases, per year, attributed to pork cuts (PC), minced meat (MM) and fermented sausage (FS), for the four case study Member States.

	MS1	MS2	MS3	MS4
No. of predicted cases by PC/year	520	9,802	1,162	1,384
No. of predicted cases by MM/year	125	11,148	182	56
No. of predicted cases by FS/year	375	4,298	165	1,246
Total no. of predicted cases (PC + MM + FS)/year	949	25,248	1,509	2686
Predicted number of cases per 100,000 habitants	12	42	4	26

At post-lairage, the output of the QMRA (average proportion of *Salmonella* positive lymph nodes) was compared to the EFSA baseline survey (EFSA, 2008). The QMRA predicts a prevalence of 1%, 20%, 0.7% and 3.5% for MS1, MS2, MS3 and MS4 respectively. The baseline survey provided estimates of 2% [1.1 – 3.6]; 21.2% [17.8 – 25]; 5.1% [3.7 – 6.9] and 5.8% [3.8 – 8.9] for MS1, MS2, MS3 and MS4 respectively. Therefore, it is concluded that, at this point of the farm-to-consumption pathway, the QMRA is producing realistic estimates for MS1, MS2 and MS4. It is unclear why the model is maybe underestimating the prevalence in MS3, but it is likely to be attributable to the model not capturing a specific aspect of MS3 at the farm and in particular within the small farm model, as MS3 has a much larger proportion of small farms than the other MSs.

Table 3 provides the predicted prevalence and microbial load at the point of retail. At the point of retail, data for validation were only available for MS1 and MS2 and these compared reasonably well to the QMRA predictions. Although it was not possible to obtain data for all product types in each MS, EFSA, 2009 provides ranges of *Salmonella* prevalence across different EU MSs. For pork cuts the prevalence ranged from 0%-6.1%, for minced meat 1.3% - 5.9% and for ready-to-eat minced meat/minced meat products (which includes fermented sausages) of 0%-3.3%. The model predictions are in the same order of magnitude, with the results from all four MSs falling within or slightly below these observed intervals. Across a number of EU MSs, studies show that contamination on retail cuts is comparatively low (scaling up to the unit of a serving commonly less than 10 CFU/portion) (Prendergast *et al.*, 2009). The average number of *Salmonella* contaminating the three product types was predicted by the QMRA to range from 1-11CFU/portion for all MS/product-type combinations. It was therefore concluded that the QMRA is producing realistic enough results at the point of retail to differentiate between MSs and provide a baseline from which to conduct an intervention analysis.

Table 3: Predicted and observed (where available) prevalence at retail level for pork cuts (PC) and minced meat (MM) and fermented sausage (FS); Predicted microbial load at retail level also for the three product types (in *Salmonella* log cfu).

Member State	Product type	Prevalence predicted (%)	Predicted average microbial load (log CFU per portion)	Observed prevalence (%)	Source of data
MS1	PC	0.18	0.57	1[(1)]	EFSA, 2009
	MM	0.20	0.92	1.6[(2)]	
	FS	0.004	0.17		
MS2	PC	4	0.69	1.9	Little et al. 2008
	MM	5	1.06		
	FS	0.09	0.66		
MS3	PC	0.07	0.44		
	MM	0.05	0.67		
	FS	0.001	0.06		
MS4	PC	0.5	0.37		
	MM	0.3	0.58		
	FS	0.01	0.17		

[(1)] Samples: 10/25 g; [(2)] Samples: 10 g;

In the draft Community Summary Report for 2008 (EFSA, 2010) a total of 2,310, 11,511, 9,149 and 10,707 cases of salmonellosis in MS1, MS2, MS3 and MS4 are reported, respectively. Although, as mentioned above, it is difficult to validate the QMRA outputs at this point due to the (often unknown and significant) level of under-reporting within each MS, the output from the QMRA seems to be an overestimation of the number of cases for each MS. This overestimation could be attributable to a number of factors. Given that the QMRA output compares reasonably to observed prevalence and microbial load data at the point of retail, factors within the Preparation & Consumption module and hazard characterisation are the most likely cause of this over-estimation. Such factors include the uncertainty associated with the consumption data, the effect of immunity, the dose-response model and many other parameters used to mathematically describe cross-contamination and cooking within the Preparation & Consumption module. In addition, the consideration of all *Salmonella* spp. within the QMRA, with no account taken for differences between *Salmonella* serovars in their ability to grow/survive in the environment or to infect humans (virulence), could have a significant impact on the estimation of the number of cases[2]. It is quite common for QMRAs to overestimate the number of cases (e.g. Hartnett, 2001; Nauta *et al.,* 2001, 2005; Havelaar et al 2008). Considering this, for any QMRA, it is important to place more emphasis on the *relative* risks (e.g. the intervention analysis) than the *absolute* risk (Havelaar *et al*., 2007).

The number of salmonellosis cases reported by each MS will not all be attributable to pork, nor will the three pork products considered here include all pork-related cases. The proportion of human *Salmonella* cases in the EU that are due to the consumption of contaminated pork/pig-meat products is unknown. In order to estimate this proportion, we originally intended to develop a source attribution model based on the microbial subtyping approach[3] (Hald *et al.,* 2004; Pires & Hald, 2009) using MS-specific animal and food data from the EU baseline surveys and human data as reported by the MS to The European Surveillance System (TESSy). It was, however, necessary to abandon this approach, since MS-specific data on the distribution of serovar and phage types in humans was not available. As an alternative, we made descriptive comparisons of animal, food and human data, which were supplemented with results from a spatial analysis and an outbreak data analysis. The conclusion that follows should, therefore, be considered as a guesstimate as it is based on very simple deductions.

[2] Within the mandate, EFSA were asked "to consider all serovars in pigs that are of human health significance". EFSA, 2006 concluded that "all *Salmonella* serovars in pork are to be regarded as a hazard for public health" and recognised that there will be variability between strains in their behaviours across the food chain. It was therefore deemed acceptable by EFSA (as stated in the call for proposals) for the QMRA to consider all types similarly and hence that a QMRA for *Salmonella* spp. would be appropriate.

[3] The principle of the subtyping method is to compare the distribution *Salmonella* subtypes in different sources (e.g., animals, food) with the distribution of subtypes in humans. The microbial subtyping approach is enabled by the identification of strong associations between some of the dominant subtypes and a specific reservoir or source, providing a heterogeneous distribution of subtypes among the sources. The approach utilises a collection of temporally and spatially related isolates from various sources, and thus it is facilitated by integrated foodborne disease surveillance programs that is focused on the collection of isolates from the major food animal reservoirs of foodborne diseases (Pires *et al.*, 2009). This method typically focuses on sporadic cases and attributes infections to the reservoir level, meaning that the original infectious source is identified, whereas the route from reservoir (primary production) to consumer is not described.

Results from the descriptive and spatial analysis were discussed in an attempt to make inferences about the most important sources of human salmonellosis in the EU. Based on this, it is assessed that 10-20% of human infections in the EU are attributable to the pig reservoir. This "guesstimate" is, however, believed to vary considerably between MSs depending on, for instance, *Salmonella* prevalence in pigs and pork, consumption patterns and preferences, pig production systems and the relative importance of other sources, such as eggs and chicken. The "guesstimate" is to some extent supported by the outbreak data analysis that indicated that meat products, particularly pork and beef, were important sources of *S.* Typhimurium infections, and this is furthermore in concordance with a recent attribution study done by Pires *et al.* (2008) and Pires (2009). In order to obtain more reliable and quantitative estimates for the importance of different sources to human salmonellosis in the EU, it is recommended to develop a model for the attribution of human salmonellosis based on the microbial subtyping approach. This will require MS-specific data on the distribution of *Salmonella* subtypes in the most important sources and in humans. Particularly, the latter data have been very difficult to obtain, which is considered most unfortunate as these data are essential for understanding the trends and sources of human salmonellosis.

During the development of the QMRA, many data gaps/deficiencies were identified. These were investigated as part of an uncertainty analysis, where we assessed the effect that parameters (with a particular lack of information) have on the model output and, in particular, the probability of illness. The MSs MS1 and MS2 were chosen for the uncertainty analysis as MS1 is a MS with a low baseline prevalence at the point of slaughter, whereas MS2 has a high baseline prevalence. From this analysis, it is concluded that the following parameters were both highly uncertain and influential on the probability of illness.

Farm:

- Prevalence of feed contamination (MS1)
- Prevalence of infection within the breeder herd (MS1 & MS2)
- Maximum mass of faeces ingested per day (finishers) (MS2)

Transport & Lairage:

- Probability of pigs being stressed during transport (MS1 & MS2)*
- Dose-response parameter α (MS1)

Slaughter & Processing:

- Amount of faeces spilled while dehairing (MS1 & MS2)

Preparation & Consumption:

- Minced meat storage time in fridge (MS1 & MS2)*
- Portion sizes of pork cuts, minced meat patties and fermented sausages (MS1 & MS2)
- pH of fermented sausage (MS1 & MS2).

Those marked with an asterisk (*) were also identified as important in the sensitivity analysis, where the impact of the variability associated with the module parameters that are described by distributions on the primary module output (e.g. for stress during transport this is the lymph node positive prevalence post transport) was investigated.

It is therefore recommended that further data generation is undertaken in order to provide improved estimates for the parameters listed above and also for the travel time between

retail store and home. The identification of such data gaps is a positive feature of any risk assessment model and many risk managers utilise such information to direct future research.

Intervention analysis

A key part of the QMRA was the investigation of interventions. In this respect, EFSA provided a number of scenarios that the QMRA needed to address. Each of these is considered below:

The expected reduction of Salmonella cases in humans (or pig meat at retail) by a reduction (e.g. 5- or 10-fold) of Salmonella prevalence in slaughter pigs (based on bacteriology or serology at slaughter)

Marked reductions in cases can be achieved by reducing slaughter pig prevalence, and indeed for MS2 and MS4 there is a strong linear relationship between slaughter pig lymph-node prevalence and the number of human cases (Figure 2). The major effect of reducing slaughter pig prevalence was to reduce the number of infected pigs with high infection/contamination loads entering the slaughterhouse, hence eventually reducing the number of highly-contaminated servings consumed by consumers.

For MS2 and MS4, the broadly linear relationship shows that factors that would be expected to introduce a non-linear relationship into the model, such as cross-contamination at the slaughterhouse, growth during retail storage and dose-response, although accounted for in the model, seem to have limited importance for the assessed relationship between pig prevalence[4] and human incidence. Indeed, data from the EFSA baseline survey support a modest linear relationship at a MS level, at least for infection and carcass contamination at evisceration. However, the results indicate that for low prevalence countries (MS1 & MS3) a 5-10% decrease in slaughter prevalence may result in a larger percentage reduction in human cases.

[4] This is based on lymph node prevalence

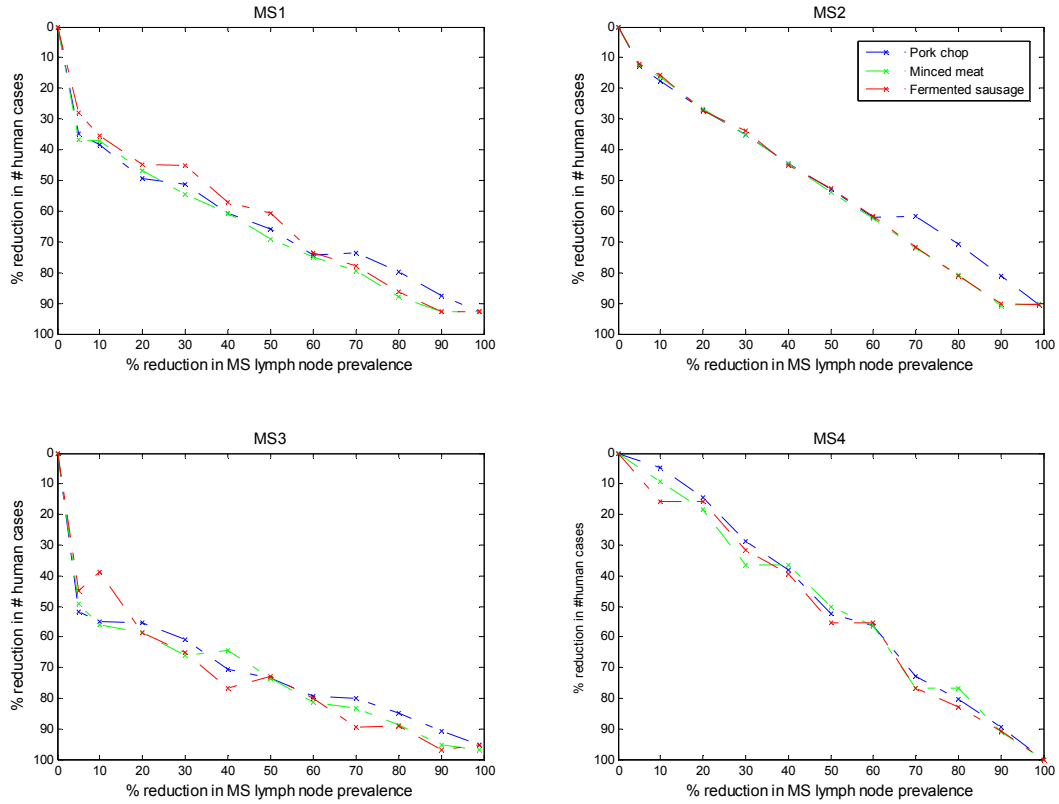

Figure 2: Effect of reducing *Salmonella* prevalence in slaughter pigs by 5 to 99% for each product type and for each case study MS (pork cuts – blue, minced meat – green and fermented sausage – red). Small variations in the downward trend can be seen, for MS1 and MS3 in particular; these are due to sampling error within the Monte-Carlo simulations (due to the low *Salmonella* prevalence in slaughter pigs).

The sources of infection for fattening pigs at farm level

We have investigated the relative importance of source of infection by simply turning off each source of infection within each MS model. The results are shown in Figure 3. The effect is striking – for MSs with a higher breeding pig herd prevalence (MS2, MS4) switching breeding pig herd prevalence to zero (hence assuming that the breeding pig herd cannot be re-infected from the finishing herd) removes the vast majority of infections at depopulation of the fattening herds. Conversely, removing feed or external contamination from the model does little to change the national fattening pig prevalence in MS2 and MS4. The reverse trend is true in MSs with low breeding pig herd prevalence (MS1, MS3) as feed contamination seems to be the most important factor for the national fattening pig prevalence in these MSs. The results from the model suggest that breeding pig herd prevalence is a strong indicator of national fattening pig prevalence – i.e. if a relatively low number of breeding pig herds are positive, national fattening pig prevalence will be relatively lower than in MSs with more infected breeding pig herds. Finally, results from the model also indicate that external sources of contamination appear to have a general low impact on the fattening pig prevalence.

The reduction of the prevalence in slaughter pigs by the most important potential treatments or control measures at farm level

Evidence that specific farm and transport interventions consistently work is sparse. This is presumably due to the more complex environment in which these interventions will have to be applied (relative to the abattoir) and the difficulty in standardising experiments to trial interventions. Hence, while the evidence for consistent effects is sparse, some farm interventions may well be effective. This was the conclusion of Denagamage *et al.* 2007 for vaccination, but no quantitative effect was able to be shown.

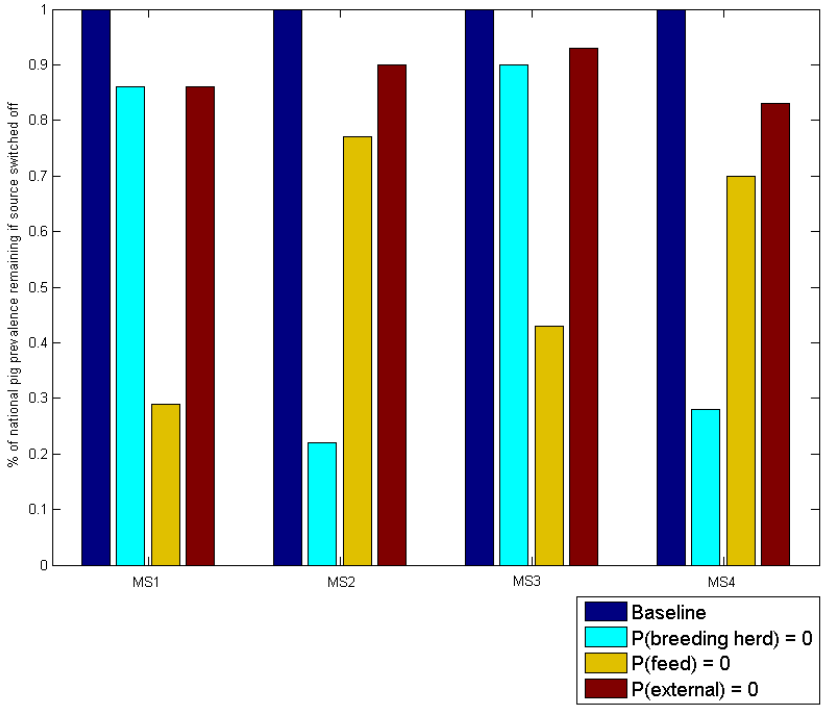

Figure 3: Relative impact on predicted *Salmonella* prevalence of slaughter pigs for each MS if each source of infection is turned off.

This lack of evidence for a consistent and/or quantitative effect meant that specific farm interventions could not be modelled. Therefore, in order to provide some assessment of farm interventions, we have modelled the effect of the varying mechanisms applied to farm interventions (e.g. modifying the dose-response for vaccination, lowering the contamination of pens due to cleaning).

Modifying the pig dose-response relationship to *Salmonella* exposure, perhaps by changing feed type, adding organic acids to feed/water, or vaccination, could have a significant effect in reducing slaughter pig prevalence within a MS, which would subsequently reduce number of cases. However, a large increase in this dose-response relationship – broadly speaking increasing the resistance of ALL of a MS's pigs such that an extra half-log to a log dose is needed to cause the same previous probability of infection – would be needed to see significant change in MS slaughter pig prevalence. This type of effect has rarely been seen in the literature and it is debatable whether such an effect could be achieved consistently at a national herd level. Cleaning and disinfection appeared to have no measurable effect.

Reducing feed contamination appears to be an effective measure in reducing slaughter pig prevalence and human cases and for large scale producers would translate into a widespread decrease in pig exposure to *Salmonella* from feed. The effect was greater in MSs with a low prevalence (MS1) of positive breeding pig herds than in MSs with relatively high breeding pig herd prevalence (MS4).

The results of the farm intervention analysis suggest that farm interventions could achieve a significant decrease in fattening pig prevalence (and hence ultimately a reduction in human cases). The choice(s) of intervention will among other things depend on the farm production type and the breeder (supplier) herd prevalence. However, the significant reductions that would be required to achieve the same effect as slaughterhouse interventions would probably be unlikely for any single farm intervention

The impact of transport, lairage and slaughter processes on contamination of carcasses

Due to the unavailability of data on the contamination of pig skin, it was not possible to model the cross-contamination of the exterior of pigs during Transport & Lairage. Therefore the contamination on the skin was estimated at the point of slaughter (using data from Davies *et al.*, 1999) and used as an input to the Slaughter & Processing module.

Within the Slaughter & Processing module, cross-contamination has been extensively modelled. The QMRA results predict that, for all four MSs, the evisceration step in a large slaughterhouse model greatly increases both the microbial load and also the prevalence of carcass contamination. This increase is due to the possibility of the gut being punctured during evisceration, therefore allowing the carcass (and subsequent carcasses on the line) to become highly contaminated. The increase in prevalence is also attributable to house flora[5], although the microbial load transferred from this source to the carcass is assessed to be low. In addition, the load and prevalence is increased during the dehairing phase (primarily due to faecal leakage) in MS2 and MS4, which had the higher infection prevalence at the point of slaughter. In the small slaughterhouses, the microbial load decreased over each phase but there was a small increase in the prevalence of contamination during the combined step of trimming/singeing.

The expected reduction of Salmonella cases in humans (or pig meat) by the most important control measures during transport, at lairage or during the slaughter process.

Transport interventions (logistic transport, increased cleaning), even assuming 100% uptake and 100% compliance/effectiveness, were assessed to have an insignificant effect in reducing the probability of human illness.

The effects of reducing concentrations on carcasses pre-chill by some decontamination step are shown in Figure 4. Marked reductions can be achieved by applying some decontamination measure, or reducing faecal leakage, at the slaughterhouse. An intervention that could consistently achieve a 1-2 log decontamination of carcasses pre-chill could reduce the number of cases by over 90% in all MSs. Further reductions can be achieved by further reducing concentrations on carcasses at pre-chill (e.g. a reduction of 3

[5] House flora is defined as the *Salmonella* contamination of the equipment, machines or other objects in the slaughterhouse that is never completely removed. It therefore acts as a permanent source of potential contamination of carcasses.

logs) with all case study MSs predicted to achieve a very high reduction (95-100%) in their number of cases Practical non-chemical interventions have been shown to produce reductions in the order of 1-2 logs (e.g. Christiansen *et al.,* 2009 and James, 2009). If such interventions are shown to be as effective when scaled up and applied across a MS's slaughterhouses, it is concluded that a control measure that reduces *Salmonella* concentrations on carcasses pre-chill would be a viable option for reducing the number of human salmonellosis cases.

The consideration of multiple interventions.

A comprehensive review of *Salmonella* in pigs (EFSA, 2006), which explored possible interventions across the farm-to-consumption pathway, concluded that it was not possible to control *Salmonella* with the adoption of just one measure. In other words, the control of *Salmonella* can only be achieved by the introduction of multiple interventions across the farm-to-consumption pathway. In order to investigate the impact of multiple interventions we considered a number of combinations of interventions; three are highlighted to show general trends from this preliminary analysis:

- Change to wet feed and 1 log decontamination post-dehair
- 1 log modification of dose-response with 1 log decontamination post-dehair
- Change to wet feed and 1 log decontamination pre-chill

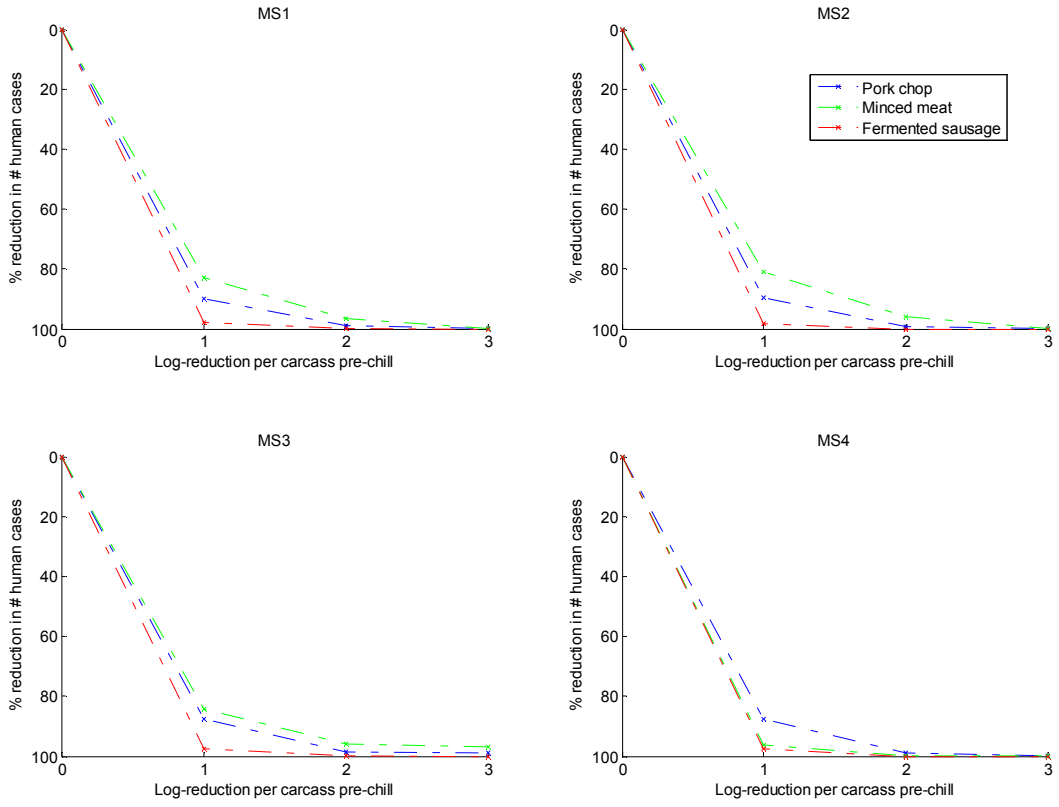

Figure 4: Effect of reducing concentrations across all contaminated carcasses in each MS by 1, 2 and 3 logs immediately before chilling of the carcass (pork cuts – blue, minced meat

– green and fermented sausage – red). For each MS, a log reduction of 1-2 logs appears to be sufficient to reduce cases by over 90%.

The analysis was carried out for MS4 only and, as predicted by EFSA, 2006, it is concluded that a combination of interventions can, if applied judiciously, produce reductions greater than the sum of the individual interventions alone. The major reason for this is that both interventions will affect the contamination level of carcasses. We also predict similar results for MS1, MS2 and MS3 although, of course, the impact of the combination of interventions that achieve the greatest reductions will be dependent on the situation within a particular MS, in particular the contamination levels of carcasses.

Summary of the intervention analysis

In summary, the farm and transport interventions are likely to vary in their ability to change slaughter pig prevalence by a sufficient amount to change numbers of salmonellosis cases. However, a combination of farm interventions applied across a large proportion of farms is likely to have a cumulative effect in reducing slaughter pig prevalence. Probably of extreme importance, but not investigated here, is the rate of uptake and correct application of interventions by farmers – if this is not universal across a MS the effect in reducing human illness will be reduced. The model results lead us to suggest that those MSs with a high breeding pig herd prevalence should focus on these herds in order to reduce the burden of infected new stock entering the weaning/growing/finishing stages However, from the results of the intervention analysis we predict that it may be more effective for MSs with a low breeding pig herd prevalence to focus their attentions on feed and other sources of infection.

From the current evidence, it would appear that specific slaughterhouse interventions are currently best placed to produce consistently large reductions in the number of human cases. For high breeding prevalence MSs, reducing infection in breeders would seem to be an important control measure as has been successfully implemented by the poultry industry. However, the hypothetical reductions and multiple interventions investigated here suggest that MSs can achieve larger reductions by targeting farm and slaughterhouse together. Reducing the prevalence at farm level is also considered important for preventing the transmission of *Salmonella* from pigs to other livestock species such as laying hens and broilers, where the prevention and control efforts are focused on the farm.

Conclusion

The farm-to-consumption QMRA developed and described here estimates the risk of salmonellosis and number of cases for three product types: pork cuts, minced meat and fermented ready-to-eat sausages. The QMRA characterises the variability between EU MSs and in particular, the variability between pig farms, slaughterhouses and consumption patterns. This was achieved by developing a generic EU model with a clearly defined set of parameters that may vary between MSs, the values of which can be easily input for any specific MS model. In addition to describing the variability between MSs, the model was designed to maximise the potential for the ability to investigate current and future interventions, which has resulted in a highly mechanistic model. Consequently, it is our opinion that this QMRA is at the forefront of methodological development at the current time. Using the QMRA to perform an intervention analysis we have shown, theoretically, that large reductions in the number of pig-meat attributable cases of *Salmonella* within a MS can be achieved via intervention at either the farm and/or slaughterhouse level.

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1 Introduction

Salmonella is a group of ubiquitous gram-negative bacteria and is a common cause of foodborne disease in the European Union (EU). In 2007 151,995 confirmed cases of human salmonellosis were reported in the EU, of which the most common serovars (81% of cases) are *Salmonella* Enteritidis and *Salmonella* Typhimurium (EFSA, 2009). *S.* Enteritidis is primarily associated with the consumption of poultry and eggs; *S.* Typhimurium is found in a range of food-producing animals, including pigs, poultry, cattle and sheep. The proportion of human *Salmonella* cases in the EU that are due to the consumption of contaminated pork/pig-meat products is unknown. However, it is known that the most common serovar for pigs is *S.* Typhimurium and hence it is widely believed that pork/pig-meat products are an important source of *Salmonella* infection in humans. The proportion of pigs/pig-meat products that are positive for *Salmonella* varies between EU countries. For example, the baseline survey for slaughter pigs carried out in the EU in 2006/2007 identified that the proportion of pigs with *Salmonella*-positive lymph nodes varied between EU Member States (MSs); it ranged from 0% to 29% (EFSA, 2008). For countries that provided data to the European Food Safety Authority (EFSA), 0 – 8.9% of samples at cutting/processing plants were contaminated with *Salmonella* and similarly 0 – 6.1% of retail samples (EFSA, 2009).

The control of *Salmonella* and other specified foodborne agents, which may pose a public health risk, is considered under Commission Regulation (EC) No 2160/2003 (EC, 2003). As part of this regulation, the Commission will set targets for the reduction of *Salmonella* at the level of primary production and where appropriate at other stages of the food chain. For poultry the target setting has already commenced and National Control Plans have been implemented. For example all MSs have been provided with a targeted reduction for *Salmonella* in laying flocks, which is dependent on the MSs *Salmonella* prevalence in the baseline survey. The provisions within EC 2160/2003 also require the setting of targets for *Salmonella* in pigs and therefore EFSA has been consulted on this matter. As a consequence of this, EFSA requested a "quantitative microbiological risk assessment (QMRA) on *Salmonella* in slaughter and breeder pigs".

Quantitative microbiological risk assessment evaluates the level of exposure and the subsequent risk to human health due to a specific pathogen (in this case *Salmonella*). The assessment process can incorporate elements of the food chain at a high resolution, and is particularly useful to evaluate the effect of interventions on human health or other end-points (e.g. point of sale). It can also be used to estimate the number of human cases that have resulted from a specific pathogen from a particular source, although the technique is considered less accurate in predicting actual public health outcomes, because of the limited availability of dose-response information. The strength of QMRA relates to its ability to assess the impact of control strategies or interventions on the risk to public health. Therefore results can be used to provide decision-makers and industry with information on which to base policies and codes of practice relating to food safety.

To date QMRAs have mainly been developed on a national basis, often as a request from a MS Government department. In order to facilitate the investigation of interventions at different points of the food chain most QMRAs take a farm-to-consumption approach; therefore modelling the prevalence of infection / contamination and the microbial load from the farm to the point of consumption (exposure). The probability of infection or illness can then be estimated by applying a dose-response model. Numerous QMRAs have been developed or are currently underway for *Salmonella* in pigs within the EU; including QMRAs

for MS2 (Hill *et al.* 2003; VLA 2009); Belgium (Bollaerts *et al.* 2009; De Sadeleer *et al.* 2009); Denmark (Alban *et al.* 2002; Hurd *et al.* 2008), Ireland (Barron *et al.,* 2009) and the Netherlands (van der Gaag *et al.* 2004). However the QMRA requested by EFSA is the first EU QMRA for *Salmonella* in Pigs, which represents numerous challenges. In particular, the variability between MSs in their pig farms, slaughter houses and consumption patterns needed to be considered.

This report documents the EFSA *Salmonella* in Pigs QMRA. The QMRA follows the framework as set by the Codex Alimentarius Commission (CAC), the international standard-setting organisation for foods in international trade, and the EU Scientific Committee for Food (CAC, 1999). Therefore, the components of the QMRA are: Hazard Identification; Exposure Assessment; Hazard Characterisation and Risk Characterisation. The challenge of modelling the EU has been overcome by developing a generic model with a clearly defined set of parameters that may vary between countries, the values of which can be easily input for any specific EU MS. To demonstrate the parameterisation and use of the model, four MSs have been selected as case studies. The QMRA is a farm-to-consumption model. The model is stochastic and highly mechanistic, i.e. mathematically describing each process at each stage of the food chain in detail, which allows flexibility for the consideration of interventions.

Chapter 2 and Chapter 3 of this report provide some context for the QMRA. Chapter 2 (Aims and Objectives of the QMRA) lists the issues/interventions identified by the EC for which the QMRA was designed and will address. In Chapter 3 (Hazard Identification and Characterisation of *Salmonella* in pork and pork products), the hazard posed by *Salmonella* to human health is characterised. Factors related to the pathogen, the human host and the vehicle (i.e. fresh pork meat) that may affect survival of *Salmonella* and lead to human illness are described. As described above, the QMRA is stochastic and therefore incorporates random chance into the model. Chapter 4 (Model Framework) describes how the generic EU model has been designed to be able to be applicable to all MSs and also the farm-to-consumption structure of the QMRA. The methodology used is described in Chapter 5 (Modelling Methodology) and, in particular, an explanation of the notation used throughout the report is given. The selection of the case studies MSs is detailed in Chapter 6. Here, the cluster analysis carried out to identify grouping of MSs within the EU and the criteria used to do this is described. Four clusters were identified and therefore 4 MSs were selected as case studies (MS1, MS2, MS3 and MS4), thus providing examples of how the QMRA can be parameterised. Although not possible to model all MSs, other MSs could parameterise the model with their own data and evaluate their own risk as required.

Chapters 7 – 10 describe the exposure assessment, in particular the probability of infection / contamination and, where possible microbial load from the farm to the point of consumption. Chapter 7 outlines the Farm model, which describes the transmission of *Salmonella* on both breeder and grower-finisher pig farms. This allows for the investigation of the role of breeder farms on the prevalence of slaughter pigs. The output of this model is the within batch prevalence of *Salmonella* for slaughter-age pigs. To allow for differences between MSs, both large and small pig herds are considered. The Transport & Lairage model is described in Chapter 8 and models the pigs from the time of leaving the farm to the point of slaughter. The model assesses the probability of further infection occurring both within and between slaughter batches of pigs. Chapter 9 describes the Slaughter & Processing part of the QMRA. A mechanistic model, similar to that developed for *Campylobacter* in broilers (Nauta *et al*, 2005), has been developed which mathematically describes the possibility of cross-contamination within and between batches. Again, to reflect the differences in EU

MSs, both a large and small slaughterhouse model have been developed. The endpoint of the Slaughter & Processing model is the *Salmonella* prevalence and microbial load on half-carcass after chilling (large slaughterhouse) and after splitting (small slaughterhouse). The further processing of the pork is modelled in Chapter 9 (Cutting Plant). Within this section of the model, the 3 pork product types to be considered are prepared; these are pork cuts; minced pork patties and fermented ready-to-eat sausage. The product types were selected to incorporate differences in product and consumption patterns within the EU. Finally, the preparation and consumption of the three pork product types by the consumer is considered in Chapter 10 (Preparation & Consumption). This model again considers the possibility of cross-contamination within the kitchen environment and also the possibility of under-cooking the product; both of which may result in human exposure to *Salmonella*.

The outcome from the exposure assessment is the probability of exposure and also the number of salmonellae ingested per serving. Chapter 11 reviews the possible dose-response models for *Salmonella* and describes how this information is combined, using the selected dose-response model, to obtain a risk of illness per serving. Combining this information with consumption data provides an estimate for the number of cases. The results of this analysis for the case study MSs are provided in Chapter 12 as well as the results for the uncertainty analysis. Model validation is an important aspect for any model and this is also described in this section, in particular the outputs from the model at the point of slaughter, at retail and also the number of human cases are compared to the relevant data.

An essential component of the QMRA was to investigate the impact of interventions. A comprehensive review of *Salmonella* in pigs (EFSA, 2006), which explored possible interventions across the farm-to-consumption pathway, concluded that it was not possible to control *Salmonella* with the adoption of just one measure. In other words, the control of the *Salmonella* can only be achieved by the introduction of multiple interventions across the farm-to-consumption pathway. Farm-to-consumption QMRAs can assess the impact of such multiple controls and this approach was adopted. The selected interventions, methods of analysis and results are presented in Chapter 13.

As mentioned previously, the proportion of human *Salmonella* cases in the EU that are due to the consumption of contaminated pork/pig-meat products is unknown. As part of this project, we aimed to investigate this using a microbial subtyping attribution model for *Salmonella* (Hald *et al*., 2004). Although it was not possible to investigate this as thoroughly as initially hoped due to the unavailability of the human data from ECDC during the time span of the project, a comparison and interpretation has been carried out for the available serovar data as well as an attribution model based on outbreak data, with particular emphasis on pigs and pork. This work is detailed in Chapter 14 (Source Attribution).

The methods and results are considered further in the discussion (Chapter 15). Here particular emphasis is placed on the results of the sensitivity and uncertainty analysis and also the identified data deficiencies/gaps. Finally, conclusions are given in Chapter 16.

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2 Aims and Objectives of the QMRA

Under Article 36 of the European Parliament and Council Regulation (EC) No 178/2002, EFSA published a call for a "Quantitative microbiological risk assessment on *Salmonella* in slaughter and breeder pigs" (EFSA, 2007) .

The objectives of the call are as follows:-

A QMRA model that covers the whole food chain is required, beginning with a baseline model for the farm-to-fork chain, including risk characterisation. While slaughter (fattening) pigs are the main object of this risk assessment, the role of piglets as a source of *Salmonella* also needs to be considered. During transport and lairage, cross-contamination might occur both between-animal and between-batches (i.e. between herds) due to carry-over of *Salmonella* on surfaces from one day to the next. The model will concentrate on primary production through to raw pig meat and raw pig meat products arriving in the kitchen. The model will also include module(s) accounting for preparation and consumption of raw pig meat and raw pig meat products, and a dose response model, thus allowing numbers of human cases to be assessed.

As a consequence of the objectives provided the VLA/RIVM/Food-DTU consortium have worked towards a full farm-to-consumption QMRA, which takes into consideration at every stage possible the opportunity of cross-contamination. To describe the cross-contamination the model needed to be highly mechanistic, which although it leads to a more complex model will allow a better examination of interventions for *Salmonella* in pigs. EFSA also provided details of the objectives raised by the EC and, in particular, are in the EC's Terms of Reference. These are as follows:

1. The expected reduction of *Salmonella* cases in humans (or pig meat at retail) by a reduction (e.g. 5- or 10-fold) of *Salmonella* prevalence in slaughter pigs (based on bacteriology or serology at slaughter)
2. The sources of infection for slaughter pigs at farm level
3. The reduction of the prevalence in slaughter pigs by the most important potential treatments or control measures at farm level.
4. The impact of transport, lairage and slaughter processes on contamination of carcasses.
5. The expected reduction of *Salmonella* cases in humans (or pig meat) by the most important control measures during transport, at lairage or during the slaughter process.

Objectives 1, 3 and 5 are considered as part of an Intervention Analysis (Chapter 13). Within this section, both hypothetical interventions (e.g. 5-fold, 10-fold reductions, etc.) and specified reductions (e.g. increase cleaning) are considered. The Farm model (Chapter 7) considers different introductory sources of *Salmonella* (Objective 2) including weaners, feed and external contamination (e.g. rodents, birds, etc.). Finally, Objective 4 is considered in Chapter 8 (Transport & Lairage) and Chapter 9 (Slaughter & Processing) and, in particular, infection within and between batches is considered at Transport & Lairage and cross-contamination is considered between pigs on the slaughter line, between batches processed on the same day and between batches processed on different days.
It is essential that the QMRA reflects as much as possible the diversity in production / consumer practices between different MSs. Within the EU, at the farm level, there may be

large variation in terms of size of farms, type of farms, size of slaughterhouses and slaughter methods. Finally, differences in the pork products consumed within the EU need to be taken into account. However this needed to be balanced with limited resources and time and, certainly, there was insufficient time to produce a QMRA for each MS. To address this issue a generic model has been developed for the EU (see Chapter 4). To demonstrate its use a number of MSs were selected as case studies. In terms of the variation in pork products consumed a small number of products were selected to represent differences in the processing of products. Information on how much of each selected product type is consumed in each MS is included as a parameter within the model.

In the call, EFSA highlighted that, between countries, there are differences in the *Salmonella* serotypes that are present in slaughter pigs. In particular, many serotypes present in pigs may not be *Salmonella* of public health significance. However, since EFSA 2006 concluded that "all *Salmonella* serovars in pork are to be regarded as a hazard for public health" a QMRA for *Salmonella* spp. was developed. It is recognised that there will be variability between strains in their behaviours across the food chain however due to the expected data gaps in the variability of serotypes to survive / grow / persist in the farm-to-consumption chain EFSA deemed it acceptable to assume that all *Salmonella* behave similarly. It is important to note that the application of this simplifying assumption will result in the risk of illness (and hence also number of cases) being too high. However, given that one of the main emphasises is the reduction in risk of illness/number of human cases from the reduction of *Salmonella* in slaughter pigs or changes at Transport, Lairage or Slaughter, this approach is still valid.

Approaches to address the above aims and objectives given above were identified by the VLA/RIVM/Food-DTU project proposal, submitted June 2007. This final report details the scientific work carried out and, in particular, focuses on the QMRA and the source attribution. The project deliverables not included here are: the proceedings from the *Salmonella* in Pigs QMRA & Data Workshop held in Copenhagen, Denmark in April 2008 and the 6, 12 and 18 month progress reports. The 3 progress reports are superseded by this final report. All project milestones have been completed.

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3 Hazard Identification and Characterisation of *Salmonella* in Pork and Pork Products

3.1 Introduction

Salmonella is an important cause of foodborne disease in humans throughout the world and is a significant cause of morbidity, mortality and economic loss (Roberts & Sockett 1994; Mead *et al.,* 1999; Adak, Long & O'Brien, 2002; Voetsch *et al.,* 2004; Schroeder *et al*., 2005). Illness can range from a mild to severe gastroenteritis and in some people, invasive disease, which can be fatal. Long term sequelae such as reactive arthritis can also result from *Salmonella* infections.

In 2007, 151,995 human cases of *Salmonella* were reported in the EU (EFSA, 2009), of which the most common serovars were *Salmonella* Enteritidis and *Salmonella* Typhimurium. *S*. Enteritidis is primarily associated with the consumption of poultry products, particularly eggs. *S.* Typhimurium is found in a range of food-producing animals, including pigs, poultry, cattle and sheep. The proportion of human *Salmonella* cases in the EU that are due to the consumption of contaminated pork products is unknown. However, it is known that the most common serovar for pigs is *S.* Typhimurium and it is widely believed that pork products are an important source of these infections in humans.

A comprehensive review of *Salmonella* in pigs (EFSA, 2006), which explored possible interventions across the farm-to-consumption pathway, concluded that it was not possible to control *Salmonella* with the adoption of just one measure, i.e. control of the hazard can only be achieved by the introduction of multiple interventions across the farm-to-consumption pathway.

Activities in EU towards management of salmonellosis, therefore calls for evaluation of disease contribution from all parts of the pork-production chain. Modelling of the entire food chain (feed, food animal production, transport, slaughter, processing, retail and consumption patterns) will support decision makers to produce science-based recommendation, best procedures, and to implement legislation to regulate production across the EU. Mathematical sensitivity analysis can be employed to evaluate intervention strategies and subsequently be used as a base for cost/benefit analysis. Ultimately, the use of modelling tools should support food safety intervention in a more cost efficient way.

The aim of this project is to develop a quantitative microbiological risk assessment (QMRA) for *Salmonella* in pigs for the EU. The model will be used to evaluate the effect of interventions on human health and other end-points (e.g. point of sale), and to estimate the number of human *Salmonella* cases that can be attributed to fresh pork products. The technique is, however, considered less accurate in predicting actual public health outcomes, because of the limited availability of dose-response information.

Hazard identification and hazard characterisation are two of the corner stones of a QMRA. In the following, the hazard posed by *Salmonella* to human health is characterised including a description of factors related to the public health outcome due to exposure to *Salmonella*. This will serve as the hazard identification and the first general part of the hazard characterisation (the dose-response relationship is also part of Hazard Characterisation, but this is considered in Chapter 11). Factors related to the pathogen, the human host and the

vehicle (i.e. fresh pork meat) that may affect survival of *Salmonella* and lead to human illness are described. Furthermore, relevant data sources (e.g. national or international databases) are presented.

3.2 The Organism

3.2.1 Classification and subtyping of *Salmonella*

Salmonella is a genus of gram-negative, aerobic, rod-shaped bacteria that can infect people, birds, reptiles, and other animals. Currently the genus *Salmonella* is divided into two species: *S. enterica* and *S. bongori.* Recently a third species, *S. subterranea* was identified (Shebolina *et al*., 2004). It was recognised in 2005, and the CDC may incorporate this species in the nomenclature system in near future. The species *S. enterica* consist of six subspecies: *S*. *enterica*, *S. salamae, S. arizonae, S. diarizonae, S. houtenae* and *S. indica* whereas no subspecies has been assigned to *S. bongori or S. subterranea* (Su and Chiu, 2007).

Based on the combination of bacterial surface-antigens the genus *Salmonella* is subdivided into 2,541 serovars (also called serotypes) (Popoff & Le Minor, 2001) For convenience the serovars are denominated by genus and serovar only (e.g. *Salmonella enterica subspecies enterica* serovar Typhimurium is called *Salmonella* Typhimurium (*S*. Typhimurium)). According to Popoff *et al*. (2004) 1,504 serovars belong to *S. enterica* ssp. e*nterica*. Most zoonotic serovars associated with human illness are in this group.

Table 3.1: Current *Salmonella* nomenclature (Su & Chiu, 2007).

Taxonomic position (writing format) and nomenclature				No. of serovars in each species or subspecies (Popoff *et al*., 2002)
Genus (capitalised, italic)	Species (italic)	Subspecies (italic)	Serovars (or serotypes) (capitalised, not italic)*	
Salmonella	*enterica*	*Enterica* (or subspecies I)	Cholerasuis, Enteritidis, Paratyphi, Typhi, Typhimurium	1504
		Salamae (or subspecies II)	9,46:z:z39	502
		arizonae (or subspecies IIIa)	43:z29:-	95
		diarizonae (or subspecies IIIb)	6,7:l,v:1,5,7	333
		houtenae (or subspecies IV)	21:m,t:-	72
		indica (or subspecies VI)	59:z36:-	13
	bongori *subterranea*	(former subsp. V)	13,22:z39:-	22

* : Some selected serotypes (serovars) are listed as examples.

All *Salmonella* serovars are considered potentially pathogenic for humans, but the degree of host adaptation varies, which affects the pathogenicity. Some serovars of *S. enterica subspecies* enterica: *S*. Typhi, *S*. Paratyphi and *S*. Sendai, are highly adapted to man (Mølbak *et al*., 2006). They cause severe systemic illness in humans characterised by fever and abdominal symptoms (enteric/ (para)typhoid fever (Miller *et al*., 1995). These serovars are usually not pathogenic to animals and are not considered to have a zoonotic potential. Therefore human infections with these serovars should not be included in a risk assessment on *Salmonella* in slaughter and breeder pigs. For the purpose of this study the serovars

denominated "non-typhoid *Salmonella*" are defined as all serovars except from the highly human specific typhoid serovars.

Non-typhoid, ubiquitous serotypes, such as *S*. Typhimurium, affect both humans and a wide range of animals, where they usually cause gastrointestinal infections of varying severity. The ability of the zoonotic serovars to infect animals and eventually infect humans via food seems to vary (Hald *et al*., 2006).

Certain zoonotic serovars appear to be more animal species specific e.g. *S*. Cholerasuis in pigs, *S*. Dublin in cattle, *S.* Abortus-ovis in sheep, and *S*. Gallinarum in poultry. They frequently cause disease in infected animal populations of the associated animal species but are only occasionally identified in cases of human infections where they may produce no, mild or serious disease (Acha & Szyfres, 1987; Mølbak *et al*., 2006). The non-host-adapted serotypes are those with principal zoonotic significance.

For some of the more common *Salmonella* serovars a subtyping system based on lysis of *Salmonella* from a panel of *Salmonella* bacteriophages (phage-typing) is available. Thus, phage typing is routinely used for the serotypes *S*. Enteritidis and *S*. Typhimurium in some MSs (EFSA, 2007a). Phage typing further subdivides serovars into phage types (PT) in *S*. Enteritidis or definitive types (DT) in *S*. Typhimurium.

Genetic typing methods (e.g. Polymerase Chain Reaction (PCR), Pulsed Field Gel Electrophoresis (PFGE) or Multi-Locus Variable-Number Tandem Repeat Analysis (MLVA)) are able to further differentiate *Salmonella*. Also plasmid profiling (typing of the transferable gene structures, plasmids) and antimicrobial susceptibility testing may be used to characterise *Salmonella* isolates.

Subtyping of *Salmonella* is used in epidemiological investigations. The high differentiation of strains obtained from genotyping is particularly useful in the investigation of outbreaks, as it helps to define groups of cases that have been infected from the same strain from the same source. (Mølbak *et al*., 2006).

3.2.2 Growth and inactivation

Reduction of *Salmonella* or prevention of its growth throughout the farm-to-consumption chain are important factors in modelling the infection risk to humans because these factors have potential as control measures against human salmonellosis.

The normal habitat of non-typhoid *Salmonella* is the gut of warm blooded animals. But also the oviduct of laying hens may harbour *Salmonella* (particularly *S.* Enteritidis) (Humphrey, 1999) and most *Salmonella* in the non-*enterica Salmonella* species are considered reptile-associated. The optimal environment and the range of physical and chemical parameters for growth of *Salmonella* reflects the adaption of the bacteria to the habitat.

Different physical and chemical measures may be used alone or in combinations (e.g. Álvarez *et al*., 2003) to control *Salmonella* at different points in the farm-to-feed chain through prevention or inhibition of the multiplication and spread of the bacteria or through reduction or elimination of existing contamination. The efficiency of these to factors to control *Salmonella* is time-dependent and *Salmonella* show some serovar variation in growth ecology which has to be considered when e.g. hurdle effects are discussed. (Bell & Kyriakides, 2002; ICMSF, 1996).

The optimum temperature for growth of *Salmonella* is 35-43°C with a growth interval of 5.2-46.2°C, and *Salmonella* will only grow slowly at 10°C. Most serotypes fail to grow at temperatures below 7°C. The resistance to heat depends on other parameters as water activity (a_w). *Salmonella* do not survive pasteurisation but is relatively resistant to freezing. (Bell & Kyriakides, 2002). In the pigs and pork production chain, reduction or kill of *Salmonella* through heat treatment is used for e.g. heat treatment of animal feed, hot-water wash of contaminated carcasses and heat treatment in processing and preparation of food. Optimal growth temperatures in the gut of animals and humans or in improperly stored food may promote growth of the bacteria, and refrigeration of carcasses, during processing and storage or in prepared food limits the growth of *Salmonella*.

The optimal pH range for growth of *Salmonella* is 7.0-7.5 and the range for growth is 3.8-9.5. Most serovars will not grow below pH 4.5 (Bell & Kyriakides, 2002; ICMSF, 1996). Reducing pH can be used to control the growth of *Salmonella.* Thus, organic acids are used as feed additive or in marinated food, and a low pH may be achieved in properly fermented wet feed and through fermentation of food (e.g. salami). A low pH in the gastrointestinal tract of animals and humans is a strong barrier against *Salmonella* infections. It is achieved from secretion of gastric acid and fermentation of carbohydrates in the contents of stomach and gut resulting in lowered pH from an increase in short-chain organic acids, and in particular their un-dissociated forms which are toxic to *Salmonella* (Hansen 2004). Feeding pigs with meal feed, fermented wet feed and coarsely grinded feed (compared to pelleted, dry and finely grinded feed) improves the fermentation in the gut of the pig, and is able to reduce *Salmonella* infections at the herd level.

The optimal water activity for *Salmonella* growth is a_w 0,99 and *Salmonella* growth stops at a water activity below approximately 0.94 depending on pH and temperature. The low water activity is utilised as a control measure for microbial growth and persistence in e.g. dry storing of feed or food, and in desiccation of surfaces in the farm and farm environment, in slaughterhouses, processing plants and kitchens. Besides from desiccation low water activity can also be achieved from e.g. high salinity or sugar contents in food. But even very low water activity (in e.g. chocolate, black pepper and peanut butter) may not eliminate *Salmonella* and sufficient cell numbers to cause infection in animals and humans can and do survive for log time periods (Bell & Kyriakides, 2002; ICMSF, 1996).

Other de-contamination procedures as gamma-irradiation, UV-irradiation or ultrasonic waves are able to reduce or eliminate bacterial contamination as well and may be considered for decontamination of e.g. animal feed, food and surfaces.

Only few chemical disinfectants are effective when applied to surfaces in e.g. farms, where the presence of some organic matter often is unavoidable (e.g. strongly alkaline disinfectants and aldehydes). For disinfection of cleaned surfaces in slaughterhouses, cutting plans, retail and in the kitchen more harmless disinfectants may be used (e.g. hypochlorite, iodine and quaternary ammonium compounds).

Table 3.2: Limits for growth of salmonellae when other conditions (e.g. temperature, pH, a_w) are near optimum (ICMSF, 1996)

Conditions	Minimum	Optimum	Maximum
Temperature (^oC)	5,2*	35-43	46.2
pH	3.8	7-7.5	9.5
a_w	0.94	0.99	>0.99

* Most serotypes fail to grow at <7 ^oC

3.3 The Disease in Humans: Salmonellosis

3.3.1 Epidemiology and pathogenesis of non-typhoid human salmonellosis

There are numerous transmission pathways through which humans can be exposed to *Salmonella* including a wide range of domestic and wild animals and a variety of foodstuffs covering both food of animal and plant origin. Infected animals will carry *Salmonella* in the faeces and the usual route of infection is through faecal-oral transmission. The epidemiology of *Salmonella* is, therefore, primarily due to direct or indirect faecal contamination of live animals, food or humans (D'Aoust, 1989). For slaughter animals including pigs, the contamination or cross-contamination of carcasses is basically a question of redistributing the *Salmonella* bacteria from the positive animals during slaughter and further processing.

The majority of human infections is believed to be acquired through the foodborne route, where exposure often occurs when the bacteria are introduced in food preparation areas and are allowed to multiply in food e.g. due to inadequate storage temperatures, or because of inadequate cooking or cross-contamination of ready-to-eat food. The organism may also be transmitted through direct contact with infected animals or faecally contaminated environments. Person-to-person transmission does also happen occasionally.

Humans are normally infected by oral uptake of *Salmonella* through contaminated food. The infective dose varies depending on strain virulence, food type involved and age and immune status of the patient. For non-adapted serotypes, there are grounds to believe that the concept of an infective dose of 10^5 to 10^7, as determined from volunteer-feeding studies no longer applies. From outbreak data it has been shown that as few as 10 cells can cause disease (D'Aoust, 1989; Hennessy *et al*., 1996; Mølbak *et al*., 2006). Furthermore, outbreak data suggest that the infective dose is lower in foods with a high fat content due to the protection of cells from the effect of the gastric acid (Kapperud *et al*., 1990; Hedberg *et al*., 1992; Hennessy *et al*., 1996; Mølbak *et al*., 2006).

The infection may be subclinical i.e. without symptoms or lead to disease (salmonellosis). Salmonellosis is caused by different virulence factors leading to diarrhoea due to increased secretion or impaired fluid uptake in the gut, phagocytosis of the bacteria into gut cells and systemic intoxication due to enterotoxins released from the bacterial cell wall during die-off of the bacteria. (Mølbak *et al*., 2006). Following an incubation period ranging from 6 to 48 hours, the first clinical symptoms appear. They are usually characterised by gastroenteritis including diarrhoea, abdominal cramps, fever, headache, myalgia, nausea, vomiting and malaise (Mølbak *et al*., 2006). Remission usually occurs within 3-4 days, but the symptoms may last for 10 days or longer (Miller *et al*., 1995). In a few percent of cases, complications such as septicaemia, endocarditis, multiple abcesses, polyarthritis, osteomyelitis, and, in

extreme cases death, may occur (Mølbak *et al.*, 2006; Miller *et al*., 1995). Case-fatality rates between 0.7% and 1.3% have been reported from the US (Cohen and Tauxe, 1986), and 1.2% in Denmark (Fisker, *et al*., 2003). A recent Danish population study based on registry data, however, estimated the 1-year mortality rate to 3.1%, suggesting that the mortality rate may be underestimated. Sequela such as reactive arthritis, Reiter's syndrome, which occur as a triad of arthritis, conjunctivitis and urethritis, and eythema nodosum are well-known late-onset complications seen in a subset of patients. Reactive arthritis occurs at an average of 10 days after the onset of diarrhoea in 2-15% of salmonellosis cases (Mølbak *et al*., 2006).

The excreta of infected persons will contain large numbers of *Salmonella* spp. at the time of onset of illness. Those numbers decrease with the passing of time. The intermittent faecal shedding that follows the acute phase of gastroenteritis may be of short duration (4-5 weeks) or may persist for more than a year (chronic carriers) (D'Aoust, 1991b; Miller *et al*., 1995; Mølbak *et al*., 2006). Some serovar-related differences occur as 90% of cases infected with *S.* Typhimurium are culture negative at nine weeks, whereas more than 20% with other serotypes are still shedding at 20 weeks (Miller *et al*., 1995). It is estimated that between 0.2 and 0.6% of cases with non-typhoid salmonellosis develops a chronic carrier state (Mølbak *et al*., 2006). Human carriers are of special concern in the food-manufacturing and food-service industries, because of the risk of contamination of foods. Several outbreaks caused by food handlers have been described (e.g. Hedberg *et al*., 1991; Anonymous, 1999a; Maguire *et al*., 2000; Ethelberg *et al*., 2004).

Demographic and societal factors contributing to human salmonellosis

Despite the many efforts to prevent and control food borne salmonellosis during the last twenty years, this pathogen continues to be one of leading causes of human gastroenteritis. There exist many factors that contribute to this development. Among these are characteristics of the population, the increasing globalisation of the food trade and changes in industrial structure, and changes in consumer behaviours.

Populations with increased susceptibility

Children and elderly people are considered to be more at risk of an infection with *Salmonella* than the average adult (D'Aoust, 1989). It is generally accepted, that immunocompromised people suffering from underlying diseases e.g. cancer, AIDS or chronic bowel disorders, are more prone to an infection than people in good health (D'Aoust, 1989; Berends *et al*., 1998). People receiving antacids have also been reported as having an increased risk of infection due to the increased pH-level in the ventricle (Miller *et al*., 1995). Since the group of both elderly and chronically diseased people is growing, this may also contribute to the explanation of the continuing high level of human salmonellosis (Altekruse *et al*., 1998).

The emergence of multi-drug resistant *Salmonella* types e.g. *S.* Typhimurium DT104, is of special concern in humans that at the time of exposure are undergoing treatment with antibiotics due to another infectious disease. The increased risk of infection in already debilitated patients has been demonstrated in several investigations, among these in an American case-control study reporting the risk factors associated with *S.* Typhimurium DT104 infections (Glynn *et al*., 1998). Consequently, the emergence of resistant *Salmonella* in combination with the use of antibiotics in humans contributes to an increased incidence of human salmonellosis. Compared with patients infected with susceptible *Salmonella* strains, patients with multi-drug resistant infections are also more likely to have a protracted course

of disease that in addition is more severe and often requires hospitalisation (Altekruse *et al*., 1998; Helms *et al*., 2002).

Salmonella infections are generally self-limiting, requiring no or only symptomatic treatment such as fluid and electrolytic replacement. However, antibiotic treatment may be required in vulnerable patients or in patients with extra-intestinal infections or severe or protracted gastroenteritis. Because most resistant *Salmonella* strains, including the typical penta-resistant *S.* Typhimurium DT104, are sensitive to fluoroquinolones, fluoroquinolones are routinely used for empiric treatment. However, infections caused by strains with reduced susceptibility to quinolones may result in reduced effect of treatment (Mølbak *et al*., 1999).

Globalisation of food trade and consolidation of food industries

A rapidly growing international trade in live animals (incl. breeding animals), animal feed stuffs, raw materials and processed foods has facilitated the introduction of new *Salmonella* types in importing countries and resulted in an increasing length and complexity of the food chain (D'Aoust, 1994). Concurrently, there has been an increase in the consolidation of food industries, including the primary production, and mass distribution. This trend toward greater geographic distribution of products from large centralised food processors carries a risk for more widespread outbreaks affecting more people (Gray & Mossel, 1992; Altekruse *et al*., 1998). The dissemination of *S.* Enteritidis in the table-egg industry is an evident example of this (Thorns, 2000; EFSA, 2006).

Consumer behaviours

There is an increasing tendency for consumers to eat more meals outside the home. This results in an increase of meals prepared for large-scale production, where improper holding temperatures, delayed serving, improper heat treatment e.g. due to a sudden demand for a special dish, or preparation of food in premises that are too small, are reported as frequently observed risk factors associated with outbreaks (Bryan, 1988; Anonymous, 2000). In addition, subclinically infected food handlers may play an important role in outbreaks from foodservice establishments (D'Aoust, 1989; Anonymous, 2000). Outbreaks that occur outside the home accounted for almost 80% of reported outbreaks in USA around 1990 (Altekruse *et al*., 1998).

Consumers have also changed their shopping habits towards less frequent, but more large-scale purchases, which consequently result in the storage of foods in the home for relatively long periods in conditions, which are also often less than ideal. So, even though, deep-freezers and refrigerators are common facilities in the modern home, their use may lead to decreased awareness of the perishability of foods (Gray and Mossel, 1992). Besides contaminated raw materials, the most important factors contributing to outbreaks in households are reported to be improper cooling, inadequate cooking, cross-contamination and preparation of food several hours before consumption (Bryan, 1988; Michanie *et al*., 1988; Anonymous, 1999b).

Traditionally, foods implicated in foodborne outbreaks have been poultry products including eggs, red meats and unpasteurised milk. In recent years, however, new types of food previously thought to be safe are considered to be hazardous. These include in particular fresh produce, which may partly be as a response to health promotion increasing the consumption of fresh fruit and vegetables that may be contaminated with animal faeces during growth, harvest and distribution. Increasing numbers of foodborne outbreaks have been traced back to these kinds of products. In particular, alfalfa sprouts has been implicated in large multi-state or -national outbreaks (Mahon *et al*., 1997; van Beneden *et*

al., 1999), and sprouts are recognised as a special problem because of the potential for pathogen growth during the sprouting process (Anonymous, 1999c).

International travel has also increased rapidly during the 20th century. In countries with a low prevalence of *Salmonella* in their domestic livestock and food this fact influences the national human statistics markedly. In Sweden and Norway, for instance, it is estimated that approximately 70-80% of all human *Salmonella* infections are acquired abroad (Kapperud & Hasseltvedt, 1999; EFSA, 2007a). Overall, around 50% of human *Salmonella* cases in EU were reported to be acquired domestically and 7% abroad in 2006. For 43% of the cases there was no available travel information (EFSA, 2008a). If travel-associated cases constitute a considerable proportion of cases this is likely to impact the expected effect of national intervention strategies and the information is therefore important from a risk assessment point of view. Unfortunately, only few countries have a systematic registration of travel history.

Finally, the recent food scares (e.g. BSE and dioxin) has shown that the widespread announcement of these stories in the media has a big influence on the behaviour of consumers for shorter or longer periods (Mitchell & Greatorex, 1992). This factor is important to consider when interpreting the trends in human food borne infections.

3.3.2 Disease incidence and burden of human salmonellosis

Statistics for the incidence of human salmonellosis (and other foodborne infections) are notoriously difficult to compare between countries and sometimes even within a country, as they depend on the definition of a case, the diagnostic method used and how the information is collected and analysed. In addition, the subjective reactions of the patients and general practitioners will influence whether a case will be diagnosed and reported. First the patient has to feel ill enough to consult a doctor, who secondly must decide to take a diagnostic sample. Thirdly, the diagnostic laboratory must recover the pathogenic organism from the sample. Finally, the result has to be reported to a central database containing data from all (or almost all) national diagnostic laboratories. Based on this, it is clear that only a minor proportion of the actual number of cases is reported and that the size of this proportion varies greatly between countries. This is also confirmed by the results of the so-called disease burden studies conducted in several countries in order to estimate the true burden of disease (Wheeler *et al*., 1999; Mead *et al*., 1999; Gallay *et al*., 2000; de Wit *et al*., 2001a, 2001b; van Pelt *et al*., 2003). The studies suggest that for every reported case of salmonellosis, between 3.8 and 38 persons in the population fell ill (Mølbak *et al*., 2006).

Human salmonellosis is the second ranking foodborne disease in EU and most European countries, only exceeded by campylobacteriosis. A large proportion of the observed difference between, for instance, Portugal, Germany and Czech Republic is undoubtedly a result of differences in reporting systems rather than a true difference in incidence rates. Still, a considerable proportion may be due to differences in food preferences and preparation, and the prevalence of *Salmonella* in animals and foods in the MSs. But even though, the actual figures cannot be compared, it is possible to compare the trends and distributions of serovars.

In 2007, the reported number of confirmed cases and incidence of human salmonellosis in EU MSs were 151,995 cases, corresponding to an incidence of 31.1 cases per 100,000 inhabitants (EFSA, 2009). Germany accounted for 36.4% of all reported cases, whereas the incidence was greatest in the Czech Republic (171.6 cases per 100,000). Salmonellosis

continues to be the second ranking zoonosis in EU after campylobacteriosis, but the incidence has decreased over the past years, and in the last four years this decrease has been statistically significant. Within each reporting MS, statistically significant and decreasing trends (2004-2007) were observed in Austria, Spain and Poland.

The two most common *Salmonella* serovars have for many years been *S*. Enteritidis and *S*. Typhimurium, representing 81% of all known types in 2007, compared to 85.7% in 2006. Poultry are the main reservoir of *S.* Enteritidis and poultry products, especially table-eggs, are recognised as the primary source of human *S.* Enteritidis infections (EFSA, 2007a). *S*. Typhimurium is endemic in domestic livestock in most countries (EFSA, 2007a; Thorns, 2000). In contrast to *S*. Enteritidis, *S.* Typhimurium is not related to a particular animal reservoir, but can infect many different hosts. However, the different subtypes (phage types) of *S*. Typhimurium are often heterogeneously distributed in the various animal reservoirs making it possible to assess the major sources of these infections as described later on in this report (Chapter 14). On this basis, it is estimated that most *S*. Typhimurium infections are caused by consumption of meat, particularly pork. The geographical distribution of human *S*. Typhimurium infections is presented in Figure 3.1.

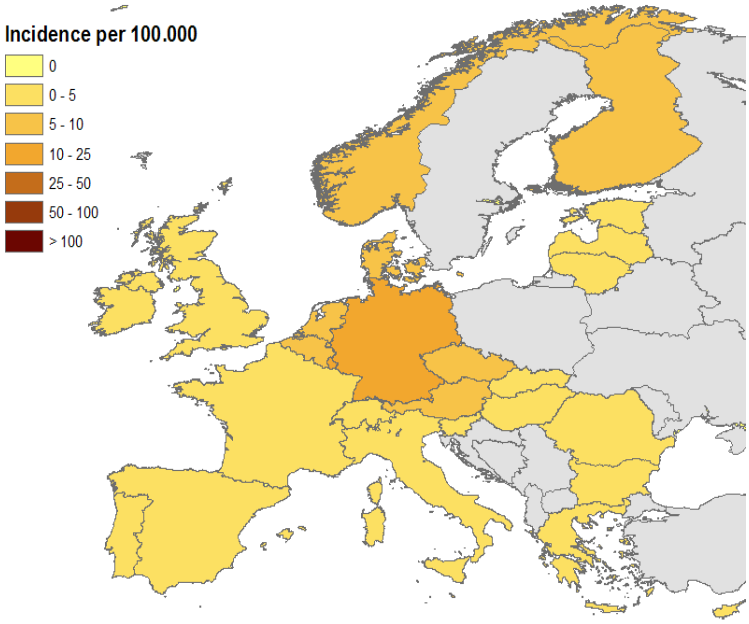

Figure 3.1: Laboratory confirmed cases of *Salmonella* Typhimurium incidences in humans in EU, 2006 (*Salmonella* Atlas: www.epigis.dk)

The cost of human salmonellosis to the society has been estimated in a study in the United States of America and in Denmark. It was found that the burden in the US was an estimated 1.4 million infections, which results in 168 000 visits to physicians, 15 000 hospitalisations and 580 deaths annually in a population of 300 million (84 % health care coverage; U.S. Census 2004). As the cost of individual cases can be estimated to be in the range of USD 40 for uncomplicated cases to USD 4.6 million for cases ending with hospitalisation and death the total annual cost is estimated to be 3 billion Dollars. Danish estimates show that the annual cost is USD 15.5 million equivalent to 0.009% of gross domestic national product. To evaluate the cost effectiveness of the Danish Salmonella control programme the cost was compared to the resulting reduction of cases concluding that the benefit amounts to USD 25.5 million annually (WHO, 2005).

3.4 The Food Product: Pork

3.4.1 Pork production

Salmonella can enter the pork production chain at multiple levels (Figure 3.2). In the following, each major step in the farm-to-consumption continuum will be presented focusing on potential sources of *Salmonella,* factors important for the transmission of *Salmonella* from one step to the next, and options for interventions. Methods applied for monitoring of *Salmonella* are also described.

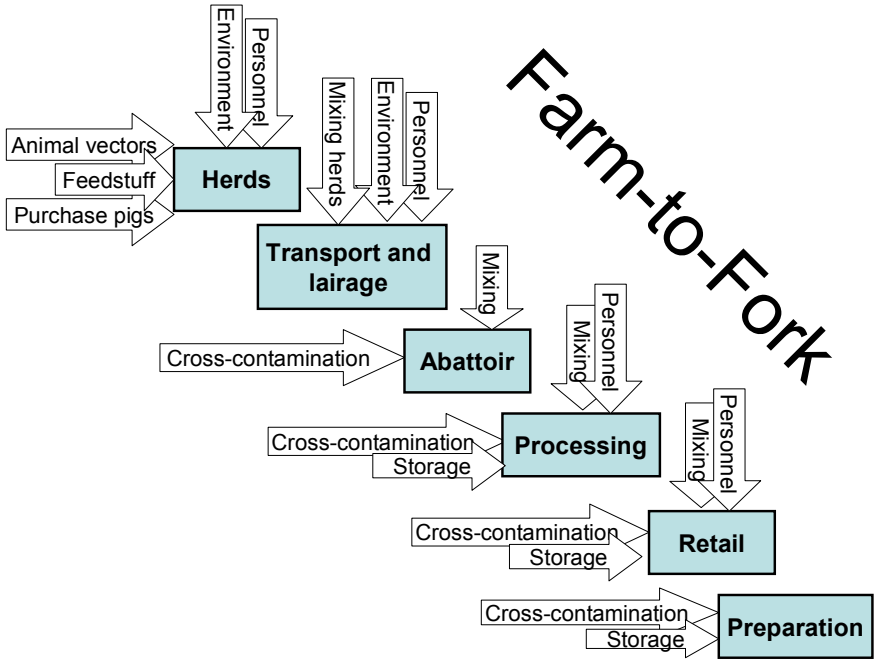

Figure 3.2: The farm-to-consumption chain of the pork production. Arrows indicate sources of introduction of *Salmonella* into the production chain.

3.4.2 Farm

Modern swine production is developing into a specialised industry, where the production steps are optimised by separation of age groups on different farms. Some farmers specialise in the genetic improvement of breeding stock, delivering young breeding animals (gilts and young boars) to sow herds. The sow herds are supplying weaners to finishing farms, where the slaughter pigs are produced. In farrow-to-finishing herds, the age groups are usually separated in segregated farm buildings with an all-in/all-out production. In contrast, the traditional swine production is predominantly run as farrow-to-finish operations, often on a family farm basis, where all the steps mentioned above is integrated at one single farm (Figure 3.3).

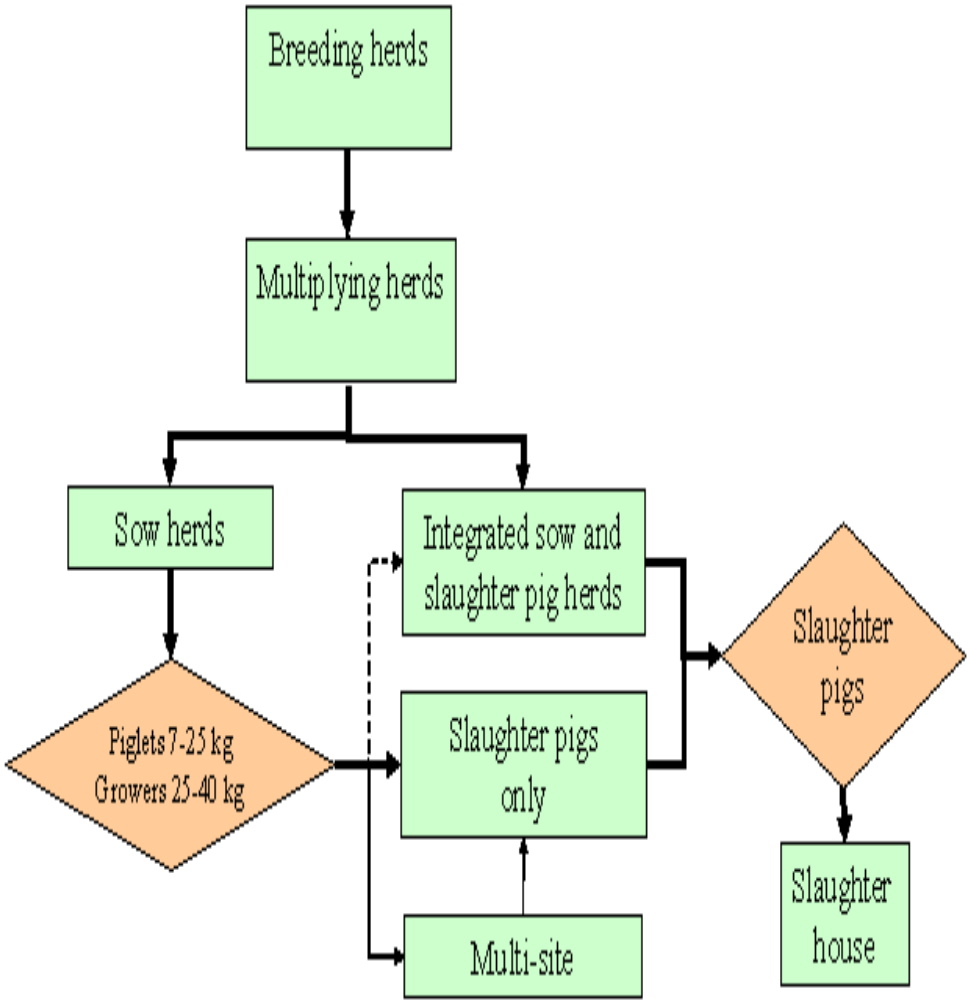

Figure 3.3: The production pyramid in the primary slaughter pig production. Sectioned rearing in a modern pig production unit. Traditional production sites will have integrated sow and slaughter pig herds.

Sources and transmission of *Salmonella* in pig herds

Pigs are reared at farms either practicing batch-production with thorough cleaning and disinfection prior to introduction of new batches of pigs or continuous production where new pigs successively are introduced to the unit after removal of pigs for slaughter. One of the most important routes for introduction of *Salmonella* into a pig herd is by purchase of infected pigs or by establishment of new herds from an infected source. In continuous production systems, pathogens such as *Salmonella* are more readily transferred from older to younger pigs thus contributing to the maintenance of infections in the herd.

Salmonella can also be introduced by the feedstuff and feed, and in particular protein containing feedstuff. Feedstuff of animal origin are more often contaminated with *Salmonella* serovars that are prevalent further up the food production chain (*S*. Typhimurium and *S*. Enteritidis), whereas *Salmonella* serotypes coming from feed components with vegetable origin often are of more diverse types (Berends, 1996; Davies, 1997) as are serovars in

feedstuff originating from countries outside EU e.g. soy beans and products hereof from South America (Hald *et al.*, 2006).

Pig manure will inevitably contaminate the farm surroundings and studies have shown that wildlife in and around farms frequently share microorganisms with the pig population. Inadequate biosecurity may therefore contribute to maintain a herd infection, and boots, clothing and tools can be vectors for introduction of infection unless properly cleaned or changed before entry to the herd.

Other vehicles of introduction are cars, tools, pets (cats, dogs with access to the herd) and wildlife such as rodents, birds, and insects (Lo Fo Wong & Hald, 2000).

Once introduced, *Salmonella* may establish and multiply in the gastrointestinal tract of susceptible pigs. From here it can be passed on to pen mates, neighbour pens and eventually to the entire herd. Sows may pass the infection on to their offspring, but often piglets weaned to cleaned and disinfected pens avoid the infection (Dahl *et al*., 1997). If continuous production is practiced, transfer of *Salmonella* via faecal material may maintain the infection in the herd. As *Salmonella* can survive in the herd environment, insufficient cleaning of pens and equipment between batches can lead to transmission of *Salmonella* to the following batches of pigs. (Lo Fo Wong *et al.*, 2002).

When the slaughter pigs reach the preferred weight (60-120 kg) in 4-5 months, they are transported by truck to the slaughterhouse.

Intervention against *Salmonella* in slaughter pig herds

- Purchase of *Salmonella* negative pigs is of particular importance in herds with low *Salmonella* infection level or no *Salmonella* infection.
- Batch (all in/all out) production enables the farmer to break infection chains between batches by cleaning and disinfecting the production sites prior to introducing new pigs.
- Feed can be formulated and treated to reduce survival and multiplication of *Salmonella,* once ingested, in the gastrointestinal tract by lowering pH and increasing the concentration of short-chain organic acids (Hansen, 2004). This may be obtained from coarse grinding of the feed, from using meal feed as opposed to pelleted feed, adding not heat treated grains to the feed ration or from feeding fermented wet feed to the pigs. Adding organic acids to the water or feed to achieve reduction of *Salmonella* in the gut has some effect as well.
- To reduce infection risk, codes for good farming practice should be followed: ensure daily routines starting in lower risk areas towards high risk areas i.e. from sections with young animal to older animals where the likelihood of an animals having been exposed to infection is higher.
- Other measures are to wash and disinfect hands continuously between infected and non-infected areas and clean/disinfect or change boots and change clothes on entry to barns
- To avoid in-farm spread by rearing pigs in smaller groups and avoiding physical contact between groups through sectioning and closed pen separations.
- To prevent *Salmonella* entering the feed and subsequently the herd, measures can be taken to transport and store the feed and feedstuff in clean environments, and implement heat treatment of the grain and/or acidification of the feed.

- Ensure high biosecurity by controlling the entrance of rodents, birds, insects, etc. and restrict traffic by personnel and pets.

Monitoring of *Salmonella* in pig herds

Infections with the zoonotic *Salmonella* serovars in pigs are usually subclinical, but in a few cases the infection may cause salmonellosis in pigs characterised by severe diarrhoea. The *Salmonella* counts in faecal material from pigs may vary from below detectable levels in carrier animals (with no proliferation of *Salmonella* in the gut) to typical counts below 10^{10} cfu/g in subclinically infected animals and counts exceeding 10^{10} cfu/g in animals with salmonellosis.

Monitoring the prevalence of *Salmonella* at the farm level has the advantage of avoiding between farm infection/cross-contamination, which can occur when sampling pigs or carcasses at the slaughterhouse. It is, however, often more laborious and resource intensive, and therefore typically applied only in breeding and multiplying herds with no continuous flow of pigs for slaughter. Pen-faecal samples and/or blood samples are the preferred material collected at the farm.

Once a herd is detected as infected, identification of the *Salmonella* serovar(s) and its distribution in the herd may point to the source of infection. On a national or regional basis, mapping of spread and shifts in *Salmonella* serovars in the primary production is important to assess the coverage of serological surveillance tools based on detection of antibodies against *Salmonella.* Finally, knowledge about the serovar distribution in pig herds may provide useful information for assessing the role of pigs and pork in human salmonellosis and for trace-back in investigations of human outbreaks.

In the last decade, monitoring programmes have been established in a number of EU MSs. Still only 9 MSs reported having monitoring of *Salmonella* in slaughter pigs at farms or slaughterhouses in 2006 (EFSA, 2007a). Most of these countries take measures as a consequence of infection or isolation of *Salmonella* in pig herds. Even less MSs reported to have monitoring of *Salmonella* in breeding and multiplying herds.

3.4.3 Transport and lairage

Sources and transmission of *Salmonella* at transport and lairage

Pigs are transported to the slaughterhouse in trucks either by professional transport companies or by the farmer. After arrival to the slaughterhouse pigs are held in pens in the lairage for varying length of time.

Transportation time may be relatively long in areas with more industrialised pig production, where large centralised slaughterhouses receive animals from distant farms. In high intensity production farms slaughter pigs are usually transported by truck separately from pigs from other farms. In less intensive production systems or in farm-slaughterhouse setups, pigs may have shorter transport distances, but as one truck may pick up pigs from several farms, the time spent on the truck may be prolonged. Poorly managed logistics may also prolong transportation time as well as the time spent in lairage.

The holding time in the lairage, preferably without any further mixing of pigs is important to ensure that the meat quality is not affected by transportation stress. On the other hand, the

lairage is a potential source of *Salmonella* exposure to the pigs, and increasing holding time will increase the risk of exposure and infection/contamination of the pigs.

Most often *Salmonella* infected pigs are subclinical carriers of *Salmonella* and will only intermittently excrete *Salmonella* bacteria in their faeces. Several studies have reported a significant increase in the number of pigs excreting *Salmonella* upon arrival at the slaughterhouse (Williams & Newell, 1970; Berends *et al.*, 1996; Rajkowski *et al.*, 1998). The reason for this increased shedding has not been entirely revealed. One explanation may be that *Salmonella* negative slaughter pigs during transportation to the slaughterhouse, may be infected from previously contaminated trucks that have not been thoroughly cleaned, or from *Salmonella* infected pigs loaded on the same truck (Williams & Newell, 1970; Rajkowski *et al.*, 1998).

There is no doubt that transportation of the pigs induces a stressful condition. During transportation, pigs are subjected to many stress factors e.g. noise, smells, mixing with "unfamiliar" pigs from other rearing pens or farms, high stocking densities, long duration of transport, change of environmental temperature and a general change of environment (Warriss *et al.*, 1992). The stress is known to increase defecation in the pigs and thus add to contamination of the environment from infected pigs. But stress may as well induce carriers to shed *Salmonella* at a higher rate or increase the susceptibility of *Salmonella*-free pigs to infection (Williams & Newell, 1970; Gronstal *et al.*, 1974; Mulder, 1995). The influence of stress on the shedding of *Salmonella* is still a subject for discussion.

Intervention against *Salmonella* at transport and lairage

- Physical separation of herds (and faecal material) during transport and lairage will prevent the spread of *Salmonella* between herds
- Clean trucks for transport and pens at lairage prior to introduction of new pigs.
- Reduce transportation time and holding time in the lairage.
- Reduction of physical and psychical stress, by careful handling and transport, by transport and holding of pigs in groups with familiar individuals. The effect of reduced stress remain to be thoroughly documented.

3.4.4 Slaughterhouse

Practices of pig slaughtering differs both within and between MSs, from small slaughterhouses with a few employees to large slaughterhouses that are highly automated; slaughtering thousands of animals per day. Evidently, practices and use of equipment varies significantly as do codes of practice, preventive measures, and monitoring schemes.

Sources and transmission of *Salmonella* in the slaughterhouse

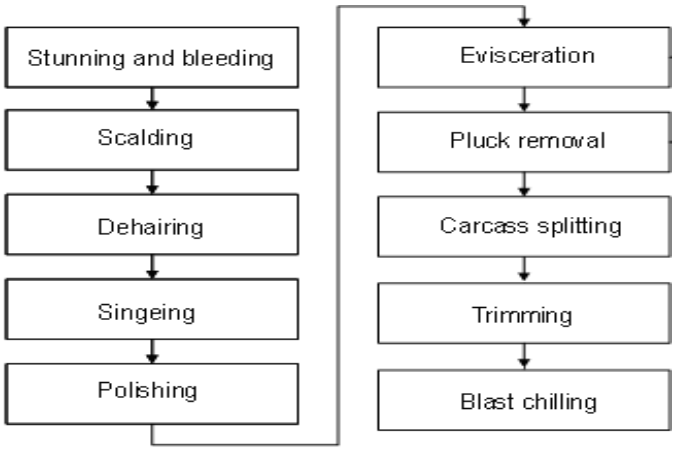

Figure 3.4: Example of a slaughter line in an abattoir

Figure 3.4 illustrates the principal construction and flow of in a typical pig slaughterhouse. ´From the lairage, the pigs are led to the stunning unit, after which they are bled and submerged in the scalding tank, where superficial skin destruction will loosen the hairs to enable the following dehairing process.

The scalding may reduce the *Salmonella* counts on the carcass surface somewhat, but even at recommended temperatures *Salmonella* can be isolated from scalding water (Chau *et al.*., 1977; Gill & Bryant, 1992; Gill & Bryant, 1993; Hald *et al.*., 2003). In particular if the temperature of the water drops below the recommended 62 °C, the water will contribute to cross-contamination of inner and outer surfaces of the carcass. At even lower temperatures the water may even serve as a growth media for *Salmonella* (Mølbak *et al*., 2006). *Salmonella* in larger coherent masses of faecal or other organic material released into the scalding water may not be killed even at proper water temperatures.

The dehairing machines are prone to contamination and are difficult to clean. The dehairing process can result in transfer of bacteria from an infected animal to an otherwise clean animal. At singeing, the outer surface of the carcass is exposed to high temperatures resulting in superficial decontamination and destruction of the skin. Certain areas of the carcass will be less exposed to the heat than others. Following singeing the carcass will be scraped in the polishing device constructed to remove the superficially burnt skin.

The dehairing and polishing may result in release of contaminated fluids and faeces from rectum, the oral cavity and the respiratory tract out onto the surface of the carcass, and the equipment may add to cross-contamination of the following carcasses. In a study of

European slaughterhouses, Hald *et al*. (2003) found the polishing process to be significantly associated with carcass contamination, and the study indicated that *Salmonella* may persist in the polishing machine for longer periods of time.

During the evisceration- and pluck removal processes, interior and exterior surfaces of the carcass will frequently be contaminated with intestinal contents either direct or indirectly from the tongue and tonsil area even if the procedures have been performed correctly. Hald *et al.*. (2003) found the pluck removal to be a significant risk factor for carcass contamination especially if the scalding tank was contaminated. The use of special measures to avoid contamination of the carcass from rectum during evisceration has proven very efficient.

Splitting of the carcass, may also contribute to contamination and cross-contamination. The saw and screens around it are difficult to clean, and considerable amounts of wet organic matter (saw ”dust”) is a potential source for growth and spread of *Salmonella* unless handled properly.

Any handling at the slaughterhouse, even trimming and traditional manual meat inspection will contribute to the contamination level on pig carcasses. In general, the contamination level on carcasses tend to be low or modest, but where accidents (e.g. puncture of intestines) have occurred, high levels of faecal contamination may be present on a number of carcasses following the accident.

Traditional small slaughterhouses tend to have more manual handling by slaughter personnel and more contact with surfaces (e.g. carcasses lying on tables or floor vs. hanging). Cleaning and disinfection is non-automated and is only carried out at the end of the day or a few times during the day. Some MSs have special slaughter procedures e.g. de-hiding and decapitation.

After dressing, the carcasses are chilled and stored at low temperatures. Many larger slaughterhouses use blast chilling of the carcasses, resulting in a superficial freezing of the skin. This freezing is not likely to influence the counts of *Salmonella* on the carcasses, as it is short lasting and *Salmonella* is relatively resistant to freezing.

Intervention against *Salmonella* at the slaughterhouse

- Batch-type slaughter, separating herds on the slaughter line.
- Logistic slaughter with special/separate handling of pigs from high risk *Salmonella* herds (high shedding)
- Temperature monitoring and alarm for scalding water and during cold storage.
- Singeing at 1300-1500 °C
- Cleaning and disinfection of equipment between batches and carcasses (knives, robots, conveyer belts etc.).
- Controlling contamination from evisceration processes: use of bung bags or similar device when removing intestinal system, careful evisceration to avoid accidental puncture of the intestine and separation of the plucks from the rest of the cutting process.
- Removal of the head before splitting of the carcass to avoid cross-contamination from the oral cavity in particular for animals from highly infected herds.
- Bacteriological end-point monitoring of *Salmonella* on carcasses, and procedures to follow up in case of increasing carcass prevalence at slaughterhouses
- Training of slaughterhouse personnel

Monitoring of *Salmonella* at the slaughterhouse

Data from monitoring of *Salmonella* at the slaughterhouses may be used for detection of animal or herd infection status, for controlling slaughter hygiene, for tracing of foodborne outbreaks, and to obtain a serovar collection from pigs or pork.

According to EFSA (2007a), approximately half of the EU MSs have implemented bacteriological monitoring programmes for *Salmonella* in pigs and pig meat at slaughterhouses or cutting plants.

At slaughter *Salmonella* from gut contents of infected animals will frequently contaminate the inner and outer surface of the carcass, and *Salmonella* may be isolated from tonsils and gastrointestinal lymph nodes, and from meat samples after cutting. By far the majority of monitoring schemes are based on culture for *Salmonella* in surface/carcass swabs. But also culture of lymph nodes or meat samples as well as serological monitoring for herd status are used routinely.

Depending on the purpose of sampling and the slaughterhouse design, different points of sampling may be preferred.

- The faeces of the pigs at the start of slaughter – indicate the introduction of *Salmonella* to the slaughterhouse, but do not provide information about the level of contamination and cross-contamination and thus the bacteriological status of the end product.
- The skin of the pigs at the start of slaughter – does primarily reflect the infection in the intestine of the pig and in the herd of origin, but also cross-contamination during transport and lairage.
- Lymph nodes – indicate that the pigs have been infected with *Salmonella*, but does not provide information about actual faecal shedding
- The carcass during the slaughter process – reflects both the infection in the intestine of the pig and in the herd of origin, and the cross-contamination and hygienic procedures in previous steps from transport until sampling
- Environmental sampling (walls, floors, equipment, etc.) – reflects the load of *Salmonella* from delivered pigs as well as the hygiene at the sampling place
- Meat cuts – reflects the infection in the intestine of the pig and in the herd of origin, but also cross-contamination and hygienic procedures in previous steps from transport until sampling. May also serve as an indicator of consumer exposure.
- Meat juice - can identify *Salmonella* carrier pigs or pigs already cleared of infection. In early phases of infection animals may be sero-negative despite considerable faecal shedding. Serology is mainly recommended for determining herd infection status.

3.4.5 Processing and retail

Sources and transmission of *Salmonella* at processing and retail

Though the processing and retail levels of pork production are very much depending on the quality of raw materials and products that entering, they too have a responsibility towards the quality of the end product and prevent contaminated products to reach the consumer. Three main factors which influence the microbiologically quality of meats are handling, time and temperature. Hygienic and sensible handling of raw materials is vital to successfully avoid cross-contamination between products, whereas time and temperature abuses may

create situations that support survival and propagation of microorganisms that may be present in foods.

At the processing and retail levels, large quantities of raw meat of different origin are handled closely together. There may be half carcasses and different size cuts of various pathogenic status present during processing and, moreover, meat from different types of production animals, creating numerous opportunities for cross-contamination or spread of pathogenic microorganisms.

Temperatures experienced during storage and display will affect the product storage/shelf life. If the temperature of meat and meat products is kept sufficiently low (below 6 °C) during storage and transport to and from the whole sale, growth of *Salmonella* can be kept to a minimum. However, retail display is possibly the weakest link in the commercial cold chain (James & Bailey, 1990), adding to the concern that *Salmonella* may proliferate to hazardous numbers during periods of temperature abuse in display cases.

Pork that is processed into special products may be preserved in order to enhance the microbiological stability. Such preservation approaches include acidification, fermentation, curing, smoking, heating, etc. Important parameters on which these preservation methods are based include: control of initial numbers of bacteria, pH, water activity, microbial competition/interaction, preservatives, oxidation reduction potential, temperature and radiation (Genigeorgis & Sofos, 1999). Preservation of the pork products is done to extend the shelf life, but the shelf life will also depend on the initial bacterial load, since the probability of e.g. *Salmonella* to survive in a hostile environment and grow to infective levels is a function of the initial numbers present. So for processed ready-to-eat products, it is imperative that the raw materials are of good microbiological quality.

For fresh pork products, there are no preservation (except for cooling) or decontamination steps at the processing and retail levels. This means that the amount of contaminated fresh product in a batch of cuts and carcasses at best will remain the same. Whereas the consumer level has been described as the last line of defence two decades ago (WHO, 1980), it is important to realise that the retail level is the last 'check-point' at which contaminated end products can be identified.

Intervention against *Salmonella* at processing and retail

As fresh pork products (per definition) are not decontaminated in any way, only preventive measures are optional for minimising the *Salmonella* contamination in the meat.

- Storage below 6 °C at all times (transport from slaughterhouse/cutting plant, storage and display)
- Limit storage time and shelf life according to the risk of breaks in the cooling chain
- Maintain clean environment
- Maintain high hygienic standards amongst personnel
- Educating the people handling the food

Monitoring *Salmonella* at processing and retail

Most processing plants and many retailers have established HACCP programmes, with standardised hygienic routines and processing procedures, and regular monitoring of e.g. storage and display temperatures. Product and equipment samples may be taken to monitor the effect of the hygienic precautions and/or to observe increases in *Salmonella* contaminated products. The number of samples to be taken depends on the prevalence of *Salmonella* and the level of safety required.

3.4.6 Preparation and consumption

The final step in the production chain is when the consumer brings the product home, stores the product and handles the product during preparation of meals.

Retail is the last step where reduction and prevention of contamination can be controlled by food authorities. The responsibility for proper and safe handling of the food in the final steps of the production chain is handled over to the consumer.

Sources and transmission of *Salmonella* at preparation of food

Salmonella prevalence at the consumer level depends on the *Salmonella* status of the meat at retail. If the meat is contaminated at the retail level and brought to home without proper cooling during transport and in-house storage growth may result in *Salmonella* concentrations above the human response level. With the exception of minced meat, only the surface of the meat is contaminated *Salmonella* as opposed to the normally sterile inner parts of the meat. If the meat undergoes sufficient heat treatment of the surfaces during cooking, the risk for acquiring a *Salmonella* infection from the meat is negligible. An exception is minced meat, for which thorough heat treatment is recommended.

Cross-contamination from pork products to ready-to-eat food e.g. salads or bread can occur in the household by transmission of *Salmonella* via raw meat juice from surfaces, equipment, and personnel carrying the bacteria, especially in kitchens where large amounts of food are being prepared and with continuously ongoing food preparation without cleaning at frequent intervals.

Intervention against *Salmonella* at preparation of food

- Disinfection of hands, cutting board, and equipment with soap and detergent
- Inactivation by cooking (raised temperature), smoking, salting (lowering water activity)
- Cold storage below 6 °C during transport from retailer and during storage. Controlled temperature during thawing
- Re-cooling of leftovers immediately after the meal
- Separate meat used for food meant for raw consumption and other medium or high risk products (e.g. minced meat)

3.4.7 Monitoring and surveillance of *Salmonella*

The basis for control programmes is monitoring the prevalence of the agent at different part of the farm-to-consumption chain. Monitoring provides information on the current status as well as trends in MSs and the EU and is an effective tool to evaluate implemented interventions. Additionally, monitoring and especially surveillance systems are used to detect disease problems and infections rapidly and respond in a timely fashion. Monitoring also produce data for scientists to design efficient surveillance systems, evaluate existing systems and ultimately produce recommendations based on solid evidence. Data generated from monitoring of different aspects of food production are used in the present project to parameterise the mathematical models being developed.

Detection methods

Two monitoring options are available for evaluation of the prevalence of *Salmonella*: bacteriology and immunology (EFSA, 2006).

The bacteriological methods express the actual infection status of the animal, including transmission or recent contamination. The actual infectious agent or agents will be isolated, which makes further characterisation of e.g. serovar and antimicrobial resistance profiles possible, and combined with enumeration methods, the microbial load (e.g. cfu/g) can be determined. However, the analytical procedure is laborious. The sensitivity of bacteriological culture can vary according to the type and contamination level of the material from which culture is attempted. The examination of individual faecal samples from pigs can have poor sensitivity. For faecal samples the sensitivity has been reported to vary between 9% (cotton swabs) and 78% (25g faeces) (Funk *et al*., 2000) and 10-80% (Hurd *et al*., 2001). Compared to faeces, lymph nodes and meat have a lower level of competitive flora, and *Salmonella* will, even when present in low numbers, be more readily isolated from such materials.

The immunological methods express a previous exposure to the infectious agent by detecting specific antibodies against *Salmonella*. The method can identify carriers or animals already cleared of infection. It detects only those serogroups included in the test and therefore newly emerging serovars may not be detected. The method can be automated, and it is less laborious, and *Salmonella* antibodies in pigs can be detected in blood serum (Nielsen *et al*., 1995) as well as meat juice (Nielsen *et al*., 1998). The sensitivity at individual level has been reported to be 80-90% (Nielsen *et al*., 1995; Chow *et al*., 2004), but depends on many factors, as described above. In reality the sensitivity may, however, be lower. For example, for modelling purposes, the minimum sensitivity of the Danish *Salmonella* mix-ELISA was assumed to be as low as 50% (Alban *et al*., 2002). The specificity of the ELISA test is defined as its ability to correctly identify as sero-negative, i.e. not infected, those pigs that do not have antibodies against the *Salmonella* serogroups incorporated in the test. It can be assumed that the specificity of the *Salmonella*-ELISAs is high at the scientific cut-off (van der Heijden *et al*., 1998).

Both methods need to be characterised and harmonised to enable comparison of data from different sources (e.g. MSs). Quality assurance has to be applied in order to produce results that can be compared with confidence between laboratories/countries. Results obtained using bacteriological methods and immunological methods, for the reasons stated above, cannot be compared directly.

Conventional bacteriological isolation methods are costly and time consuming. Therefore, much effort has been made to develop rapid methods for the detection of *Salmonella*. In general, the principle of such alternative methods is to enable a rapid screening of all samples by which means the suspect positive samples can be identified. The screening performed in these alternative methods can be either immunologically based or Polymerase Chain Reaction (PCR) based. In the former test only certain serovars will be detected, while in the latter all serovars will be detected. Before use, however, alternative methods have to be formally validated in relation to the specific material to be sampled and tested in the course of the investigations/surveillance. DNA microarray-based methods potentially address identification of family, genus, species, subspecies, strain and genotypic characterisation, as well as the presence of several crucial genetic markers such as those coding for antibiotic resistance and virulence.

3.4.8 Prevalence at different stages of production

Occurrence of *Salmonella* is monitored by sampling at different locations throughout the production process and the findings are reported to EFSA on an annual basis. Table 3.1 shows the reported number of samples taken from fresh pig meat in 2006 in EU MSs. The majority of samples are taken at the slaughterhouses (carcass samples), cutting plants, and at retail. The MSs listed in the table are the only countries that reported figures for 2006. Table 3.2 lists the results from samples of minced meat taken at the processing plant or at retail. For a substantial number of samples, the sampling location was not stated.

By comparing the reported serovar distributions, it can be seen that several serovars are commonly found in both pigs (Table 3.5), pork (Table 3.6), and humans (Table 3.7). However, to make interpretation of the role of pigs and pork as a source to human salmonellosis, serovar data on other major sources such as poultry and poultry products are needed. In Chapter 14, available serovar data from food-animals, food and humans were analysed with the purpose to estimate role of pork.

3.4.9 Diversity of pork production in the EU

The structure of pig producing units varies greatly in the EU. Some MSs have almost entirely large pig producing farms illustrated in Figure 3.5 (EuroStat 2007b). It is evident from the figure that for example Poland and Romania belongs to a category with many small holding and from the raw data it can furthermore be seen that Poland also has a number of very large holdings indicating that Poland not only has small scale non-intensive production but also intensively driven units.

An evolution from many small holding towards larger and more intensive units has been the trend for most countries during the last decades, particularly the countries with very high production figures (EFSA 2006, EuroStat 2008, Fowler 2004). It should be expected that MSs with low intensive production systems will join this trend unless development be skewed by subsidies.

Some MSs are major producers of pork products in the EU, mainly large countries and countries specialised in pig production. Import and export data furthermore describes Germany, Spain, Denmark and The Netherlands as major suppliers to many MSs. It seems like there are different structures of import amongst eastern and western countries. Countries in the east tend to have a higher degree of import from other eastern countries whereas EU 15 mostly imports from Germany and Netherland or a neighbour. This trend may be due to traditions, import restrictions or to a “neighbour effect”. Even though this pattern might be true there is a large trade between east and west.

Table 3.3: *Salmonella* in fresh pig meat (Adapted from EFSA 2007a)

	2006		2005		2004		2003		2002	
	N	% Pos	N	% Pos	N	% Pos	N	% Pos	N	% Pos
Pigs (sample based data) - carcass swabs - at slaughterhouse										
Belgium[1]	-	-	442	9.3	374	12.3	287	14.6	298	15.4
Denmark[2]	27,892	1.9	30,730	1	33,890	1.3	34,250	1.4	36,690	1.4
Estonia	683	0.1	671	0	648	0	-	-	-	-
Finland	6,454	0	6,609	0	6,576	<0.1	6,186	<0.1	6,260	<0.1
Sweden	3,151	0	5,764	<0.1	594	0	6,281	0	6,420	<0.1
Spain	297	6.4								
Norway	3,122	0	3,157	0	2,456	0	2,947	0	2,615	0
Fresh pig meat at cutting plants										
Belgium[1]	328	2.4	307	7.2	374	12.3	278	6.1	224	11.2
CzechRepublic[3]	4,077	0.2	2,445	1.9	-	-				
Estonia	351	0	457	0	442	0.2	-	-	-	-
Finland [1]	2,311	0	3,226	0	3,092	0	2,826	0.1	1,840	0.1
Slovenia[1]	159	0	113	0	188	0	-	-	-	-
Spain	88	0	263	4.9	-	-				
Sweden[6]	-	-	4,119	0	4,474	0	4,411	0	4,478	0
Pig meat at retail										
Belgium[4]	-	-	155	6.5	166	12.7	181	9.4	184	13.0
Latvia[5]	-	-	47	0	30	0	-	-	-	-
Sweden[6]	-	-	1,052	0.3	1,262	0	1,272	0.4	1,125	1.9
Spain	227	11.5								
EU Total	**4,6018**	**1.3**	**56,400**	**0.8**	**52,110**	**1.0**	**55,972**	**1.0**	**57519.0**	**1.0**

Table 3.4: *Salmonella* in minced meat, meat preparation, and product samples. (After EFSA 2007a)

	Description	Sample unit	Amount of sample /swabbing area	N	% Pos
At retail					
Spain	Meat product	Single	25g	367	3.3
At processing plant					
Ireland	Meat product	Single	Varies	3,509	1.3
Slovenia	Meat product	Single	25g	159	0
Spain	Meat product	Single	25g	713	2.2
Stage of sampling not stated					
Austria[2]	Meat preparation	Single	25g	90	2.2
Czech Republic	Minced meat	Batch	25g	26	0
Estonia	Meat preparation	Single	10g	110	0.9
Germany[2]	Minced meat	Single	25g	1,261	3.8
	Meat preparation	Single	25g	1,055	4.0
Hungary	Minced meat	Single	25g	2,777	2.7
	Minced meat	Single	10g	360	4.7
Italy	Meat product	Single	25g	1,094	2.9
Italy[2]	Meat preparation	Single	25g	1,509	2.8
	Minced meat	Single	25g	562	4.8
Luxembourg	Meat preparation	Single	25g	49	0
Netherlands	Minced meat	Single	25g	69	2.9
	Meat preparation	Single	25g	76	2.6
Poland	Meat product	Single	25g	4,672	0.5
Poland[2]	Meat preparation	Single	25g	2,116	0.7
	Minced meat	Single	25g	7,524	0.2
Portugal	Meat preparation	Single	25g	186	2.7
Slovakia	Meat preparation	Batch	25g	199	0.5
	Minced meat	Batch	25g	151	0
Sweden	Meat product	Single	25g	339	0
EU Total				**28,973**	**1.4**
Romania	Meat preparation	Single	25g	123	0
	Meat product	Single	25g	1,038	0.1
	Minced meat	Single	25g	1,080	1.5

Table 3.5: Ranking of the ten most reported serovars in pigs. (After EFSA 2007a)

	No. of isolates serotyped	S. Typhimurium incl. var. Copenhagen	S. Derby	S. 1,4,5,12:-:i	S. Anatum	S. group B	S. Livingstone	S. London	S. Enteritidis	S. Infantis	S. Brandenburg	Other serotypes
EU total, No. of isolates	2,253	1,235	265	96	77	43	36	31	31	17	14	408
Austria	26	0	15.4	0	0	0	0	0	3.8	3.8	0	76.9
Belgium	271	68.3	8.9	0	1.1	4.1	1.8	1.1	2.2	1.8	2.6	8.1
Czech Republic	36	69.4	11.1	0	0	0	0.0	0.0	5.6	5.6	0	8.3
Estonia	10	10.0	0	0	0	0	0	0	50.0	0	0	40.0
Finland	6	83.3	0	0	0	0	0	0	16.7	0	0	0
Germany	644	79.3	3.4	0	0.2	4.8	0.3	4.2	0.9	0.6	0.5	5.7
Greece	13	15.4	23.1	0	0	0	0	0	7.7	0	0	53.8
Hungary	27	40.7	40.7	0	0	0	0	0	7.4	0	0	11.1
Italy	807	25.5	18.8	10	8.9	0	2.6	0.0	0.4	0.0	0.0	34.0
Lithuania	11	0.0	27.3	0	0	9.1	0	0	0	0	0	63.6
Luxembourg	96	54.2	27.1	14.6	0	0	0	0	1.0	0	0	3.1
Netherlands	140	60.7	9.3	2.9	0.7	0	5.7	0.7	0.7	2.9	2.9	13.6
Poland	1	0	0	0	0	0	0	0	0	0	0	100
Slovakia	11	27.3	27.3	0	0	0	0	0	9.1	0	0	36.4
Slovenia	5	60.0	0	0	0	0	0	0	20.0	20.0	0	0
Sweden	10	60.0	0	0	0	0	0	0	0	0	0	40.0
United Kingdom	140	100	0	0	0	0	0	0	0	0	0	0
% EU		**54.8**	**11.8**	**4.3**	**3.4**	**1.9**	**1.6**	**1.4**	**1.4**	**0.8**	**0.6**	**18.1**

Table 3.6: Ranking of serovars samples taken from pork after slaughter. (After EFSA 2007a)

	No. of isolates serotyped	S. Typhimurium	S. Derby	S. 1,4,5,12:-:i	S. Rissen	S. Infantis	S. London	S. Enteritidis	S. Bredeney	S. Kentucky	S. group B	Other serotypes
EU Total, No. of Isolates	**1,454**	**525**	**368**	**102**	**69**	**50**	**48**	**29**	**24**	**8**	**7**	**224**
Austria	6	16.7	16.7	0	0	0	0	50.0	0	0	0	16.7
Belgium	11	27.3	72.7	0	0	0	0	0.0	0	0	0	0
Czech Republic	10	30.0	50	0	0	0	0	0.0	0	0	0	20
Denmark	156	38.5	23.1	0	0	6.4	0	0.0	0	0	0	32.1
Estonia	4	75.0	0	0	0	0	0	0.0	0	0	25.0	0
France	284	50.4	40.8	0	4.93	3.9	0	0.0	0	0	0	0
Germany	57	57.9	15.8	0	0	3.5	3.5	0.0	0	0	10.5	8.8
Hungary	129	41.1	18.6	10.1	0	20.9	5.4	0.8	0	0	0	3.1
Ireland	89	52.8	19.1	0	0	0	3.4	0.0	4.5	9.0	0	11.2
Italy	636	24.1	23.11	14.0	7.7	0	5.7	0.6	2.8	0	0	22.0
Latvia	16	0	6.3	0	0	0	0	93.8	0	0	0	0
Netherlands	14	50.0	14.3	0	0	0	0	7.1	0	0	0	28.6
Poland	5	80.0	0	0	0	0	0	0.0	0	0	0	20
Portugal	4	0	0	0	25	0	0	25.0	0	0	0	50
Slovakia	5	20.0	20	0	0	0	0	0.0	40	0	0	20
Slovenia	1	100	0	0	0	0	0	0.0	0	0	0	0
Spain	28	46.4	3.6	0	17.9	0	0	14.3	0	0	0	17.9
% EU		**36.1**	**25.3**	**7.0**	**4.7**	**3.4**	**3.3**	**2.0**	**1.7**	**0.6**	**0.5**	**15.4**
Norway	1	0	0	0	0	0	0	0.0	0	0	0	100
Romania	48	4.2	52.1	0	0	6.3	6.3	6.3	4.2	2.1	0	18.8

1. Include isolates from fresh meat, meat products, meat preparations and unspecified meat

Table 3.7: Ranking of serovars in humans (Community Summary Reports 2005-2008)

Serovar	Year							
	2005 (N=23 MS + 2)		2006 (N=24 MS + 4)		2007 (N=26 MS + 3)		2008 (N=26 MS + 3)	
	N	%	N	%	N	%	N	%
S. Enteritidis	86,536	53.7	90,362	71.0	81,472	64.5	70,091	58.0
S. Typhimurium	15,058	9.3	18,685	14.7	20,781	16.5	26,423	21.9
S. Infantis	1,354	0.8	1,246	1.0	1,310	1.0	1,317	1.1
S. Bovismorbificans	621	0.4	-	-	-	-	501	0.4
S. Hadar	577	0.4	713	0.6	479	0.4	-	-
S. Virchow	535	0.3	1,056	0.8	1,068	0.8	860	0.7
S. Derby	259	0.2	477	0.4	469	0.4	624	0.5
S. Newport	245	0.2	730	0.6	733	0.6	787	0.7
S. Stanley	-	-	522	0.4	589	0.5	529	0.4
S. Agona	-	-	367	0.3	387	0.3	636	0.5
S. Anatum	179	0.1	-	-	-	-	-	-
S. Goldcoast	173	0.1	-	-	-	-	-	-
S. Kentucky	-	-	357	0.3	431	0.3	497	0.4
Other	55,619	34.5	12,790	10.0	18,562	14.7	18,495	15.3
Total	161,156		127,305		126,281		120,760	
Unknown	56,619		17,359		9,814		6,636	

The variation in pork production in the EU described above constitutes challenges to the modelling of microbial risks. In Chapter 6, we attempt to address this by grouping MSs by their production systems and figures (a cluster analysis), thus simplifying modelling procedures.

3.5 Data Sources

Human salmonellosis is a notifiable disease in all EU MSs, but there is a great variation in when and how cases should be reported to central registers. Some MSs only report cases that have had more than one week of diarrhoea or are hospitalised cases whereas other MSs report more readily.

There are as many sampling locations as there are steps in the food production chain and differences in testing locations and procedures complicates the comparison and use of data. This is a challenge to the parameterisation of the risk models which must be taken into account when employing the models for decision making and a factor to consider in variation and sensitivity/uncertainty analysis.

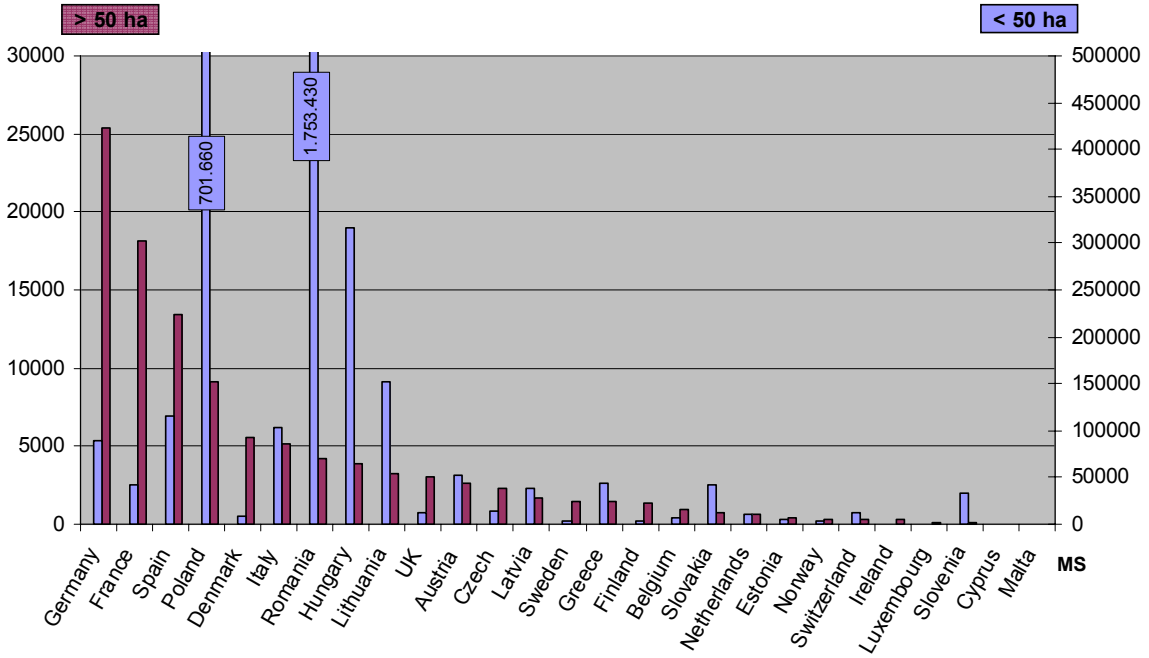

Figure 3.5: Number of pig producing farms less or more than 50 ha. Some MS have comparably high numbers of farms less than 50 ha indicating that they have small and low intensive farming.

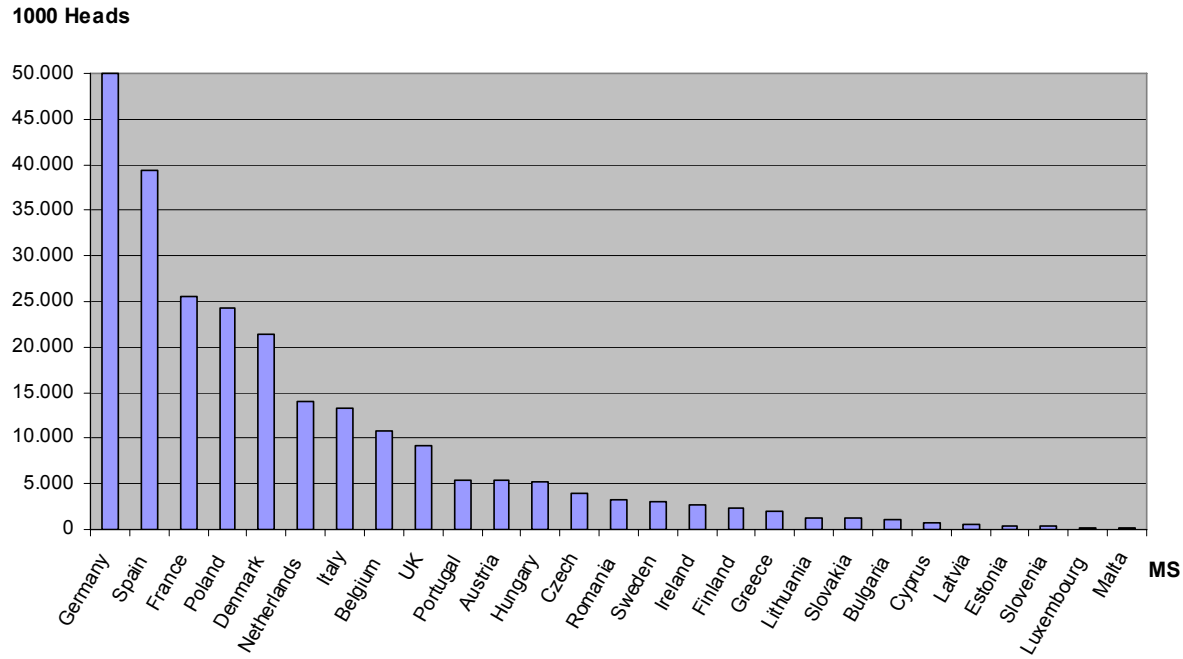

Figure 3.6: shows the MS specific amount of slaughtered pigs in 2006. The total number for the EU 27 is 237 million. (EuroStat 2007a).

3.5.1 Reporting of data on human salmonellosis

TESSy (The European Surveillance System)

The TESSy database is hosted by ECDC and collects data from all MSs on human cases of salmonellosis and the responsible serovars. Aggregation and comparison of MS specific prevalence is difficult because case definitions, reporting requirements, surveillance systems, and microbiological methods are not necessarily consistent across the EU.
The data base provides data on:

- Total number of cases
- Incidence
- Age groups (0-4, 5-14, 15-24, 25-44, 44-64, ≥ 65)
- Seasonality (monthly number of cases)
- Travel associated cases (including country of origin when possible)
- Distribution of cases according to serovars
- Distribution of cases according to phage types

TESSy data are aggregated cases of salmonellosis that has been approved by each MS, while Enter-Net data may come directly from reference laboratories or from epidemiologists.

Enter-Net

Enter-Net is a laboratory and epidemiologic surveillance network for *Salmonella* (and other Zoonoses). The objective is to increasingly integrate Enter-Net into TESSy.

The database provides data on:

- Total number of cases
- Incidence
- Age groups (0-4, 5-14, 15-24, 25-44, 44-64, ≥ 65)
- Seasonality (monthly number of cases)
- Travel associated cases (including country of origin when possible)
- Distribution of cases according to serovars
- Distribution of cases according to phage types
- Antimicrobial resistance in *S*.Typhimurium

Enter-net has been the most used reporting system but TESSy is likely to be the system used in the future because less variables have to be entered and the software reporting platform is easy to use.

Resource: http://www.ecdc.europa.eu/Activities/surveillance/ENTER_NET/index.html

BSN (Basic Surveillance Network)

BSN started in 2000 and provides easy access to case based data on salmonellosis and a list of 40 diseases. Participants of the network have access to an internal web site were all the data are presented in tables and graphs.
The data base provides data on :

- Total number of cases
- Incidence
- Date of onset
- Age

- Gender
- Immunisation status

Resource: http://ecdc.europa.eu/Activities/surveillance/BSN.html

3.5.2 Reporting of data on *Salmonella* in feed, food-producing animals and food in EU

Monitoring and/or surveillance programmes are implemented in all MSs, by decree of directive (2003/99/EC2), and are reported to EFSA on an annual basis (Figure 3.6). Each year, EFSA prepare a Community Summary Report (CSR) presenting and discussing the trends and sources of zoonotic diseases in EU (e.g. EFSA 2007a; EFSA 2009). There are differences in how the MSs monitor and report findings of *Salmonella* making it difficult to compare data between MSs and often also from year to year within the same MS. Guidelines on harmonising the reporting have recently been drafted by EFSA (EFSA 2007b). Currently, data input for monitoring programmes coming from samples taken at different locations in the food chain depends on the sampling protocol in the respective MS: routine sampling at slaughterhouses, notifiable diseases reported by veterinarians, sampling in the transport and lairage link, self testing at retailers etc. Furthermore, monitoring efforts can be scaled up and down at any part of the food chain should increased *Salmonella* prevalences occur.

Feed

Data on *Salmonella* in feed stuffs in MSs is derived from different surveillance programmes and not all MSs report each year. Data are, therefore, not comparable between MSs and cannot be considered as national prevalences. Results, including the serovar, of sampling both feeding material and compound feeding stuff are included The results generally indicates that fish meal and oil seeds like soybean, rape, sunflower and products thereof, probably are the most likely sources of *Salmonella* in animal feed.

Animals

Data on *Salmonella* prevalence at different stages of the pork production is provided in the CSRs and includes samples taken from faeces, blood, meat juice, litter, feed, lymph nodes, pen faecal samples, and carcass swabs, and represent both fattening herds at farm level and fattening herds at slaughter.

Food

Salmonella in foodstuffs is reported to EFSA from MS monitoring programmes. The reporting is mandatory as of 2008. Data are taken from different sources across the EU: official monitoring, targeted sampling, random sampling, monitoring, targeted and routine sampling, and own control in the industry. Samples are: part of product (minced meat, fresh meat), surface swabs from the meat, and environmental swabs. The database provides data on serovar and phage types.

3.5.3 Consumption data

The EuroStat database provides numbers on amount of consumed pork and products thereof in terms of production numbers.
The database provides data on:

- Pigs meat kg/head
- “Other fresh or chilled pig meat” (Kg)
- “Frozen carcases and half-carcases of pig meat” (Kg)

- "Frozen ham" (Kg)
- "Ham"
- "Bellies and cuts thereof: salted; in brine; dried or smoked" (Kg)
- "Pig meat in brine, dried or smoked" (Kg)
- "Preparation of pork (incl. fats)"
- Amount consumed (kg) (EuroStat, 2007d)

3.5.4 Other data sources

EU baseline study

The European Commission has initiated activities for collection of comparable data on *Salmonella* prevalence in food producing animals in order to provide a baseline for setting *Salmonella* targets in specific animal productions. The report from the slaughter pig baseline survey was published in 2008 (EFSA, 2008b; EFSA 2008c), whereas the draft report from the breeding pig survey was still under preparation when finishing this report. However, the preliminary results were made available for the project and were used in the QMRA.

Data call

A call for data specifically targeting the data required for this risk assessment was developed by the project team and published on the EFSA website in March 2008. The call was advertised widely via EFSA, EU and project networks, e.g. via MS zoonoses representatives. The call was published at: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178696473049.htm. Further clarification on the call for data was provided at the data workshop and ultimately over half of EU MSs (15/27) provided full responses, including published and unpublished data, papers, opinion and statistics. With the exception of two MS, all responses were submitted online using the EFSA website. The project team then used the information, data and questionnaires within the project, collaborating with respondents to clarify issues or elicit further opinion.

Data workshop

A data workshop organised by the project consortium was held in Copenhagen in April 2008. Invited experts identified or brought with them available data on, for instance farming and production practices, prevalence and concentrations figures in pigs and pork, and data on human consumption of pork products. During the workshop Excel spread sheets provided for the working groups were filled in with available data or by expert opinions as given by the participants. Details of the outcome and discussions can be found in the Workshop proceedings, which were distributed to all participants after the workshop and can be obtained by request to the consortium.

Literature

Relevant research, surveys and interventions studies published in the international peer-reviewed literature were used as appropriate.

Expert opinion

Expert opinion was used when no other relevant data was available. Experts were scientists attending the workshop held in April 2008 and scientists employed at the institutes in the consortium, but not directly part of the work. Due to time constraints, no formal expert elicitation was undertaken.

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4 Model Framework

This Chapter describes, in some detail, the framework of the QMRA in which constituent modules of the model are embedded. In particular, special attention is given to the inputs and outputs of each module.

The model uses Monte Carlo sampling as a means for dealing with variability in the parameters. Thus, several iterations are run until the results (probabilities of illness) have converged. It should be kept in mind that the discussion of Section 4.1 concerns one iteration only, the final result is the average of the results of many iterations (Section 4.1.7).

In the following sections we briefly describe how the modules are linked together, starting at the farm and resulting in a probability of illness.

4.1 The QMRA Modules

4.1.1 Farm

Inputs

The Farm module has no specific inputs related to Salmonella infection. However, it does have several parameters that determine the dynamics and sources of infection. Such parameters, however, are not what we consider to be inputs. The major input to each iteration of the farm model is the farm type (e.g. whether it uses wet feed, solid flooring), which is randomly sampled from a multinomial distribution, using the relative weighting of farm types taken from the EFSA baseline surveys and other data. This is determined for both the small and large farm models.

Model

Each iteration of the large and small farm model represents one farm, and the production of batches of slaughter-age pigs. A farm is run for 500 days, during which several opportunities arise for infection of the pigs (via sows, feed or external contamination). This infection of pigs may then lead to transmission of infection to other pigs. The model is described in detail in Chapter 7.

Unit

The basic unit is the lymph-node positive status of the pigs (together with the associated magnitude of shedding).

Outputs

For each iteration of the small and large farm model, the model output is the lymph-node status and the concentration of *Salmonella* in the faeces for each individual pig within the 72 large farm and 3 small farm batches sent to slaughter from each farm during each iteration. Thus, we have a two-dimensional array of numbers (which we refer to as the *farm matrices*).

The Farm module has an extremely long running time, in the order of days. Therefore, it was decided to run the farm model outside of the main code by default. The resulting farm matrices are written to a file, and may be read by the following modules.

4.1.2 Transport & lairage

Inputs

The Transport & Lairage module couples the farm to the slaughterhouse. For each iteration of the model, a days worth of pigs to be slaughtered in a "large" and "small" slaughterhouse are selected, where size relates to the number of pigs slaughtered per day (a small slaughterhouse slaughters up to 400 pigs a day while a large slaughterhouse slaughters more than 400 and as many as 15000 pigs a day, see Chapter 9). We assume (due to lack of data to the contrary) that pigs from large farms will go to large slaughterhouses and pigs from small farms will go to small slaughterhouses. The model accounts for variation between slaughterhouses. The farm matrices, as described above, are usually pre-calculated.

Model

For each iteration the model is first run for the small slaughterhouse and then independently for the large slaughterhouse. The selected slaughterhouse (large or small) is assigned a specified number (or 'capacity') of pigs to be slaughtered (determined by MS slaughterhouse capacity data). The model then randomly selects batches of pigs from the output of the Farm module, until the capacity of the slaughterhouse is reached. These batches of pigs then enter the Transport & Lairage model, where the transmission of *Salmonella* within these batches is modelled on an individual pig basis.

Increased shedding due to transport stress and infection via the environment (both at transport and lairage) may occur. The model is described in detail in Chapter 8.

Unit

The basic unit during transport and lairage is the lymph-node positive and gut contamination status of the individual pigs. The batch lymph-node positive prevalence is also calculated. Note that at lairage the batch may be split up into multiple lairage pens and thus the batch of pigs entering the slaughter line may be a subset of the batch of pigs that leave the farm. After lairage, the prevalence and concentration of *Salmonella* on the hides of individual pigs is also estimated.

Outputs

For each iteration of the model the output is the numbers of *Salmonella* in the gut of individual pigs at the end of lairage and the concentrations of *Salmonella* on the skin of individual pigs at the end of lairage. Data from batches of pigs that occupy the same lairage pen (at the same time) are grouped together in a vector. From this, a These batches are sorted in the order that the pigs enter the slaughter process to provide an ordered list of *Salmonella* numbers, in the gut and on the skin.

4.1.3 Slaughterhouse

Inputs

The large and small slaughterhouses take lists of *Salmonella* numbers, in the gut and on the skin, as in input from the Transport & Lairage module. Note that negative (i.e. no contamination or infection) pigs are not treated differently from positive pigs. Thus, the input lists contain many zeros for negative pigs.

Model
In the slaughter models, the pig and later the carcasses go through the slaughter line in the order that they are delivered from the Transport & Lairage module. At each phase one or more microbiological processes may take place, e.g. inactivation, partitioning, cross-contamination with the environment, etc. Due to this cross-contamination pigs may contaminate other pigs further down the line via slaughter machinery. Chapter 9 describes the slaughter model in detail.

Unit
At the start of the slaughter line the unit is 'pig', after slaughter 'carcass', and after the splitting phase 'half carcass'. For pigs and carcasses, *Salmonella* numbers are considered both on the skin and in the gut. At the point of half-carcasses, only *Salmonella* on the skin is taken into account, since the intestines are removed at the evisceration phase.

Outputs
The final output is the number of *Salmonella* on each half carcass (provided as a list). Necessarily, this list is twice the length of the input list of *Salmonella* on pigs.

4.1.4 The cutting plant

Inputs
We do not distinguish between cutting plants that accept half-carcasses from large slaughterhouses and cutting plants that accept half-carcasses from small slaughterhouses. Therefore, the inputs are two lists: the *Salmonella* numbers on the half-carcasses from the small slaughterhouse and the numbers on the half-carcasses from the large slaughterhouse (a much larger list).

Model
The cutting plant model has two main functions. Firstly, it combines the half-carcasses from both the large and the small slaughterhouse into three lists of half-carcasses. Each combined list contains the half-carcasses that will be processed into a specific food product: pork cuts, minced meat, or fermented sausage. The number of half-carcasses in each list is 10,000, corresponding to the 10,000 portions that will be produced. Each of the lists is populated by randomly sampling from the large and small slaughterhouse, choosing between them in such a way that the true ratio of productions from large and small slaughterhouses is approximated.

The second main function of the cutting plant is producing pork products from the half-carcasses. During this process, cross-contamination is taken into account. Note that the fermentation process is handled later in the model, at this point the portions of fermented sausage are produced in the same way as the minced meat portions (since this is the basic ingredient). The cutting plant is described in detail in Chapter 9 as it is part of the Slaughter & Processing module.

Unit
The unit changes from 'half-carcass' to 'portion of pork cuts/minced meat/minced meat for sausage production' during the Cutting Plant module. Also, portion sizes are taken into account, according to consumption data of the MS under investigation.

Outputs
For each of the three food products a list of 10,000 entries of *Salmonella* numbers is produced.

4.1.5 Preparation & consumption

Inputs
The output lists of the Cutting Plant module are the input to the consumer models, i.e. the number of *Salmonella* on each portion of pork cuts, minced meat and minced meat for sausage production.

Model
The consumer phase consists of three models that are parallel to each other; pork cuts, minced meat and fermented sausage are modelled independently. In each of the models there may be growth, inactivation or cross-contamination (not between products, but between product and environment, e.g. knife or salad). The fermented sausage is somewhat different from the other products in the sense that in the consumer phase the fermentation process is modelled (otherwise, the consumer phase would be empty as there is no preparation and the sausage is eaten raw). Chapter 10 describes the models in detail.

Unit
The units are consumer portions, for 10,000 of each of the products.

Outputs
The number of *Salmonella* ingested (i.e. the *dose*) for each of the 10,000 portions for each of the three products.

4.1.6 Dose-Response

Inputs
The input for the dose-response model is the number of *Salmonella* ingested from each of the products from the consumer phases.

Model
Using a dose-relationship between the dose and the probability of illness, each of the doses yields a probability. A binomial trial converts these probabilities into lists of ones and zeros, representing the occurrence or absence of illness resulting from each probability. Finally, for each of the three products we calculate the average, and interpret this again as a probability of illness. The dose-response model is further elaborated in Chapter 11.

Unit
We start with doses (number of *Salmonella* per portion of pork cuts / minced meat patty and fermented sausage), ending with dimensionless probabilities.

Outputs
Three numbers are output, the probability of illness given consumption for minced meat, pork cuts and fermented sausage. It is those numbers that must converge over many iterations of the Monte Carlo procedure.

4.1.7 Summary of modules

As a summary of the preceding sections one can say that Salmonella introduced at the farm level is tracked through the production chain, keeping track of numbers on individual units, until finally an average probability of illness is found.

The following figure (Figure 4.1) illustrates the various modules present in the model.

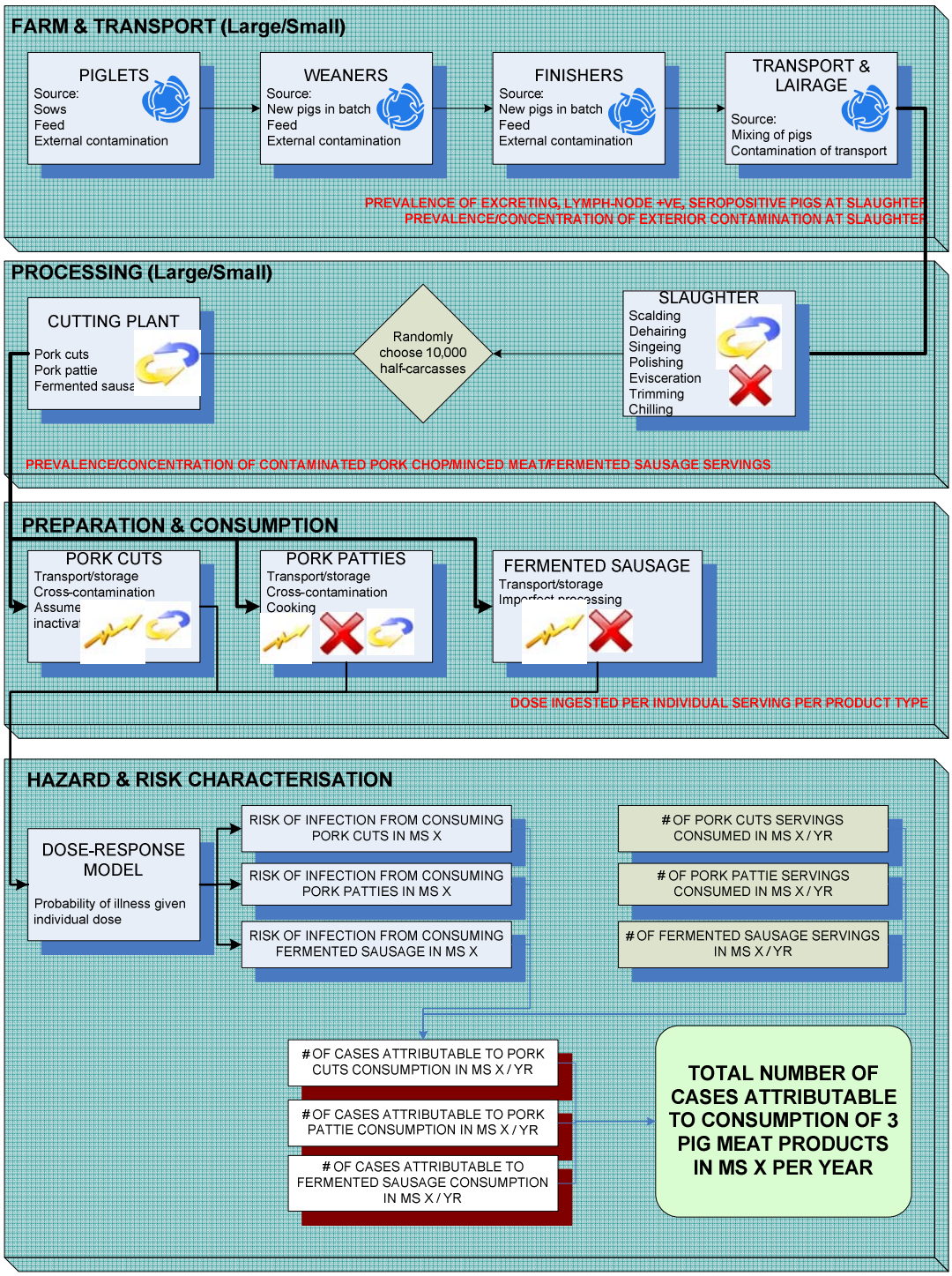

Figure 4.1: An overview of the modules within the farm-to-consumption QMRA. Icons represent the relevant microbiological processes: all-blue – transmission; blue-yellow arrows – cross-contamination, X - inactivation, bolt – growth.

Note that Figure 4.1 one iteration. Yet, there is already variability within this one iteration - variability between pigs, carcasses or products exists. One can think of e.g. the mass of the pigs, or the size of the consumer portions. This variability can still be seen in the distribution of doses, but is no longer of relevance in the final probability.
In addition to this within-iteration variability, there is also variability over the iterations. This variability is for the largest part variability in parameters describing production characteristics. For example, the temperature of the scalding water is a slaughterhouse characteristic which has variation.

The final probability of illness is an average of an average, firstly within each iteration it is the average of a number of probabilities resulting from doses, secondly, this average is averaged over iterations representing variability. But, this variability is not explicitly presented in the results, since it is not of prime importance for answering the main research question: the relative impact of interventions.

It is important to stress that the outcome of the model is an average probability of illness (for each product), given consumption. This probability can however easily be used to calculate the expected number of cases of illness. For such an exercise one needs to take into account the consumption patterns and population size of the MS under investigation. See Chapter 12 for further elaboration and results.

4.2 Modelling the EU

The model, as described in the previous sections, has a fixed chain of modules, and fixed processing steps within the modules. However, the parameters governing the dynamics can be modified by the user. We have determined, for each MS under investigation, suitable parameters. The estimation of parameters for each MS is described in detail in the chapters on modelling.

We have selected four representative MS. This was done according to a clustering scheme, thereby grouping MS having similar production practices. Within each of the four groups we selected one representative MS based on data availability. This clustering process is described in detail in Chapter 6. Ultimately, results for the four MS are meant to represent not only MS specific results, but also indicate the variability of the hazard of *Salmonella* in pork over the EU.

All parameters related to a single MS are grouped together in one single file, this file may then be used as input to the main code. Additional MSs can be implemented in a user friendly way, by using a current implementation of a MS as a template, and modifying the appropriate parameters.

4.3 Intervention and uncertainty analysis

Comparison of results of model runs[6] is required for intervention and uncertainty analysis. In an uncertainty analysis the impact that certain parameters, and particularly those that are deemed to be uncertain, have on the probability of illness are investigated. This provides insight into the reliability of the results predicted by the model. It also provides an indication

[6] A model run is synonymous to a simulation in our vocabulary. But please be aware that some software packages use the term simulation where we use iteration.

of key data gaps, which will be important in the prioritisation of future data collection by EFSA and the MSs should they wish to reduce the (unquantified) uncertainty in the model output. For both the intervention and uncertainty analysis the model has to be run a number of times and the final results recorded. In some cases it is not explicitly needed to re-run the farm modules, for example when assessing slaughterhouse interventions. In this case the farm matrices can be re-used for multiple runs of the model.

The intervention and uncertainty analyses have been made user friendly by allowing easy modification of the requested interventions, or parameter values, in specified user files. Also, results (figures and data structures) are automatically written to an archive, allowing convenient access to previous model runs or analyses.

We refer to Chapter 12 for the presentation and further discussion of the baseline results and uncertainty analyses. The sensitivity analysis methodology is described in Chapter 5 and the results are provided in the individual module chapters (Chapters 7 – 10). The results from the intervention analysis are given in Chapter 13.

5 Modelling Methodology

5.1 Overview

As required by EFSA (see Chapter 2), our modelling efforts are targeted towards a QMRA, implying that the model inputs are quantitative numbers and hence so too are the model outputs. In our case the basic unit is the number of *Salmonella* per unit under investigation (pig, carcass, etc.). By adjusting input parameters several real-world scenarios can be simulated, opening the possibility for comparison between those scenarios. In particular, interventions (adjustments in the baseline scenario that reflect possible interventions or controls that hopefully lead to a reduction in the risk of illness) may be modelled. By comparing model results with and without interventions (relative) risks may be assessed.

Quantitative risk assessments may be classified as either deterministic or stochastic. Deterministic risk assessment uses point-values as model inputs and therefore the model outputs are also point values. Stochastic risk assessment, however, incorporates uncertainty and/or variability into a model, which are both now defined. Variability represents a true heterogeneity in a population, e.g. the weight of a pig will vary between pigs and the fact that we cannot assign a fixed number to the weight has nothing to do with our incomplete knowledge; it is inherent to the population. On the other hand, uncertainty reflects our lack of knowledge on the exact value of a parameter. For example, the inactivation of *Salmonella* when subjected to high temperatures may be modelled by an exponential decay, dependent on time and an inactivation parameter. This inactivation parameter is hard to measure and therefore not known exactly.

In a stochastic model variability and uncertainty can be modelled using statistical probability distributions, instead of fixed parameter values. Incorporating distributions into the model results in a distribution for the model output; hence providing more information compared to the deterministic approach. Due to time constraints, we do not take uncertainty into account explicitly within this QMRA. We do however perform an uncertainty analysis, in order to ascertain whether uncertainty might play a major role. Variability however, in its various incarnations (between pig, batch, slaughterhouse, etc.) will be explicitly modelled. The specific choices made during the process, on the type of distribution representing the variability and the estimation of parameters will be thoroughly discussed in the model descriptions.

Further, more detailed, information on quantitative risk assessment – and in particular stochastic risk assessment - is available in e.g. Vose 2000 or Haas, Rose *et al.* 1999.

Appendices A5.1, A5.2 and A5.3 to this chapter give some common notation and conventions used throughout the report.

5.2 Monte Carlo Simulation

Since variability plays an important role, a Monte Carlo modelling framework is used. The basic idea behind a Monte Carlo procedure is that the result of the simulation is iteratively refined during a number of steps, also termed iterations. Each iteration represents a feasible representation of a real-world situation during which numbers are sampled from the probability distributions representing the variability. The number of iterations is preset, or dependent on the convergence behaviour of the simulation. The results of each iteration are

stored. At the end, statistical measures can be extracted from the results. For example, means, standard deviations or percentiles may be insightful to the modeller or risk assessor.

The description above is somewhat simplified, as it does not incorporate variability that exists also within iterations. This is best illustrated using an example. A slaughterhouse is defined by a number of parameters (e.g. line speed, temperature of scalding water, etc.) and each iteration of a Monte Carlo procedure will result in a realisation of a slaughterhouse. However, within this single slaughterhouse, the processed pigs will have their own variability (e.g. length, mass). This variability is likewise modelled using a Monte Carlo procedure and we end up with a multi-level model containing nested iterations. Such a nested method can lead to a severe computational burden when not implemented efficiently.

However, we do not use such a multi-level method. Rather, within each iteration we work with vectors of quantities (e.g. vectors of pig lengths), instead of using an extra loop. Formally these approaches are equivalent. However, we use Matlab R2008b (© Mathworks Ltd, USA) for implementation of the model, and Matlab vector operations are extremely efficient. During a Monte Carlo simulation, several parameters are re-sampled from variability distributions during the procedure. We will now discuss our terminology and notation for the use of distributions and probabilities.

A sample space is a set of possible outcomes of an experiment. For example the infectious state of a pig is represented by a sample space $\Omega = \{\text{infected}, \text{not infected}\}$[7]. A random variable maps a member of the sample space to a real number. For example, we may define the random variable $X : \Omega \to \mathbb{R}$ as $X(\text{infected}) = 1,\ X(\text{not infected}) = 0.$

A random variable is always associated to a distribution. The distribution in itself is not a mathematical 'formula', but a convenient way of expressing how a random variable behaves. For example, if X is distributed according to the Bernoulli distribution[8] with parameter p, we write

$$X \sim B(1, p). \qquad (5.1)$$

A distribution is always coupled to a probability mass function (for discrete random variables) or a probability density function (for continuous random variables), specifying the probability of a certain outcome. A probability is a number between zero and one, where zero stands for 'not occurring', while one means 'always occurring'. Therefore a probability mass function is a function $f : \mathbb{R} \to [0, 1]$. The probability mass function belonging to the Bernoulli distribution is given by

$$f(x; p) = \begin{cases} p & for \quad x = 1, \\ 1 - p & for \quad x = 0, \\ 0 & otherwise. \end{cases} \qquad (5.2)$$

[7] Please refer to Appendix A5.1 and A5.2 for a list of distributions and of mathematical notation and conventions used throughout this report.

[8] We treat Bernoulli distributions as binomial distributions with one trial, hence the notation B(1,p) below.

The probability mass function represents the probability that X equals x, given a distribution (for discrete random variables). This is often written shorthand as e.g. $P(X = 1) = p$ or, for the example given above, $P(\text{infected}) = p$.

In Monte Carlo simulation one needs a random sample (also called a realisation) from a distribution. For example, if we generate n samples from a Bernoulli distribution with $P(\text{infected}) = p$ and $P(\text{not infected}) = 1 - p$, then our sampling algorithm must produce the number 1 approximately np times and the number 0 approximately $n(1-p)$ times. In general, the histogram of the random samples (scaled to an area of one) will approximate the graph of the probability mass function, implying also that the average of the samples will tend to the mean of the distribution.

Now, the above is much too convoluted for use in our following discussion and we introduce the following notation for a realisation from a distribution B with parameter p, for pig i,

$$\Re(B(1,p),i). \qquad (5.3)$$

Thus, this realisation takes the values 0 and 1, with relative frequencies tending to $1-p$ and p. When realisations vary over iterations, not over pigs or products, we omit the index,

$$\Re(B(1,p)) \qquad (5.4)$$

Now that the rather technical concept of a realisation is handled, we discuss the simpler notion of a fraction. In contrast to a realisation, which is used inside of an iteration, yielding a physically meaningful state (such as, being infected, bacterial numbers, etc.), a fraction is a fixed number representing a fractional part of a quantity. For example, we have fractions α in the scalding stage, representing the part of the bacteria moving from a carcass to the scalding water. Note that an extra index i is not needed. If we have a fraction of time, we will also use the term rate.

5.3 Modelling Limitations

When interpreting model results, one should keep in mind that a model is always a simplification of reality. A typical model usually contains a large number of assumptions, simplifications and abstractions. It would, however, not do the model justice to regard these as modelling 'errors'. Let us briefly discuss some of the most relevant modelling issues.

Firstly, the modeller will distinguish a number of determining factors for the specific process that needs to be modelled. For example, pathogen inactivation will often be a function of time, temperature and the roughness of the surface. A number of factors, that are supposed to have a negligible impact, will be discarded.

Also, entities will usually be abstracted by defining them in terms of a limited number of parameters. For example, a typical carcass will be defined by a weight and be assumed to be circular.

Relations between entities and factors are cast in the form of mathematical equations, which may take the form of differential equations, difference equations, algebraic equations, etc. Obviously, this is also a crucial step and the mathematical expressions should be well founded by the modeller.

These two simplifications lead to a description of reality in terms of data and this brings us to two important aspects: data availability and data accuracy. Unfortunately, much of the needed data are unavailable. In this case, the modeller needs to somehow find a substitute for the lacking data. There are several options

- Use data from a similar case
  When data are available that refers to a similar situation, one may choose to use these data instead. Of course, it should be clearly stated why inclusion is justified. For example, pathogen inactivation rates on pig carcasses are not reported in the literature. However, such data are available for poultry. Assuming that inactivation rates for *Salmonella* on poultry and pig skin are similar, we use these data.

- Use expert opinion
  Experts may be consulted for their opinion on data. Obviously, this leads to some subjectiveness in the results. Use of a formal expert study overcomes this disadvantage to some degree (see e.g. Meyer & Booker 1991, Fels-Klerx, Cooke *et a*l. 2005). Time constraints unfortunately do not allow us to conduct such a formal elicitation study.

- Use a black-box model
  By a black-box model we will understand a simple input-output relation that does not take into account any of the complexities of the situation in reality. Such a model may be used when the process is simply too complex and not well understood, or parameters can not reasonably be determined by any of the above mentioned cases. A black-box model will typically link the output to the input using a simple relation involving a small number of parameters. If input and output data are known from e.g. experimental studies, the parameters can be estimated using standard statistical techniques. Such a model is also known as an 'empirical model'.

After abstraction and filling the data gaps, we have a mathematical model which may be used to perform simulations. Mistakes in parameter estimation, incorrect mathematical modelling and plain mistakes are minimised by using, as much as possible, information from peer-reviewed journals, which have been scrutinised by the scientific community. Additionally, we rely on internal and external reviews of the reports and model.

Since our method of choice is a Monte Carlo simulation we also have to deal with statistical errors and misinterpretations. Given input distributions and parameters, the simulation yields output distributions. However, if the number of iterations is not sufficient, the results will not have converged to a stable outcome and will not be reliable. We will monitor the convergence at several points in the simulation, thus gaining confidence in the final result.

Other mathematical sources of error are rounding errors, introduced by using finite precision arithmetic and errors in numerical solvers. We will not be concerned with rounding errors, an effect which is mostly insignificant.

5.4 Sensitivity Analysis

To determine the extent to which the variability of the model parameters affects the model, we conducted a one-way analysis of variance (ANOVA) test. This tests the parameters of the model that incorporate variability (i.e. are estimated using a statistical distribution to

describe the variability (e.g. duration of transport) against a response variable. Note that the sensitivity analysis only considers the variability of the parameter values which are part of the baseline model. The uncertainty associated with the parameter values is investigated in the uncertainty analysis (Chapter 12). The ANOVA method has previously been used as a method for sensitivity analysis of food safety risk assessments (Carlucci *et al.* 1999, Patil & Frey, 2004) and the methodology is discussed in Frey & Patil (2002).

The choice of the response variable is important. If we were to choose the probability of illness, the analysis would tell us the effect that our parameters have in relation to all the parameters in the whole model (i.e. including, farm, slaughter, processing, retail and consumption parameters), because probability of illness is dependent on the whole model. However, there are two concerns with this approach; 1) this would not tell us anything about the effect that the parameters are having at the stage they are implemented and 2) it is not straightforward, within the model, to associate the variability of parameters at earlier stages of the module with the risk of illness from a particular product. For example we would have a duration of transport for a batch of pigs. This batch may get split up in lairage. The individual pigs then go through the slaughterhouse and are split into half carcasses at the splitting stage. Carcasses are then picked at random from which to make the products. These changes in the unit of interest, particularly the random selection of carcasses at the processing stage, make it very difficult to determine which truck during transport a particular product was on in order to link the duration of transport with the risk of illness. This problem of sensitivity analysis across modules where aggregation occurs is mentioned as an issue in Frey & Patil (2002).

Therefore, for this model we conduct independent sensitivity analyses for the Farm, Transport, Lairage, Slaughterhouse, Cutting Plant and three analyses at Preparation & Consumption for the different product types (pork cuts, minced meat and fermented sausage). We do not consider the Dose-Response module in this analysis. Each analysis is conducted on 200,000 units (e.g. batches of pigs during transport, carcasses at the slaughterhouse and products at preparation and consumption).

We show the results in the form of bar graphs, where we plot the F value associated with the parameter. The importance of the parameters are assessed in terms of the relative magnitude of the F values, so that the bigger the bar the more significant the variation in the parameter is on the variation in the response variable. When interpreting the graphs it should be noted that a large difference in the F values of the parameters should be observed before it can be safely assumed that one parameter is more significant than another (there is no statistical test to determine the magnitude of this difference). It should also be noted that the sensitivity analysis assesses the importance of the variation in the input parameters on the response variable, it does not give an indication on how important a variable is on the absolute value. To take an extreme example; assume that you have a parameter in your model that reduces the number of *Salmonella* on the product by 99%, immediately before your response variable value is taken. This is clearly going to have a very big impact on the number of *Salmonella* on the carcass. However, this reduction is applied to all products, there is no variation about this parameter. Therefore, the sensitivity analysis will report that it is completely insignificant (i.e. it will have a F value of 0). However, if this parameter had been included in the uncertainty analysis (e.g by changing the magnitude of reduction to different values between 0%-100%) then it would have been identified as being significant.

Due to the complexity of the model, some modules have a lot of parameters with distributions associated with them and to show them all on a single graph would get confusing. For these modules we only show the most significant parameters on the graph.

5.5 References

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Appendix 5.1: List of Quantities and Variables

For convenience, we here list all quantities and variables used throughout this report. We make use of the following distributions,

Table A5.1: List of distributions

Symbol	Name	Type	Probability mass function
$B(n,p)$	Binomial with parameters n and p	Discrete	$f(x;n,p)=\binom{n}{x}p^x(1-p)^{n-x}$ for $x\in[0,n]$ $f(x;n,p)=0$ for $x\notin[0,n]$
$B(1,p)$	Bernoulli with parameter p	Discrete	$f(x;p)=p$,for $x=1$, $f(x;p)=1-p$, for $x=0$,
$U(a,b)$	Uniform distribution between a and b.	Continuous	$f(x;a,b)=1/(b-a)$, for $x\in[a,b]$, $f(x;a,b)=0$, for $x\notin[a,b]$.
$N(\mu,\sigma^2)$	Normal distribution with mean μ and standard deviation σ^2.	Continuous	$f(x;\mu,\sigma)=\frac{1}{\sqrt{2\pi\sigma^2}}e^{\frac{-(x-\mu)^2}{(2\sigma^2)}}$
$DU(a)$	Discrete uniform distributions with values $a\in\mathbb{R}^n$	Discrete	$f(x;a)=1/n$ for $x=a_i$
$DG(a,b)$	Discrete general distribution with values $a\in\mathbb{R}^n$ and associated probabilities $b\in\mathbb{R}^n$.	Discrete	$f(x;a,b)=b_i$ for $x=a_i$, note $\sum_{i=1}^{n}b_i=1$.
$G(a,b)$	General distribution with categories $a\in\mathbb{R}^n$ and $b\in\mathbb{R}^{n-1}$	Continuous	$f(x;a,b)=b_i$ for $x\in[a_{i+1},a_i]$ note $\sum_{i=1}^{n-1}b_i=1$.
$\Gamma(a,b)$	Gamma distribution with parameters a,b	Continuous	$f(x;a,b)=x^{k-1}\frac{e^{-x/b}}{b^k\Gamma(k)}$ for $x>0$ and $a,b>0$
$Beta(a,b)$	Beta distribution with parameters *a*, *b*	Continuous	$f(x;a,b)\propto x^{a-1}(1-x)^{b-1}$ for $x\in[0,1]$ and *a*,*b* > 0

Symbol	Name	Type	Probability mass function
$BP(a,b,c)$	BetaPert Distribution with parameters a, b, c; minimum, most likely, maximum.	Continuous	See Vose 2000
$EV(a,b)$	Extreme Value distribution	Continuous	$f(x;a,b) = (\frac{1}{b})e^{-\frac{x-a}{b}}e^{-e^{-\frac{x-a}{b}}}$ for $x \in [a,b]$
Po(λ)	Poisson distribution	Discrete	$f(x;\lambda) = \frac{\lambda^x e^{-\lambda}}{x!}$
Mn(x,p)	Multinomial distribution	Discrete	$f(x_1..x_k;n,p_1..p_k) = \begin{cases} \frac{n!}{x_1!...x_k!}{p_1}^{x_1}...{p_k}^{x_k} & \sum_{i=1}^{k} x_i = n \\ 0 & otherwise \end{cases}$
LogNormal(μ, σ)	Log Normal distribution	Continuous	$f(x;\mu,\sigma) = \frac{1}{x\sigma\sqrt{2\pi}}\exp\left[-\frac{(\ln(x)-\mu)^2}{2\sigma^2}\right]$
Weibull(α, β)	Weibull distribution	Continuous	$f(x;\alpha,\beta) = \beta\alpha^{-\beta}x^{\beta-1}\exp\left[-\left(\frac{x}{\alpha}\right)^{\beta}\right]$
GPareto(K,σ,θ)	Generalised pareto distribution	Continuous	$f(K,\sigma,\theta) = \frac{1}{\sigma}\left(1+K\frac{x-\theta}{\sigma}\right)^{-\left(\frac{1}{K}+1\right)}$

Appendix 5.2: Mathematical Notations and Conventions

We use the following mathematical notation and conventions

Table A5.2: Mathematical notation.

Notation	Explanation
$k = N, \ldots, M$	the expression is valid for all k from N up to M
$\{a, b, c, \ldots\}$	the set (unordered list) containing the elements a, b, c etc.
$a \in A$	any element a from the set A
$\mathbb{N}$	the set of natural numbers: $\mathbb{N} = \{1, 2, 3, \ldots\}$
$k \in \mathbb{N}$	k is a natural number (an element from the set $\mathbb{N}$).
$\mathbb{R}$	the set of real numbers (i.e. numbers from the continuum, having arbitrary precision)
$\mathbb{R}^+$	$\mathbb{R}$, restricted to the positive numbers including zero, $\mathbb{R}^+ = \{x \vert x \in \mathbb{R}, x \geq 0\}$.
$[a, b]$	the set of real numbers (i.e. numbers from a continuous range, with arbitrary precision) between and including a and b
$[a, b)$	the set of real numbers between a and b, excluding b
$[a, \infty)$	the set of real numbers greater or equal than a
$\sum_{i=n}^{m} a_i$	sum the numbers a_n to a_m, the sum equals zero for $m \leq n$.
$\lfloor x \rfloor$	round x down to the nearest integer
$\log$	the base 10 logarithm
$\ln$	the natural (base e) logarithm

6 Cluster Analysis - Definition of EU regions and Selection of a Representative MS within each Region.

6.1 Introduction

6.1.1 Background

An 'average' EU model for modelling the risk of human salmonellosis attributed to pork will not describe the variability between MSs and modelling 27 individual MS is not feasible within the given time frame. Therefore, efforts were made to aggregate MSs into groups (clusters) of countries with similar patterns of factors related to the risk of *Salmonella* from pork. A detailed risk assessment is then conducted in one MS from each cluster. The MSs were selected according to data availability.

6.1.2 Aim

In order to select the MSs as case studies a cluster analysis was carried out. Its aim was to identify meaningful regions (clusters of MS) by using available register data related to the pork production and consumption in each country.

Initially, the project group defined a number of criteria in the farm-to-consumption chain important for the risk of *Salmonella* infection. After evaluating the available MS-specific data, the project group decided which data should be included in the cluster analysis.

The data were used for combining countries into clusters such that:

- Countries in each cluster are similar to each other with regard to the selected information
- Each cluster is different from the other clusters with regard to the selected information

6.2 Material and Methods

6.2.1 Data

Data for the cluster analysis were defined by the four criteria listed in Table 6.1. This list was first put forward, in its original form, by the QMRA project group during a workshop on 21-24 April in Copenhagen, where 40 experts representing 13 MSs and EFSA representatives discussed and commented on its applicability for the QMRA.

Data exploration led to identification of only a limited amount of suitable data that both matched the defined criteria and at the same time had data for the majority of the MSs. No direct measures were available, and it was necessary to use 'proxy-data' as information - e.g. size of slaughter-pig holdings was used to describe the type of production (modern with relatively high biosecurity (large holdings) versus traditional with relatively low biosecurity (small holdings).

Table 6.1 Criteria used for quantitative description of the farm-to-consumption food chain, and identified data.

Criteria	Data available	Value used in the cluster analysis	Data source
Production	Size of holdings (heads)	Ratio of big holdings/small holdings	EuroStat
Slaughter	Slaughterhouse capacity (heads)	Ratio of output from big SH / small SH	EU baseline study
Consumption	Pig meat consumed per capita Relative consumption of sausages	Amount pig meat consumed per capita (kg) Relative consumption of sausages	FAOSTAT EuroStat

Production – size of holdings

Many factors related to management of pig farms including biosecurity measures influence the possibility for transmission of *Salmonella* within and between pig farms. However, such risk factors are numerous and very often hard to measure. Information on these factors is therefore only available from specific research studies from a few countries and not at the MS level. As a proxy for these factors, information on the number of holdings and size of holdings was obtained from EuroStat[9], under the assumption that smaller holdings will not have as strictly implemented biosecurity measures as larger holdings. According to expert opinion, production units having less than 400 animals (stock at any given time) are holdings with other biosecurity measures related to purchase of animals and within herd prevention of transmission of infections compared to larger production units. These differences may very well influence the *Salmonella* risk. The data are provided in Table 6.2.

Slaughter – capacity

Factors related to the possibility for introduction of *Salmonella* into a slaughterhouse and cross-contamination between carcasses within the slaughterhouse are considered to be an important influence on the risk of salmonellosis. However, the number of factors (typically slaughtering processes and hygiene factors) are numerous and very often hard to measure. Therefore, no information related to these factors is available at the MS-level. As a proxy for these factors we used information on the capacity of slaughterhouses in the different MSs, which were reported in a baseline survey on *Salmonella* in pigs (see Table 6.3)[10]. The data are

[9] The data in the EuroStat database are collected and validated following standardised procedures (Council Regulation (EC) No 322/97, Council Regulation (Euratom, EEC) No 1588/90, Commission Regulation (EC) No 1444/2002). The meta data describe the quality of each reporting/survey. This means that the data are "trustworthy" in terms of reporting though it says nothing about the quality of the data input. It is believed that the data in this dataset are of high quality based on earlier data sets and on data from other reports.

[10] Unpublished raw data from "Baseline survey on the prevalence of S*almonella* in slaughter pigs" (Commission Regulation (EC) No 1444/2002). An outline for data collection and reporting procedure is described in a Commission Decision (Eurostat Metadata). In the baseline studies are Member States asked to report the slaughter capacity of at least 80% of their gross national production. This reporting implies that there may be inconsistency in the reporting: some Member States may have chosen to mainly include output from large slaughterhouses whereas other Member Stases has included more small slaughterhouses.

divided into two categories: slaughterhouses with an output of more than 100,000 whole carcasses per year (large) and slaughterhouses with an output of less than 100,000 carcasses per year (small). It was assumed that smaller slaughterhouses will not have as strictly implemented slaughtering processes and hygiene measures as larger slaughterhouses, thereby having higher risk of introducing *Salmonella* and a higher risk of cross-contamination due to more contact with the environment. Larger slaughterhouses are often newer and more modern, with an infrastructure to avoid cross-contamination e.g. hanging carcasses, decontamination facilities, biosecurity).

Table 6.2: Production as expressed by number of 1,000 heads in the production from small farms (1-399 animals) and large farms (more than 400 animals) reported for 2003. In the right column percentages of production from small farms are presented.

Member State	Small [#]	Large [#]	Small/(Small+Large) [%]
Austria	1 863	1 382	57%
Belgium	528	6 011	8%
Bulgaria	703	330	68%
Cyprus	6	483	1%
Czech Republic	282	1 705	14%
Germany	5 578	20 756	21%
Denmark	551	12 398	4%
Estonia	44	313	12%
Spain	3 057	20 996	13%
Finland	466	928	33%
France	1 303	13 947	9%
Greece	287	707	29%
Hungary	2 163	2 750	44%
Ireland	25	1 707	1%
Italy	913	8 245	10%
Lithuania	583	474	55%
Luxembourg	17	59	22%
Latvia	231	206	53%
Malta	20	54	27%
Netherlands	769	10 400	7%
Poland	15 124	3 481	81%
Portugal	593	1 656	26%
Romania	3 929	922	81%
Sweden	229	1 675	12%
Slovenia	392	229	63%
Slovakia	319	1 124	22%
UK	323	4 518	7%

Table 6.3: Total number of slaughterhouses covering 80% of the pig slaughters in each Member State and the percentage of slaughters from slaughterhouses with more than 100,000 per year.

Member State	Total [#]	Large [%]
Austria	27	48
Belgium	20	100
Bulgaria	6	0
Cyprus	3	67
Czech Republic	45	24
Germany	78	64
Denmark	9	100
Estonia	8	13
Spain	19	100
Finland	7	100
France	21	100
Greece	39	3
Hungary	101	12
Ireland	5	100
Italy	17	100
Lithuania	11	18
Luxembourg	3	0
Latvia	13	0
Malta		
The Netherlands	10	100
Poland	401	14
Portugal	36	22
Romania		
Sweden	8	100
Slovenia	7	29
Slovakia	23	0
UK	18	94

The cut-off point of 100,000 was dictated by the data availability but has shown to be somewhat supported by the distribution of the capacity of slaughterhouses in general. Slaughterhouses tend to fall in two categories with an aggregate of small slaughterhouses slaughtering from a few hundred animals per year to some tens of thousands. The other category was slaughterhouses well above 100,000 slaughters per year. The assumption that smaller slaughterhouses are old is partly validated by changes to the infrastructure of slaughterhouses in recent decades, where many small slaughterhouses have disappeared and the number of large slaughterhouses has increased.

Consumption – Consumed pork per capita

The risk of contracting salmonellosis from pork is directly correlated with *Salmonella* prevalence. The FAOSTAT database (FAOSTAT, Consumption, Livestock and Fish Primary Equivalent) contains data on consumption of pork per capita in 2003[11]. The source from

[11] From the data in the EuroStat database, which contains entries for fewer Member States than the FAO data, but for more years it can be seen that the numbers between 1991 to 2005 do not change

where FAOSTAT obtain the data is not given. (The data base EuroStat data contains information of consumption for some MSs in 2005. However, because the EuroStat data base lacks data for ten of the 27 MSs we use data from 2003 registered in the FAOSTAT data base in the cluster analyses.). The data used in the cluster analysis are provided in Table 6.4.

Consumption – sausages consumed per capita

Fermented sausages[12] are included in this risk assessment because they has been known to cause outbreaks of salmonellosis (Emberland *et al*. 2006; Nygard *et al*. 2007, Luzzi *et al*. 2007, Cowden *et al*. 1989, Gilsdorf *et al.* 2005, Bremmer *et al.* 2004, Pontello *et al.* 1998) and EFSA specifically asked that an example product, that is not prepared at the consumer phase nor heat-treated during production, be included within the model.

very much. This indicates that even though the FAO data are five years old (2003) they should still be valid. One concern though is that the numbers from FAO are generally 1-10% higher (Austria, Netherlands and Belgium with 22, 22 and 48% respectively). It has not been possible to find the reason for this. This could be due to inclusion of more products (EuroStat does not include products made from e.g. offal in the data set used). While Austria is in top three of most consumed pig meat in both data sets, Netherlands and Belgium are ranked markedly different. This would evidently result in different clusters.

[12] Fermented or cured sausages are produced with starter culture (bacteria) or gluconodlacton to control the pH decline. The process is initiated at room temperature and high humidity. The end products contain 10% salt in water and pH is approximately 5.0. No heat treatment is involved (Alban *et al.* 2002). Fermented sausages are produced in all Member States are made using different recipes.

Table 6.4: Amount of pig meat consumed per capita per year (2003).

Member State	Amount of pig meat consumed (kg)
Austria	74
Belgium	35
Bulgaria	35
Cyprus	45
Czech Republic	43
Germany	54
Denmark	63
Estonia	30
Spain	66
Finland	33
France	38
Greece	27
Hungary	52
Ireland	44
Italy	43
Lithuania	33
Luxembourg	
Latvia	25
Malta	32
The Netherlands	36
Poland	50
Portugal	42
Romania	28
Sweden	37
Slovenia	39
Slovakia	32
UK	25

It has not been possible to obtain data on consumption of fermented sausages containing pork specifically. The consumption data on sausages (all sausages) in the EuroStat database consist of sausages containing pork, beef and poultry meat (Sold production and external trade of foodstuffs, EuroStat Data Shop Handbook). In order to use this dataset as a proxy for consumption of sausages containing pork, the numbers should only be used as relative measure. It is assumed that the proportion of sausages containing pork as well as the fraction of fermented sausages is the same across the EU. It is likely that less sausages containing pork are eaten in some MSs due to cultural and religious differences. This effect is not evaluated in this project and we assume that the proportion of persons not eating pork products out of religious beliefs are relatively low and equal in the MSs.

Table 6.4: Amount of sausages consumed per capita per year (2006).

Member State	Amount of sausages consumed/(capita*year) (kg)
Austria	19.4
Belgium	7.5
Bulgaria	11.5
Cyprus	
Czech Republic	
Germany	17.2
Denmark	12.1
Estonia	26.7
Spain	11.2
Finland	23.9
France	6.2
Greece	
Hungary	16.6
Ireland	
Italy	4.2
Lithuania	16.6
Luxembourg	
Latvia	19.1
Malta	
The Netherlands	9.1
Poland	13.1
Portugal	3.1
Romania	9.7
Sweden	
Slovenia	
Slovakia	
UK	7.4

6.2.2 Cluster Analysis

Algorithm

To identify meaningful clusters of EU MSs we performed a cluster analysis[13] based on the available data sources. The *k-means* clustering method was applied.

Briefly, the *K-means* algorithm starts by partitioning the input data into *k* initial clusters. It then calculates the mean point, or centroid, of each group and constructs a new partition by associating each observation with the closest centroid. Then the centroids are recalculated for the new clusters, and the algorithm repeated by alternate application of these two steps until convergence, which is obtained when the points no longer switch clusters.

[13] Cluster analysis is a tool frequently used to handle the heterogeneity of datasets and where the hoped-for result is a small number of groups (clusters). Each cluster should consist of a number of relatively homogeneous objects with a within-group variation considerably smaller than the total variation in the full data set (Lattin & Carroll, 2003).

The cluster analysis was done using the FASTCLUS procedure in SAS (Cary, 1999). This procedure uses by default Euclidean distances so that the clusters centroids are based on the least-squares estimation. Each iteration reduces the least-squares criterion until convergence is achieved, which is equivalent to the situation where none of the observations change cluster.

The results from a cluster analysis are influenced by variances in the dataset caused, for example, by the different units of the data. To eliminate this effect, all variables utilised in the analysis were standardised (mean=0 and STD=1) prior to the analysis using the STANDARD procedure in SAS (Cary, 1999).

Throughout the analysis, the cluster solution was obtained for 3, 4 and 5 clusters.

Evaluation of the cluster solution and determining the number of clusters

The cluster solution was evaluated using the following 5 relevant statistical parameters:

- *Overall R-square* is a ratio calculated as sum of squares between the cluster centroids ("a measure of the extent to which clusters are different from each other") / (sum of squares within clusters ("a measure of the extent to which observations within a cluster are similar") + sum of squares between the cluster centroids). The value can range between 0 and 1: 1 indicating that the clusters are homogeneous and well separated, 0 indicating that the clusters are heterogeneous and not very well separated (Cary, 1999).

- *Overall within-STD (Standard Deviation) divided by total STD.* Low values suggesting that the resulting clusters are quite homogeneous.

- *Pseudo-F-statistic* is the ratio of the mean sum of squares between clusters to the mean sum of squares within clusters, and so accounts for the degrees of freedom. The degrees of freedom are function of *k* (number of clusters). Usually, the larger the *pseudo-F*, the more efficient the partition is, in reducing within-group heterogeneity (Lattin & Carroll, 2003).

- *Cubic clustering criterion* (*CCC*) is a comparative measure of the deviation of the clusters from the distribution expected if data points were obtained from a uniform distribution. Usually, larger positive values of *CCC* indicate a better solution as it shows a larger difference from a uniform (no clusters) distribution (Lim *et al.*, 2006). Values of CCC greater than 2-3 indicate good clusters. Values between 0 and 2 indicate potential clusters, but they should be taken with caution (SAS Institute, 1999).

- Performing a canonical discriminant analysis and plotting the canonical variables. The canonical discriminant analysis was performed using PROC CANDISC in SAS (SAS Institute, 1999). The plots illustrate the spatial separation between clusters and the variation of observations within clusters.

Different scenarios for the cluster analysis

Using the available data and according to suggestions and advice given by the EFSA Working Group, different scenarios for the cluster analysis were proposed:

- All the criteria have the same weight in the analysis: Since there are two datasets representing the consumption criterion, the standardised data-values of '*Total pig meat*

consumed (kg)' and '*Consumption of sausages (kg)*' have to be multiplied by a numeric factor (0.5) ensuring that all criteria in the pathway have the same influence in the calculation of clusters.

- Include/not include the "consumption of sausages" data in the analysis: Sausages are fermented products that are ready-to-eat and require less handling by the consumer than fresh pig meat. Still, the processing can be critical for the survival and growth of *Salmonella* due to suboptimal heating and/or preservation. It was therefore deemed relevant to do the analysis both including and not including this dataset. Another option is to run the analysis using both the "amount of pig meat consumed per capita" and a "ratio between the consumption of sausages and the total amount of pig meat consumed."

- Put more weight on the consumption criterion relative to the other criteria in the analysis: In this scenario, extra weight is "shifted towards" the consumption criterion, as the amount of consumption is considered to be more important for the description of the EU MSs with regard to *Salmonella* risk from pork. So, in contrary to the previous scenarios, the criteria of consumption (with and without sausages) will be given twice the weight of each of the other criteria in the calculation of clusters.

- Include the data on prevalence of infected carcasses from baseline studies: The main reason to include this dataset is to increase the amount of information whereupon we differentiate MS into different clusters. However, it would be redundant to use this dataset in an analysis, since further on, the model is supposed to predict prevalence for the infection of *Salmonella* in pig meat. The cluster analysis was done with and without using this dataset.

6.2.4 Mapping

Results from the cluster analysis were colour coded and georeferenced to present clusters visually, using ArcView program (Lim *et al.*, 2006). Colours do not represent the risk of salmonellosis in any way.

6.2.5 Interpretation of the cluster solution

The cluster solution (the output of the cluster analysis) was used to describe the profile of each cluster. In the cluster solution, each cluster is labelled by the cluster's centre for each criterion. Given the standardisation of data to the overall mean for each criterion before the analysis, the interpretation of the cluster's centres was undertaken relative to the mean of the criteria for the EU countries.

6.3 Results

The results from the cluster analysis for the different scenarios were discussed at the meeting between modellers in RIVM, 4-6 November, 2008, and it was agreed that the analysis should focus only on the most relevant scenarios and datasets:

- Focus the analysis in the scenarios with equal weight to all criteria and twice the weight to the consumption criterion relative to each of the other data criteria;
- Include data for the production criterion (size of holdings), for the slaughter criterion (slaughter capacity) and for the consumption criterion (the amount of pig meat consumed of pig meat and the ratio between the consumption of sausages and consumption of pig meat);
- Run the analysis only with 3, 4, and 5 clusters;

6.3.1 The pseudo-F statistics, cubic clustering criterion (CCC) and the number of clusters

Figure 6.1 represents the variation *pseudo F statistics* and *Cubic Clustering Criterion* (CCC) according to the number of clusters for both the scenarios with and without weight towards consumption.

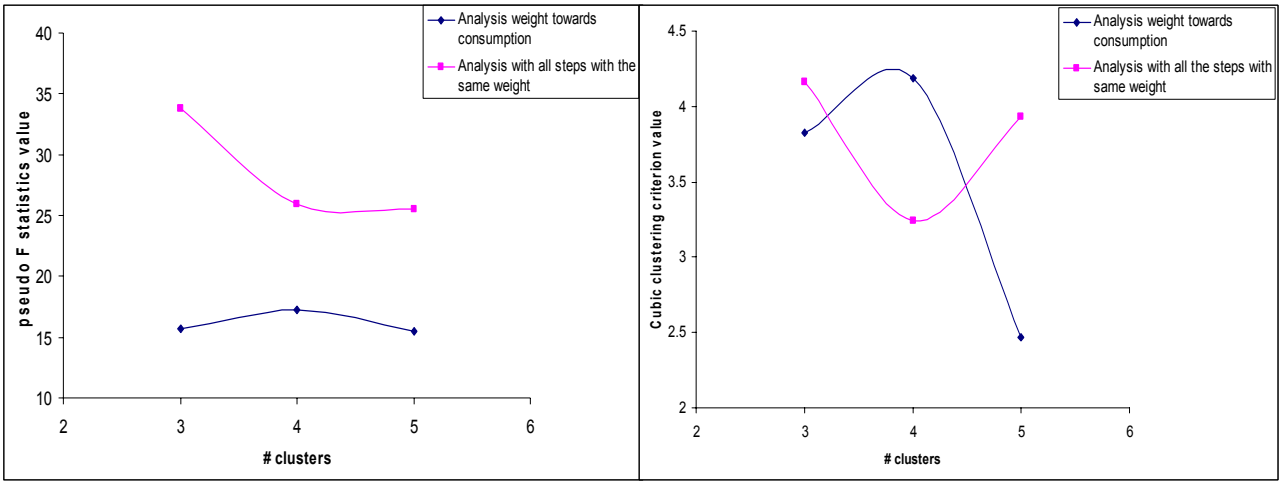

Figure 6.1: Representation of the pseudo-F and CCC values for *k*=3, 4 and 5. Analysis with equal weight to all criteria and with twice without weight towards consumption

Considering the results for these 2 parameters, the optimal number of clusters for the scenario with twice the weight on consumption relatively to each of the other criteria (weight "towards" consumption) would be 4 clusters, while for the scenario where all the criteria have the same weight 3 clusters would be optimal.

The *Overall R-squared* values also show a good cluster distribution for these cluster scenarios:

- Overall R squared– 0.57 (weight "towards" consumption, *k* = 4);

- Overall R squared – 0.60 (same weight, *k* = 3);

In terms of *within-STD by overall STD parameter*, for these cluster solutions, the values are:

- 0.59 (Analysis with weight "towards" consumption criterion and *k*=4);
- 0.54 (Analysis with all the criteria having the same weight and *k*=3);

6.3.2 Results of scenarios with twice the weight on consumption relatively to each of the other criteria

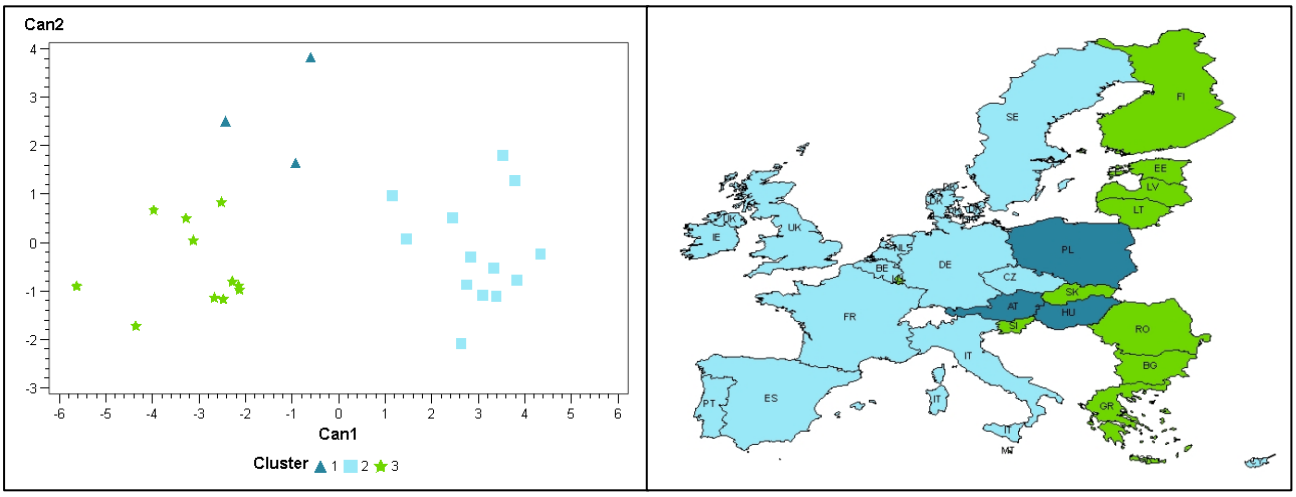

Figure 6.2: The canonical variable plot and maps with the results for the analysis of the scenario with twice the weight on consumption relatively to each of the other criteria and with a 3-cluster solution

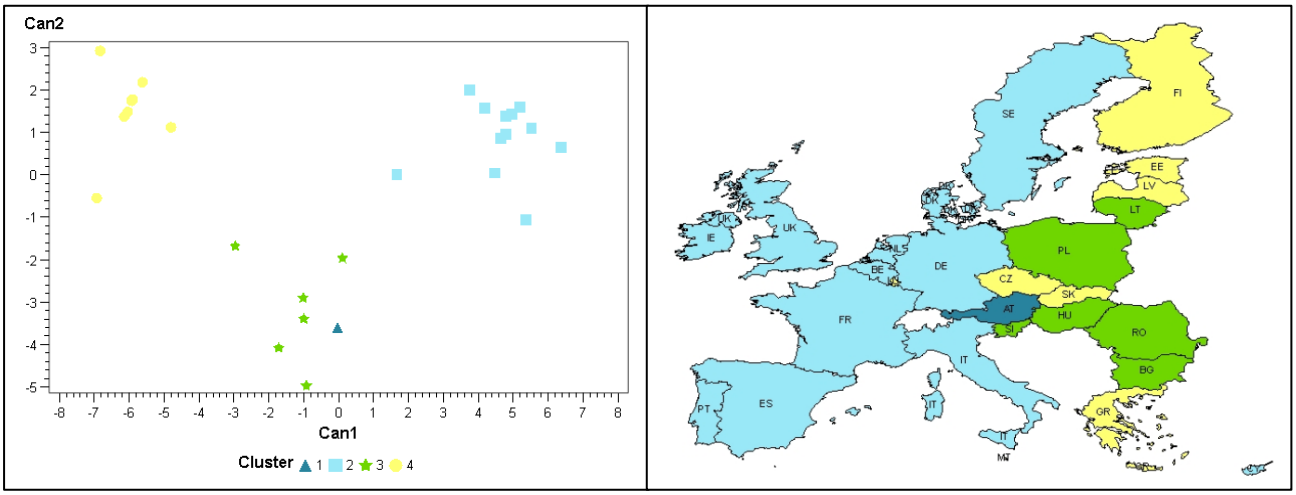

Figure 6.3: The canonical variable plot and maps with the results for the analysis of the scenario with twice the weight on consumption relatively to each of the other criteria and with a 4-cluster solution

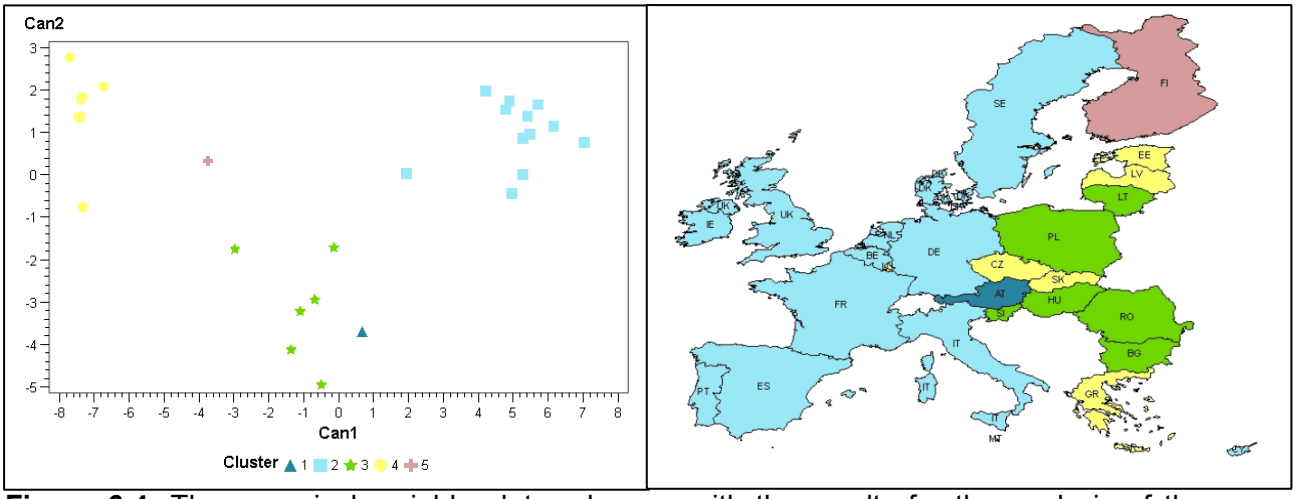

Figure 6.4: The canonical variable plot and maps with the results for the analysis of the scenario with twice the weight on consumption relatively to each of the other criteria and with a 5-cluster solution

6.3.3 Results of scenario with all criteria having the same weight

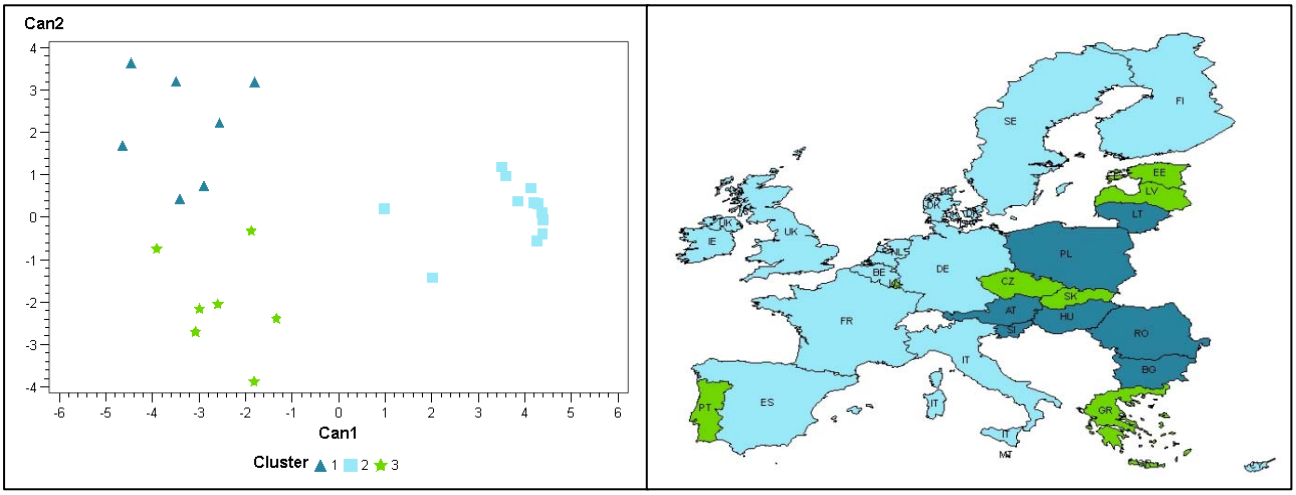

Figure 6.5: The canonical variable plot and maps with the results for the analysis of the scenario with no weight and with a 3-cluster solution

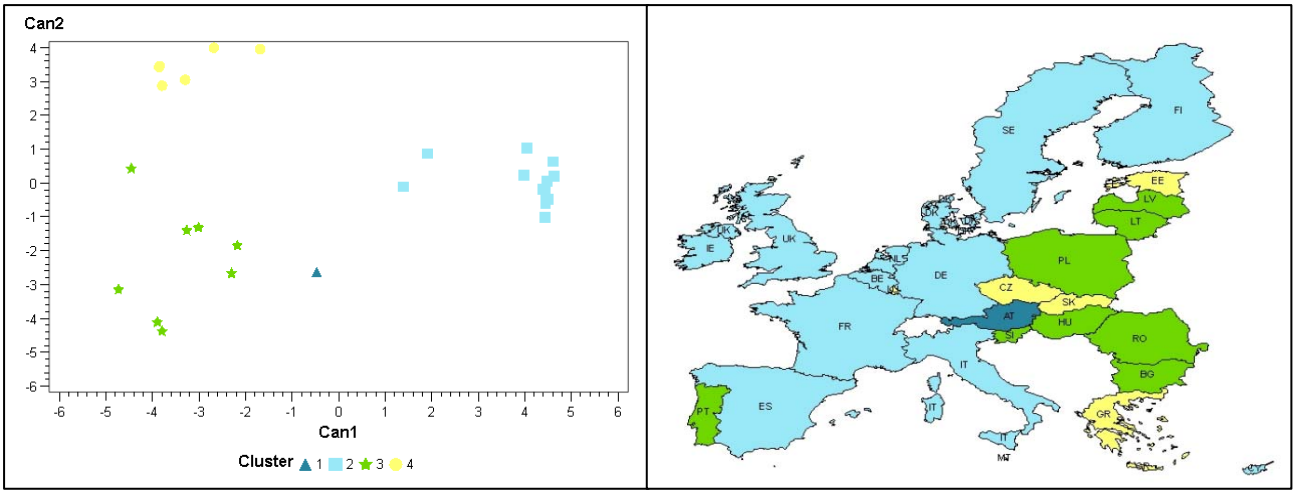

Figure 6.6: The canonical variable plot and maps with the results for the analysis of the scenario with no weight and with a 4-cluster solution:

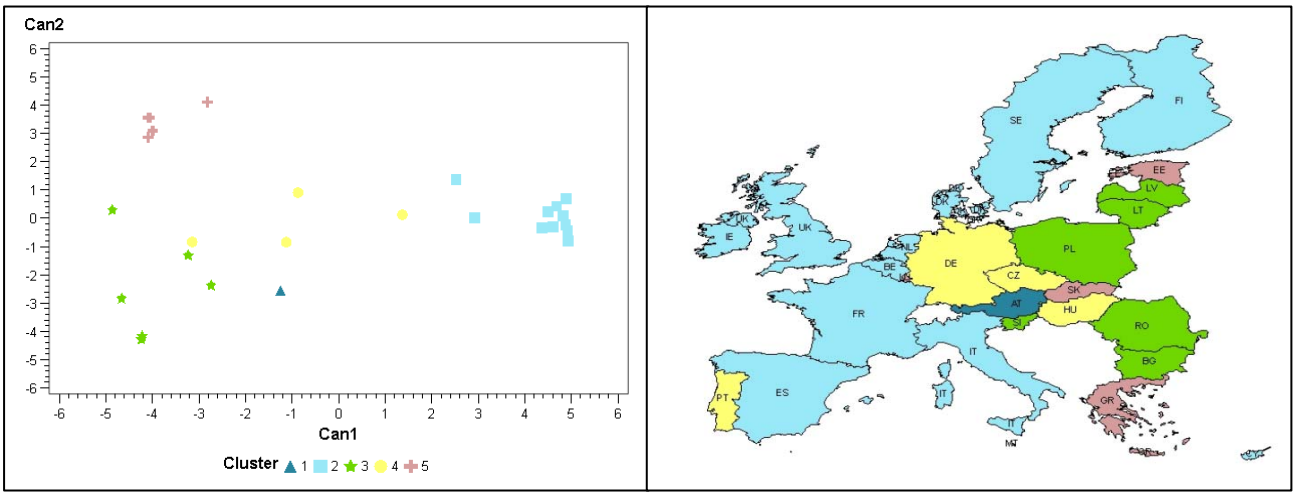

Figure 6.7: The canonical variable plot and maps with the results for the analysis of the scenario with no weight and with a 5-cluster solution

6.3.4 Choice of the final EU Member States grouping

The following criteria were used to decide which scenario should be used to define the clusters:

- the statistical measures (e.g. *pseudo F* and *CCC*)
- the cluster separation represented by the canonical plots
- which countries-grouping made more sense, according to expert opinion

The final decision fell upon the scenario with twice the weight on consumption relatively to each of the other criteria and MSs divided in 4 clusters. The MSs within each cluster and the description of the clusters are presented in Table 6.5.

Table 6.5: Description of the clusters for the final cluster solution.

Cluster	Example Member State	Description of the cluster
1	Austria	Relatively high proportion of small holdings, average proportion of large slaughterhouses, very high consumption of pork meat but relatively low consumption of sausage
2	Belgium, Cyprus, Germany, Denmark, Spain, France, Ireland, Italy, the Netherlands, Portugal, Sweden and United Kingdom	Relatively high proportion of large holdings and large slaughterhouses, medium consumption of pork meat and relatively low consumption of sausage
3	Bulgaria, Hungary, Lithuania, Poland, Romania, Slovenia	Relatively low proportion of large holdings and high proportion of small slaughterhouses, medium consumption of pork meat and relatively medium consumption of sausage
4	Czech Republic, Estonia, Finland, Greece, Luxembourg, Latvia, Malta, Slovakia	Medium proportion of large holdings, large proportion of small slaughterhouses, medium consumption of pork meat, very high consumption of sausage (however, 5 out of the 8 MS in this cluster have no data for sausage consumption).

6.3.5 Analysis of the seed influence for the chosen cluster solution

The initial population of the clusters with MSs (which MS that belongs to which cluster - initial cluster seeds) can have influence on the results of a cluster analysis. To evaluate whether or not this effect impacts the validity of the analysis, the analysis for a 4-cluster solution was rerun using different initial seeds. By changing the initial cluster seeds randomly (sorting the data according to random generated values), the cluster analysis was rerun 10 times (with 10 random groups of initial seeds) and the results analysed.

In the re-run analyses, following discrepancies in the repeated clustering solution were revealed:

- Denmark and Germany switched cluster twice away from cluster 2 (but kept belonging to the same cluster);
- Finland switched cluster 3 times;
- Spain and Portugal both switched cluster 2 times;

These results from the re-analysis indicated that the initial seeds did have an influence on the cluster solution. However, for the majority of MSs, the variation in the initial cluster seeds did not influence the cluster to which the MS belonged. The result of the cluster solution obtained when Austria, Belgium, Bulgaria and Germany were used as initial cluster seeds was used for the final evaluation of the cluster solution.

6.3.6 Effect of missing data

Nine MSs failed to report values for the consumption of sausages. In addition Luxembourg had not reported consumption of pork meat and Malta had not reported slaughterhouse capacity. This means that these two countries' assignment to groups is only based on two data entries.

6.4 Discussion

When comparing the results, by changing the number of clusters, some countries switch groups. For instance, in the 3-cluster solution of weight towards consumption data (Figure 6.2) all the three Baltic States group together with Finland, whereas in the 4-cluster analysis Lithuania groups with Poland instead (Figure 6.3).

However according to these results, for most of the groupings the cluster solution is quite robust, and changing the number of clusters does not have a significant influence on which countries usually group together.

It is worth mentioning, that although no geographical information was included in the analysis, the clusters to some extent represent geographical regions in EU.

6.5 Selection of countries representing respectively cluster

Within each cluster, the criterion for selection of a MS for a detailed risk assessment was the expected availability of data. The MS selected for a detailed risk assessment were named MS1, MS2, MS3 and MS4.

Throughout the risk assessment, efforts will be made to use data from the other countries within the cluster if data lacks for the selected country.

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7 Farm Module

7.1 Introduction

The EFSA Terms of Reference (ToRs) specifically state the inclusion of control options at the farm level within the risk assessment, as well as an assessment of the sources of infection. Hence, the inclusion of a farm model of some description is necessary in order to consider these ToRs.

Salmonella infection in pigs has been described widely in the literature. Pig morbidity or mortality because of *Salmonella* infection is rare, and is more commonly associated with *Salmonella* Choleraesuis, rather than serotypes associated with human illness. Hence pig infection is primarily a food safety issue (meaning we can largely disregard any effects on pig health with the model). Studies (Lo Fo Wong & Hald 2000; VLA 2009) have shown that transmission of *Salmonella* infection in pigs is a complex process involving many factors, which we cannot identify and include all within a quantitative transmission model. For example, we have been requested to treat all *Salmonella* serotypes the same, but an interesting factor is that broadly speaking the most common serotype isolated from the western states of the EU is *Salmonella* Typhimurium, but *Salmonella* Enteritidis is more commonly isolated in the eastern MSs (EFSA 2008a). Clearly there are reasons for these differences the investigation of which are not within the scope of the project. Therefore, we have modelled those factors that we judge to be, from the published literature and expert opinion, the most important in determining the prevalence of infection within pigs at slaughter, within and between MSs.

Infectious disease transmission models have been developed for a wide variety of animal diseases, including *Salmonella* in pigs (Hill *et al.* 2008; Lurette *et al.* 2008; Soumpasis & Butler 2009). Typically these models have become more detailed over time, abandoning the traditional use of transmission parameters. Transmission parameters represent a simple "black-box" approach that describes the force of infection to pigs because of *Salmonella* in the environment. The final estimation of these transmission parameters represents many different factors, including the resistance of the pig to infection and the level of contamination in the environment. In order to investigate interventions (such as vaccination, organic acids, etc.) a more detailed model is necessary to differentiate between those factors which increase/reduce the contamination of the environment and those factors which increase/reduce the resistance of the pig to infection.

Of critical importance to the success of an EU-wide model is the consideration of varying management practices within the EU. As described in Chapter 4 we have approached the modelling of the EU by taking a generic approach and then parameterising for an individual MS. No farm transmission model, at least for *Salmonella* in pigs, has yet dealt with management practices in sufficient detail to differentiate between farm types. Hence, while we are able to use the previous models listed above to inform the development of the transmission dynamics between pigs, we must apply the novel methodology described in Chapter 4 to include varying management practices.

7.2 Aims and Objectives of the Farm Module

The objective of the Farm module is to mathematically describe the management of farms within the EU and the associated transmission of *Salmonella* between pigs, such that the farm interventions specified by EFSA can be assessed. Specifically, we will model the chosen case study MSs (although the farm model is intended to be flexible enough to be adapted for other MSs). The primary output of the farm model is the prevalence of infection within a batch of pigs, at the point of depopulation for slaughter. This prevalence of infection is naturally variable, as well as being directly affected by the farm management systems in place. This output (prevalence of infection within a batch of pigs) is the input to the transport module of the risk assessment model, and is a natural point to assess the effect of the specified interventions.

7.3 Overview of Farm Management and *Salmonella* Transmission between Pigs

Management, microbiological and transmission literature searches were conducted in parallel, each informing the other.

The management of pig production is extremely variable, both within and between MSs. While every effort has been made to understand the differences in pig management, we cannot model every variation that exists, and more importantly the scope of this model only extends to the effect of interventions on slaughter pigs (we differentiate between the sows and the progeny intended solely for meat production; therefore where referenced slaughter pigs means all pigs within a farm primarily intended for meat production. Sows are hence not included in this terminology). Therefore, we only include in this overview management systems and practices that we deem relevant to this risk assessment. This overview is a summary of all the data collected, of which there was a vast amount (some of it relevant, a lot of it not); for transparency only key references are added in this section, but important assumptions are referenced and supported in the description of the model or the parameter estimation section (Section 7.4).

Based on discussion from the Data Workshop held in Copenhagen in April 2008, we have chosen to delineate pig production into two categories (large and small farms), with the threshold being 400 pigs slaughtered per year. The rationale for this cut-off point is that farms larger than this size will probably produce pigs along the lines of modern conventional production practices (e.g. splitting different aged-pigs into different rooms/buildings) and hence there should be a difference in *Salmonella* transmission according to large and small management practices.

7.3.1 Large farm management

Modern intensive pig farming has evolved into a sophisticated technology, and much of the pig meat produced in the EU will be produced in this way, especially in those MSs which produce the majority of the EU's pig meat (e.g. Denmark, Germany and France) (http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/).

Like all pig management systems, intensive production comes in many different forms. However, here we only consider differences that affect *Salmonella introduction and/or transmission*. We can therefore initially approach intensive pig farming at a low resolution to gain an overview of the main factors that should be included within the model.
First, the main stages of production are defined as:

Farrowing: Between 8-15 piglets born to a sow, each sow and litter within its own pen; around 15-50 pens within each compartment. The piglets from all the sows farrowing simultaneously in a compartment can also be grouped together in a large pen. Piglets weaned between 21-42 days of age.

Weaning: Pigs moved into specialist accommodation. Litters of piglets grouped together into pens of around 1050 pigs each. Pigs are moved onto either dry or wet feed, and stay within these pens (depending on whether there is a growing stage or not) until 8-12 weeks of age.

Growing: Pigs moved into specialist accommodation. Becoming less common in modern systems, this intermediate stage will generally see relatively little mixing of pigs from different pens, and pigs will stay here for approximately 6-8 weeks.

Finishing: Pigs moved into specialist accommodation. These farms/buildings tend to be larger, as pigs are fattened to slaughter weight over a period of 8-16 weeks. Contract finishing farms may source their stock from a number of nursery or grower farms.

Not all farmers will practice all of these stages of rearing, and differences may be found in mixing patterns and the age of pigs within each system. Information relating to the transmission of *Salmonella* between different ages of pigs is limited, therefore we conclude that it is sufficient to differentiate between these rearing groups rather than specific age groups.

The main management difference between farms relates to how the farmer manages the transfer of pigs through the different stages of rearing. There are many different ways to organise the serving of sows, mixing of pigs etc, but the main difference will be if pigs are raised in an all-in-all-out (AIAO) or a continuous system, with the assumption being that AIAO limits the number of pigs that contact each other, and whether or not there is any movement of pigs between farms.

A special form of AIAO production and of crucial importance for the *Salmonella* status is whether farms apply batch production, and how this is applied through the production chain. Batching can be from letting 20-50 sows farrow simultaneously (i.e. within a few days) in one compartment, and later keeping all the piglets born by those sows in one compartment up to slaughter without introducing or allowing contacts with other pigs. Within that system the piglets from the same litter can also be kept together in same pen up to slaughter. Batching is perceived as beneficial because of the ability for the farmer to plan ahead and reduce peaks and troughs in labour demands, and also has productivity gains. Batching of sows into groups can be done on either a 1, 2 or 3 weekly-cycle, such that groups of sows give birth within a defined weekly period. In addition pigs produced in these systems reach slaughter up to one month earlier than in old traditional systems with a continuous production which is considered to be as a result of improved health status. Of basic importance for the efficacy of this system is that a cleaning and disinfection procedure is applied between batches.

In discussion with pig farming experts (industry, vets etc) true AIAO production (i.e. AIAO by building) will be at a compartment level (where cohorts of similarly-aged pigs are moved into and out of a room/section of a building separately from other cohorts). This complicates parameter estimation as it is unclear how farmers perceive AIAO or continuous (for example from the management data collected via the EFSA breeding survey). This survey, and other information collected from individual MSs, suggests that AIAO production is slightly more common than continuous production in the four case study MSs; in the absence of any other information we will assume that the figures from the EFSA breeding survey represent our AIAO-by-room definition.

Harder to define, but a crucial difference between farms, is the biosecurity of the farm. We define biosecurity as anything that provides a barrier between the *Salmonella*-free pig on the farm and the (possibly) *Salmonella*-positive environment outside (or indeed inside) the pigs' dwelling, including any cleaning and disinfection routines. Biosecurity would include the maintenance of any pig housing, good hygiene during production (in particular good manure management that decreases pig exposure to manure (apart from floor type described below), cleaning and disinfection between batches of pigs, and storage of feed to prevent access of birds and rodents (e.g. open storage/non silos). We can model the batching of pigs and the associated cleaning and disinfection, but currently the only available data relates to rodent control, and thus we have focused only on cleaning/disinfection and rodent control to provide some quantification of "good" versus "bad" biosecurity. Also important, and related to biosecurity, is whether the pig is kept indoors or outdoors. Outdoor production has become more popular for large-scale production within the last couple of decades (especially in MS2) and has particular differences to inside production that could affect *Salmonella* introduction and transmission, for example exposure to wildlife including birds and rodents, mixing of sows and type of feed. According to the EFSA breeding survey (not yet published) large-scale outside production is still quite rare for pigs beyond the stage of weaning, and therefore we only include the farrowing stage as a possible outside production stage.

The above factors are probably important to consider regardless of the particular infectious organism. However, for *Salmonella* introduction and transmission we are interested in at least two other factors: feed and flooring.

Feed can be both a source of *Salmonella* infection in pigs and a factor in reducing the level of transmission. Clearly contaminated feed poses a risk to pigs, and has been highlighted as probably the main cause of infection in regions where *Salmonella* infection in pigs is low (e.g. some Scandinavian countries) (EFSA 2008b), but the relationship between feed and *Salmonella* infection in pigs is complex. The serotypes commonly associated with feed contamination are not usually those – especially *S*. Typhimurium – which are commonly associated with pig infection (EFSA 2008b); although we assume here that all salmonellae are capable of infecting pigs and are of zoonotic potential (as prescribed in the EFSA ToRs).

As with management systems, feeding systems are variable between farms. There will be variation in the type of food used, the additives used, and how the feed is presented to the pigs (meal/mash/pellets/grinding). All of these factors affect the ecology of the pig gut. The main factor with relevance to *Salmonella* transmission appears to be the way in which the feed affects the pH and content of organic acids in the pig gut (O'Connor *et al.* 2008; Wales *et al.* 2009). The lower the pH the more hostile the environment for any *Salmonella*, and hence infection is less likely. Of particular importance is whether the feed is presented as a

dry or wet form, or whether it is pelleted or non-pelleted (Lo Fo Wong & Hald 2000; O'Connor *et al.* 2008). Risk factors studies highlight the effect of different feeds on *Salmonella* infection, but these do not provide enough information to model the relative protective effect of individual types of feed. Therefore we concentrate on describing the dynamics of *Salmonella* transmission between pigs given consumption of wet or dry feed, where there is some information on the relative effect, and good information on whether a farmer uses wet/dry feed from the EFSA baseline survey. This is an important simplifying assumption for the model, but one made because of a lack of *quantitative* data describing the effect of feed in changing the pig response to *Salmonella* infection.

While the evidence for flooring type affecting *Salmonella* transmission is varied (some studies point to it as a risk factor, most don't) (Lo Fo Wong *et al.* 2004; Nollet *et al.* 2004), logical thinking suggests that slatted flooring may well have some effect as it will remove faeces/*Salmonella* from the pig environment. The inclusion of this factor is relatively straightforward, and so we include it to investigate whether this factor is important or not. Again, there are many flooring types (partially slatted, bare concrete, straw-laden), but it is not possible to differentiate between individual types of flooring, and hence we consider only the distinction between slatted and solid flooring (assuming, given the propensity for pigs to earmark a particular area for defecation, that partially slatted flooring is equivalent to fully slatted flooring).

Based on the previous discussion, the five main factors considered for large pig farm management are: *rearing stages; AIAO vs continuous production; feed; flooring and finally inside vs outside production*. There are other factors that may influence *Salmonella* introduction and transmission, but these have either not yet been proven to be important or are not possible to model with current data, in particular as biosecurity and hygiene factors (e.g. stocking density, age of building, storage of feed). One important example is herd size, which in a number of studies has been shown to be related to prevalence of infection (although we do capture this at a very broad level by considering large and small farms). However, this relationship is far from universally proven, and the underlying drivers of why herd size is related to prevalence of infection (e.g. stocking density, sharing of equipment between farms) are unlikely to be captured within the current model. Therefore, herd size is a factor judged not to warrant further inclusion at this stage - especially as it is unlikely that farmers can change herd size as an intervention measure.

The broad overview gained by the above review is the foundation of the generic farm model, which then forms part of the larger generic risk assessment model. Finer resolution can be achieved by considering parameter estimation at a MS level.

7.3.2 Small farm management

Information on smaller farms is extremely limited. The only reliable data we were able to find was the EFSA breeding pig survey on the number of farms with less than 20 sows (However, only for three of the four case study MSs, MS1, MS3 and MS4 – MS2 did not sample any small farms as we have defined them).

Based on expert opinion and miscellaneous evidence from the literature (although this evidence cannot be relied upon to be representative of all small EU pig farming), we have modified the management system for large farms to describe small farms.

The main differences between large and small farm management are that we assume small farms will not have enough stock (maximum of 20 sows) to warrant grouping of pigs, and so we assume that this group of up to 20 sows is serviced, and hence farrowed, at the same time. This produces a large group of piglets (say 200), which is then weaned at the same time and placed within a single block of accommodation. The weaned pigs then stay in this same accommodation until they reach slaughter weight, by which time the next group of piglets should be about ready to be weaned. We consider all of the same factors as for large farms above, i.e. inside/outside production, feed type and flooring type.

By its nature, small farm management is likely to be varied, and the structure described above will only be applicable to a certain percentage of small EU farms. More information on the small farm model can be found in Section 7.4.1.

7.3.3 Source of infection

Previous information (Nollet *et al.* 2005) suggests that infection (from any route) in piglets born to a seropositive sow is relatively lower than piglets born to seronegative sows. The source of infection for most farms is thought to be the introduction of new stock or contaminated feed (EFSA 2006; Lo Fo Wong & Hald 2000), for which one possibility is that a small number of "seeder" pigs may initiate widespread transmission in the event of a period of mixing and feed change such as weaning. Therefore, one source of infection within the model is the prevalence of infection in sows from the EFSA breeding survey, which is used to estimate the amount of *Salmonella* that might be excreted into the farrowing pen. Any piglets infected then pose an increasing threat to other pigs once they have entered the weaning stage. As described above, feed and external contamination (primarily due to rodents) are also included as sources.

7.3.4 Transmission

Transmission between pigs has been shown by a number of studies (Jensen *et al.* 2006; Kranker *et al.* 2003; Nollet *et al.* 2005). However, transmission studies relevant to the risk assessment, using "natural" modes of infection (i.e. not deliberately inoculating pigs with a large dose to ensure infection) are lower in number and usually smaller. Transmission in these observational studies shows intermittent shedding at low levels (usually less than 100 CFU/g of faeces) and a fairly low incidence of infection, apart from the period immediately post-weaning, when there is typically a distinct increase in incidence/prevalence (Jensen *et al.* 2006; Nollet *et al.* 2005).

From the studies mentioned above, transmission is highly variable, and different *Salmonella* serotypes will intermittently contaminate/infect a pen of pigs over the course of time (Davies *et al.* 1999; VLA 2009). This variability will come not only from the management system used, but is also inherent in the transmission of infectious diseases; therefore the model developed must capture this variability. In order to account for interventions, the environment and the resistance of the pig must be included within the transmission model. Hence, the transmission model focuses on two main factors: the amount of *Salmonella* in the environment (predominantly through the excretion of *Salmonella* in the faeces of infected pigs) and the dose-response relationship for the infection of a pig (which can be varied to simulate greater immunity due to vaccination or feed).

7.4 Methodology

7.4.1 Simulation modelling

The farm model was developed in Matlab R2008b (© Mathworks Ltd, USA). The overall farm model is made up of two distinct models, the large and small farm models. Each MS is simulated separately. The model for each MS is identical except for the parameter estimation of each. For each MS model, the following chronology of model events applies:

1. Set number of iterations/farms for both small and large farm models. Each iteration of the model approximates the management of pigs on one farm throughout a 500-day cycle[14].
2. Set up management system of large and small farms. Input management and transmission parameter values for large and small farm models.
3. Allocate farm type to each farm (iteration) of the small or large farm simulation. Farm type is allocated according to proportion of farm types found for MS within the management data collected from the EFSA breeding survey baseline survey. In all there are 56 farm types, each with a different combination of management factors, e.g. large/small farm, feed type, AIAO/continuous production.
4. Run large and small farm simulation models.
5. At iteration level: track movement and birth of pigs over 500-day cycle.
6. At iteration level: determine whether infected breeding pig herd or not. If so, allocate sows that are *Salmonella*-positive.
7. At iteration level: seed any *Salmonella* into environment via sows, feed or external contamination.
8. At iteration level: if/when infection in slaughter pigs occurs (via sow, feed or environment), transmission is modelled.

As stated there are separate large and small farm models, and the above chronology applies to both. A graphical representation of this management model is shown below in Figure 7.1.

We now go through each of the steps in turn and in more detail.

Number of iterations

The number of iterations represent the number of farms included within a MS model. We have chosen 1,000 iterations as a suitable number for the baseline model, in order to ensure convergence of the national slaughter pig prevalence for each MS (derived from the ~70,000 batches generated from a 1000-iteration simulation). Explicitly, the small farm model is run for 1,000 iterations, and the large farm model is also run for 1,000 iterations.

Large and small farm management and transmission setup

For the purposes of the model, we assume that all slaughter pigs will go through four main stages of rearing: farrowing, weaning, growing and finishing (fattening); and will be moved into specialist accommodation for each stage of rearing (and can be transported between farms at the end of weaning if on a multi-site farm). At the beginning of the model (t=1) we populate each pen/room/building with pigs, except for one farrowing building, which is left

[14] 500 days was chosen as the best balance between i) running the model for long enough to produce enough batches of pigs that will track through all farm stages (thus making the results more realistic) and ii) reducing the runtime to a manageable level.

empty for cleaning and disinfection for one week. Assuming most large systems will raise pigs using some form of weekly/fortnightly batching, we assume the model system described below in Figure 7.2 is applicable for all MSs (small adjustments to the parameter estimates are possible to reflect a MS more accurately).

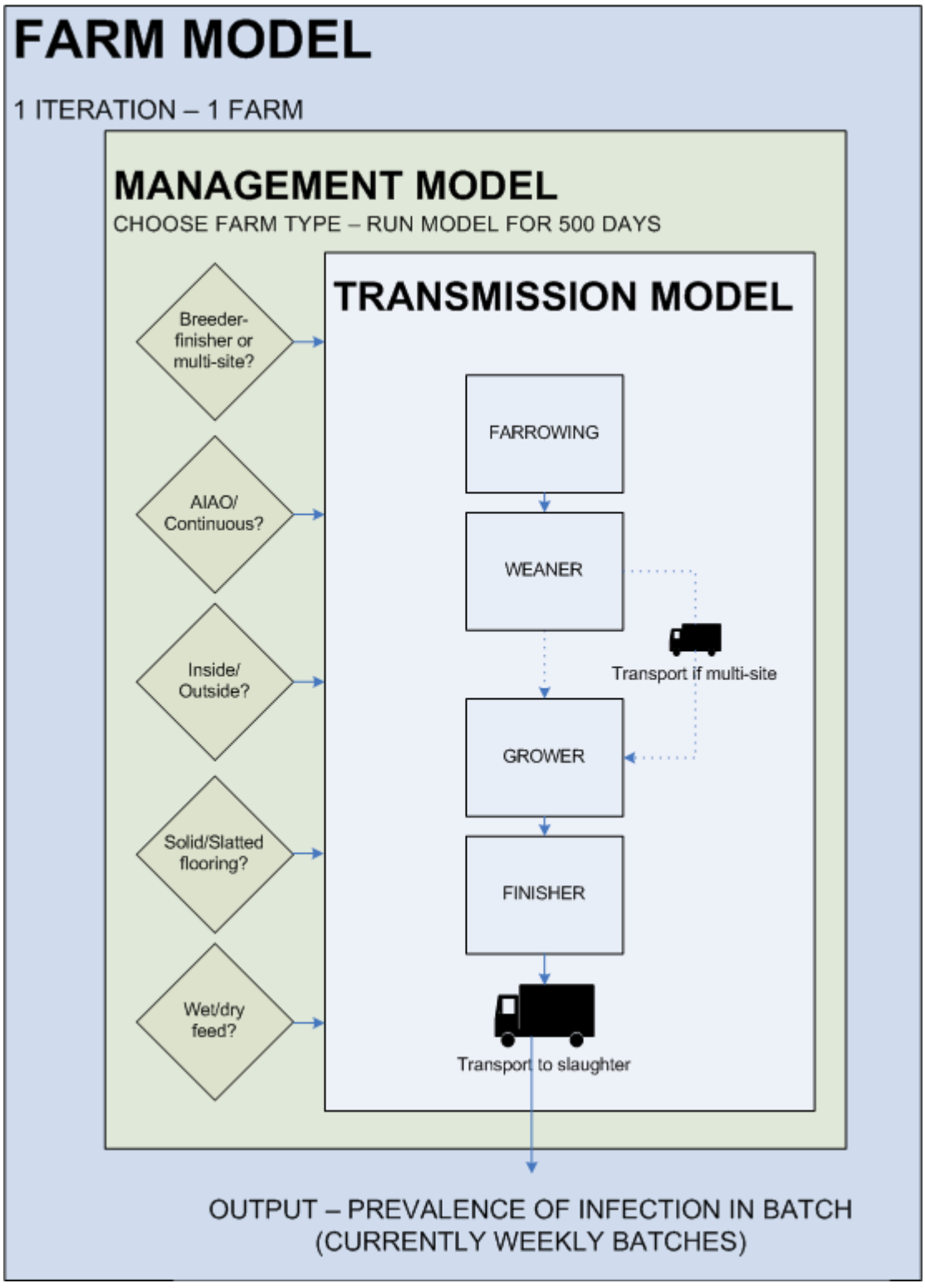


Figure 7.1: Overview of model flow.
The management model predicts the movement of pigs over 500 days. Each iteration of the simulation model represents one farm; the characteristics of the farm are chosen at the start of the iteration, e.g. inside AIAO breeder-finisher farm producing pigs on solid flooring using dry feed. The transmission model is initiated when the first infection occurs.

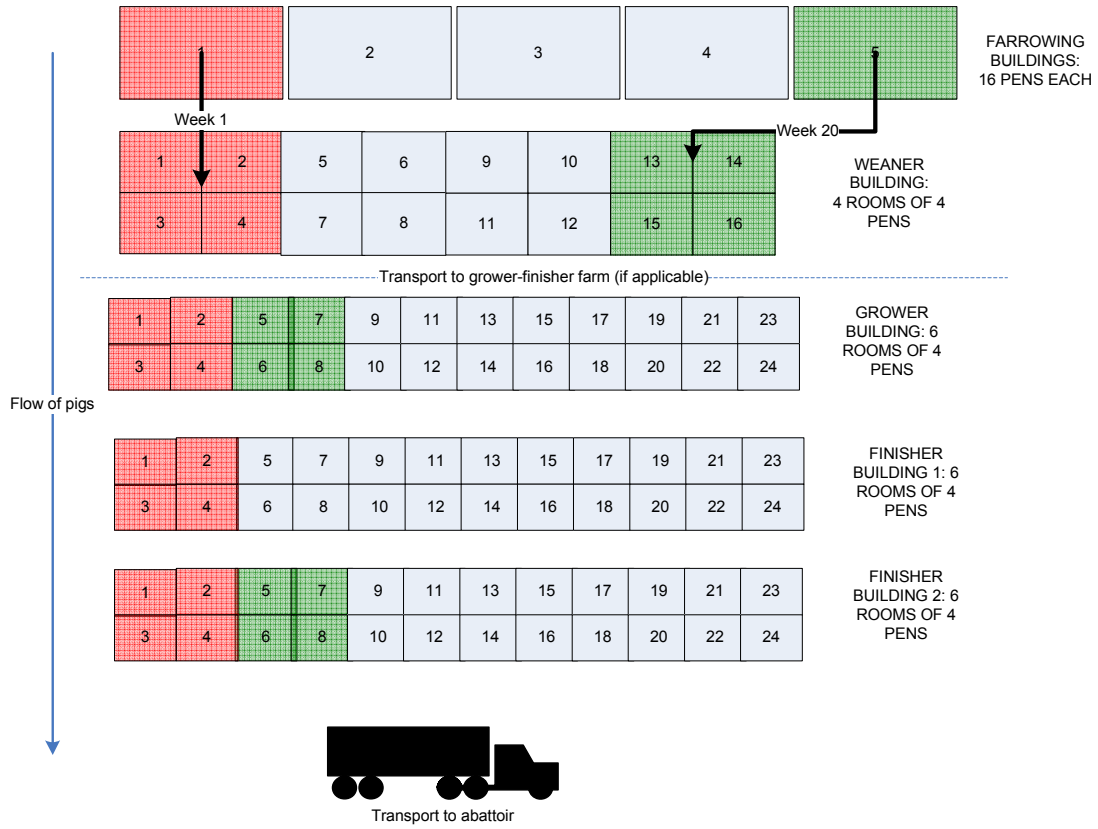


Figure 7.2: Schematic of pig flow through generic large farm system as modelled.
Pigs are reared through 4 distinct stages: farrowing (4 weeks - upon which one batch of pigs from farrowing building is mixed into 1 room of 4 pens in weaner building), weaning (4 weeks), growing (6 weeks) and finishing (12 weeks). Examples of flow are given by coloured annotations: red: piglets are weaned and grouped into batch of 4 pens within one weaner room at the start of Week 1, moved to growing accommodation on Week 5, finishing accommodation on Week 11 and slaughtered on Week 23; green: New group of sows moved into vacated farrowing building 5 on Week 16; piglets are weaned at start of Week 20 and pass through rooms in subsequent accommodation as they become empty at the time where movement occurs.

This system is relatively flexible: the schematic was described first for an all-in-all-out inside, breeder-finisher production (where 4 pens within the weaning, growing and finishing buildings are assumed to represent one room with adequate screening between other rooms to provide a biosecure area), but can be modified with relative ease for other systems such as continuous or outside production. The number of pens/rooms within a building, the number of buildings, and the number of pigs within a pen can all be modified too (although for simplicity we assume the number of weaners/growers/finishers within a pen must be a multiple of the number of pigs within a pen from the previous stage).

As discussed herd size is not included as a variable within a MS-specific model. We have taken a typical large farm herd size and applied it to the framework shown in Figure 7.2. Therefore, the large farm model is for a herd of 460 sows or alternatively 1600 finishers; while the latter is still relatively small for some countries (UK, Denmark), the dynamics of *Salmonella* transmission are reasonably captured, while reducing the computational effort required to run the model.

The overall framework shown in Figure 7.2 remains fundamental for any large farm type, but with the following modifications:

Breeder-finisher (BF): original framework as in Figure 7.2. Model accounts for different flooring types/feed types between farrowing/weaning stages and growing/finishing stages.

Breeder-weaner/Grower-finisher(BWGF): as for breeder-finisher, but transport (see Chapter 8) between weaner and grower stages. (Effect on *Salmonella* transmission because of the grower-finisher farm sourcing pigs from more than one farm is investigated as a scenario analysis).

Outside production: applies only to farrowing stage (assume all pigs moved indoors from weaning onwards). Farrowing transmission model parameters are modified to allow for decreased removal of faeces and increased cross-contamination of faecal material between sow crates (less biosecurity).

Continuous: Framework and flow as described in Figure 7.2, except no sectioning of weaning, growing or finishing buildings into rooms, i.e. number of rooms set to 1 (hence cross-contamination of faecal material between pens containing different cohorts of varying-aged pigs possible).

Flooring/Feed: As for outside production, differences in flooring/feed are achieved by modifying the values of transmission model parameters (i.e. removal of faecal material, dose response parameters).

The small farm model is a modification of the large farm model described above. We essentially reduce the large farm model down to one "cohort" of the large farm. The model framework is shown in Figure 7.3.

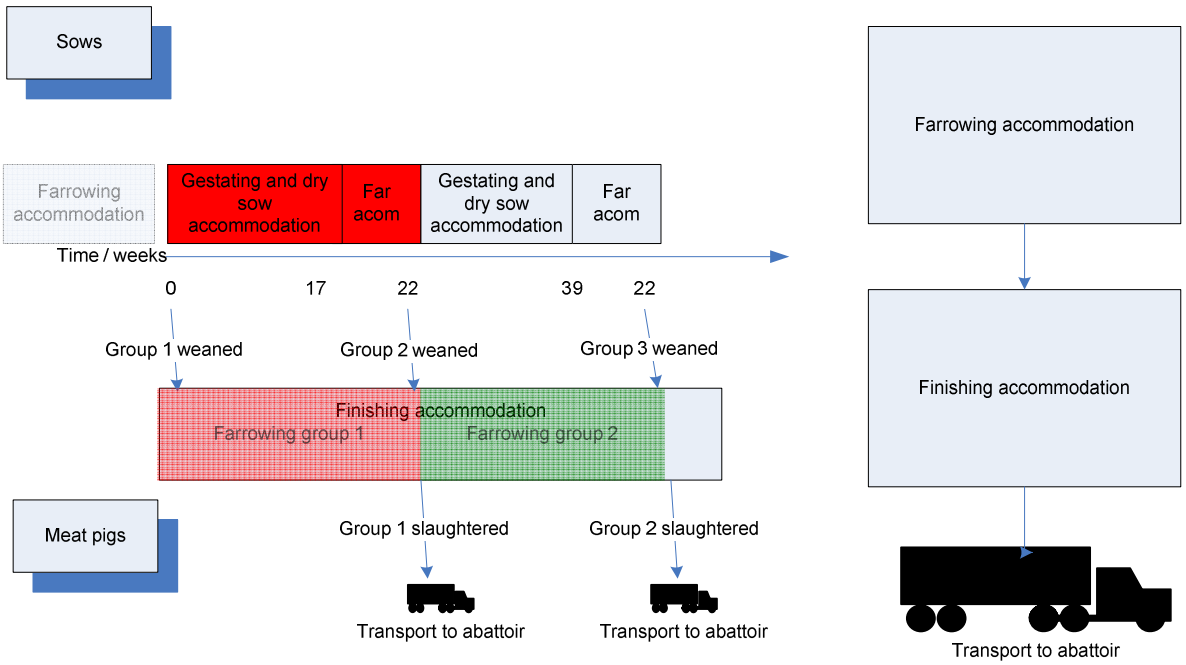


Figure 7.3: Model framework for small farm model.

Within the small farm model we model the maximum capacity for a small farm, 20 sows. Figure 7.3 shows that for a batch of up to 20 sows, then up to 200 pigs will be slaughtered every 22 weeks. There are only two accommodation blocks that slaughter pigs reside in, the farrowing and finishing accommodation. We assume all small farms are breeder-finisher farms.

As with large farms, each iteration of the small farm model represents one farm; the farm characteristics of each are selected according to the weighting taken from the EFSA breeding survey (similar to the large farm model). Transmission and introduction of *Salmonella* are treated the same as within the large farm model, where parameter estimation is determined according to the farm type.

Allocate farm types to 1,000 farms (iterations)

As described above, pig production in each MS is characterised by a heterogeneous mix of production systems. Therefore, as discussed above, five main factors of pig production are ascribed to each farm/iteration of the large and small farm model:

1. Breeder-finisher versus breeder-weaner/grower-finisher production
2. Inside versus outside production (breeding pig herds only)
3. All-in-all-out versus continuous production
4. Solid versus slatted flooring
5. Dry versus wet feed.

More detailed definitions of each of the types mentioned above are given in Table 7.1.

Table 7.1: Definitions of farm types used within the EU farm model.

Farm type	**Definition**
Breeder-finisher	Farm rearing slaughter pigs from birth to slaughter weight
Breeder-weaner	Farm rearing pigs from birth to approximately 8 weeks old (large farm only)
Grower-finisher	Farm rearing pigs from approximately 8 weeks old birth to slaughter weight (large farm only)
All-in-all-out (AIAO)	Farms producing pigs on strict batch system, where pigs within a batch are of same age, and with a barrier between other batches (e.g. within the model, we assume batches are kept in separate rooms) (large farm only)
Continuous	Any system that does not meet the criteria of AIAO above
Slatted flooring	Any flooring that contains slatting of some kind (be it partial or full)
Soild flooring	Any flooring that does not meet the criteria of slatted flooring
Wet feed	Any moisture-added feed Iinside production only)
Dry feed	Any feed that does not fit into wet feed category, including pelleted or compound feed.

From the above table definitions we consider a total of 56 farm types (see Table 7.2).

Table 7.2: Farm types included within the farm model. The proportions of each farm type are set from the EFSA breeding pig survey and other sources of data (VLA 2009).

Large farm			**Small farm**
Breeder-finisher	*Breeder-weaner*	*Grower-finisher*	*Breeder-finisher*
Inside AIAO Solid Dry	Inside AIAO Solid Dry	Inside AIAO Solid Dry	Inside Solid Dry
Inside AIAO Solid Wet	Inside AIAO Solid Wet	Inside AIAO Solid Wet	Inside Solid Wet
Inside AIAO Slat Dry	Inside AIAO Slat Dry	Inside AIAO Slat Dry	Inside Slat Dry
Inside AIAO Slat Wet	Inside AIAO Slat Wet	Inside AIAO Slat Wet	Inside Slat Wet
Inside Cont Solid Dry	Inside Cont Solid Dry	Inside Cont Solid Dry	Outside Solid Dry
Inside Cont Solid Wet	Inside Cont Solid Wet	Inside Cont Solid Wet	Outside Solid Wet
Inside Cont Slat Dry	Inside Cont Slat Dry	Inside Cont Slat Dry	Outside Slat Dry
Inside Cont Slat Wet	Inside Cont Slat Wet	Inside Cont Slat Wet	Outside Slat Wet
Outside AIAO Solid Dry	Outside AIAO Solid Dry	Outside AIAO Solid Dry	
Outside AIAO Solid Wet	Outside AIAO Solid Wet	Outside AIAO Solid Wet	
Outside AIAO Slat Dry	Outside AIAO Slat Dry	Outside AIAO Slat Dry	
Outside AIAO Slat Wet	Outside AIAO Slat Wet	Outside AIAO Slat Wet	
Outside Cont Solid Dry	Outside Cont Solid Dry	Outside Cont Solid Dry	
Outside Cont Solid Wet	Outside Cont Solid Wet	Outside Cont Solid Wet	
Outside Cont Slat Dry	Outside Cont Slat Dry	Outside Cont Slat Dry	
Outside Cont Slat Wet	Outside Cont Slat Wet	Outside Cont Slat Wet	

For each MS, the proportions of each farm type are estimated from the EFSA breeding pig survey and other sources of data (VLA 2009).

The framework of the model does not change according to farm type, only the parameter estimates assigned to each MS model.

Run large and small farm models

As stated the small and large farm models are run independently of each other for each MS, each being run for 1000 iterations, where each iteration is run over a 500-day cycle. A selection of these batches is then taken (weighted according to the proportion of pig production from large and small farms) to use an input to the Transport & Lairage model.

Anderson and May 1979 state that the timestep of an Susceptible-Infected-Recovered model (of which the farm model is a modified version) should be similar to the latent period

of infection, which is the time it takes from ingestion to infection. For pigs, this is estimated to be around 24-48 hours; hence the model is run on a 1-day timescale.

For the large farm, a batch of pigs is sent to slaughter every week. Therefore, over 500 days there are 72 batches sent to slaughter. These 72 batches represent the saved output of each farm/iteration. For the small farm a batch will be sent to slaughter approximately every 26 weeks, and so there are 3 batches sent to slaughter within the 500-day cycle of each farm.

At the start of each iteration, the slaughter pigs present on that farm are all susceptible, and hence not shedding any *Salmonella* into the pig environment. However, sows may be *Salmonella*-positive, and so may shed *Salmonella* into the environment (explained in more detail in Section 7.4.3). There is also the potential for *Salmonella* to be ingested by slaughter pigs through contaminated feed and/or the external environment. This ingestion of *Salmonella* is highly variable and infection may (or may not) occur at any time point in the 500-day cycle.

The random nature of the seeding of infection also means that the time at which the model is started is also completely arbitrary. In reality most farms are old and will have been infected at some point in time, but we cannot run the model from the initial startup of a farm (this would require running the model for years of production, currently not feasible).

Management and movement of pigs

For all stages of production we use the following notation for the *k*th pig in the *j*th pen of building/room *l*: $k = \{1,2,...,n_{pig}\}$, $j = \{1,2,...,n_{pen}\}$ and $l = \{1,2,...,n_{room}\}$ where n_{pig} is the number of pigs within a pen, n_{pen} the number of pens within a farrowing house or a room of a weaning, growing or finishing house, and n_{room} the number of rooms/buildings within the stage of production (e.g. there are 5 buildings of 16 pens within farrowing, and 6 rooms of 4 pens within growing production). For finishing production there are 6 rooms (of 4 pens each) within two finishing buildings. For ease of notation we consider the two finisher buildings as one building (i.e. there are 12 rooms within the finishing building).

For farrowing, there is one sow in each pen. Each sow gives birth to a constant number of n_{pig} piglets (we assume that *Salmonella* transmission is insensitive to the number of piglets in a litter surviving to weaning). Piglets are weaned *wa* days after birth. Assuming weekly slaughter batches, one batch (i.e. one building) of piglets are weaned at the beginning of each week (e.g. *t* = 1 or 8) and the piglets from the *j* pens in building *l* are mixed into large groups for placement into weaner accommodation.

There is one weaner building; weaners will spend *ga* days in the weaner accommodation before being moved as intact pen groups into the grower accommodation. For the grower accommodation, growers will spend *fa* days in this accommodation before being moved as intact pen groups into the final stage of finishing. Finishing is identical to the growing accommodation, except there are two buildings instead of one. Finishing pigs spend *sa* days in this accommodation before being sent to slaughter.

One point to make explicitly clear is the difference between AIAO and continuous production. For AIAO production, within the weaning/growing/finishing building there will be 4/4/6 distinct compartments/rooms where pigs are raised. For continuous production, there is no barrier preventing cross-contamination of pens etc, and the weaning/growing/finishing building is assumed to represent one compartment.

At t = 1, four of the five farrowing buildings are occupied (the remaining pen is empty for cleaning and drying). Within all other stages, all pens are occupied (in discussion with experts, it seems unlikely that many farmers would practice downtime beyond the farrowing stage). The *Salmonella* status of each slaughter pig k (i.e. not sow) in pen j of room/building l at time t, $\Omega(k,j,l,t) = 0 \; \forall \; k, j, l$, where 0 represents susceptible status, 1 represents infected status) (see Section 7.4.3 for further description). Movement of pigs from one production stage to another, and to slaughter, takes place on specific days within the model. This is the set **t'** = {1,8,15,… 498}. Each individual movement time is denoted *t'*.

We start movement of pigs from one production stage using building or room 1. Therefore, on day 1 the following movements occur:

a. Piglets from farrowing building 1 grouped into 4 pens → moved into weaner room 1. Sows from farrowing building 1 moved back to service/gestation accommodation. Farrowing building 1 left empty for one week. New batch of sows ready to give birth moved into farrowing building 5.
b. Weaners from room 1 moved to growing room 1.
c. Growers moved from growing room 1 into finishing room 1 of finishing house 1.
d. Finishers within finishing room 1 (building 1) transported to slaughter.

These same movements occur, but for different rooms/buildings, on days **t'**. Production is staggered sequentially; for example, on day 8, piglets are moved out of farrowing building 2 and moved into weaner room 2, and on day 15 piglets are moved out of farrowing building 3 and moved into weaner room 3. I

For slaughter pigs that are finished on a grower-finisher farm, it is assumed that they were reared on a breeder-weaner farm and transported to the grower-finisher farm. Within some studies transport has been highlighted as a risk factor for *Salmonella* transmission between pigs (Berends *et al.* 1996), and hence transport is included within the BWGF model. Transport between farms is assumed to be almost identical to transport between the finishing house and slaughterhouse, hence the model we use here is largely based on the transport model (Chapter 8). There are important similarities and exceptions, outlined below:

1. As for the main transport model, we assume one batch is transported in one vehicle.
2. Only one batch is transported from the weaner to growing stage at a time.
3. Unless direct data available, assume duration of travel has a similar distribution as transport to slaughterhouse.

7.4.2 Summary of inputs and outputs

There is no specific input to the model in terms of *Salmonella*. The main input is the type of farms which will be included within each MS model, which is derived from the EFSA breeding survey management data and other sources. *Salmonella* is seeded into the environment over the 500 day cycle of each farm within a MS simulation model from three different potential sources – the sow, feed and external contamination. However, infection of slaughter pigs does not necessarily occur.

The output of the farm model is the within-batch lymph-node prevalence of pigs at the point of depopulation (represented by the set b). For the large farm there will be approximately 72,000 batches from 1000 farms, and for small farms 3000 batches from 1000 farms. It is assumed that this database of batch prevalence represents national production, so that the average prevalence over all batches represents the national slaughter pig prevalence (before transport to the slaughterhouse) for that MS. As stated above, a selection of these batches is then taken (weighted according to the proportion of pig production from large and small farms) to use an input to the Transport & Lairage model.

7.4.3 Transmission model

<u>Faecal shedding</u>

For the rest of this section we write generally for all stages of production (farrowing, weaning etc) unless explicitly stated. We define the amount of faecal material shed by a pig in any one timestep (one day) as $\Phi(k,j,l,t)$, where k represents an individual pig within pen j of room l and $\Phi(k,j,l,t) = \Re\left(N\left(\mu_\Phi, \sigma_\Phi^2\right)\right)$ (for farrowing pen – sow is defined as k = 1, piglets k={2,3,..11}).

Therefore, the amount of faecal material shed into pen j in room l on day t is given by:

$$F_{pig}(j,l,t) = \sum_{k=1}^{k=n_{pig}} f(k,j,l,t)$$

except at the farrowing building where $F_{pig}(j,l,t) = \sum_{k=1}^{k=n_{pig}} f(k,j,l,t) + f_s(k,j,l,t)$.

n_{pig} is equal to 40 for weaning growing and farrowing stages, and 11 (1 sow and 10 piglets) for farrowing.

We also define $\beta_{F,day}$ and $\beta_{xc,day}$ as the proportional factors with which faecal material shed <u>on day *t*</u> is removed from the pen via slatted flooring/cleaning and cross-contamination of <u>adjacent</u> pens respectively. These are samples from beta distributions and consequently we define $\beta_{f,day}(j,l,t) = \Re\left(Beta\left(\alpha_{\beta_{f,day}}, \beta_{\beta_{f,day}}\right)\right)$ and $\beta_{xc,day}(j,l,t) = \Re\left(Beta\left(\alpha_{\beta_{xc,day}}, \beta_{\beta_{xc,day}}\right)\right)$.

Therefore, the amount of faecal material shed in pen j on day t and available to be ingested by pigs within pen j, $f_{day}(j,l,t)$, is given by:

$$F_{day}(j,l,t) = F_{day}(j,l,t) \cdot \beta_{f,day}(j,l,t) \cdot \beta_{xc,day}(j,l,t) \qquad (7.1)$$

The amount of faecal material that was present on day [*t*-1] removed by cleaning/slatted flooring etc at the end of day *t*, $F_{old}(j,l,t)$, can be calculated as:

$$F_{old}(j,l,t) = F(j,l,t-1) \cdot \beta_{f,old}(j,l,t) \qquad (7.2)$$

Where $F(j,l,t-1)$ is the amount of faecal material at the end of day [*t*-1] and $\beta_{F,old}$ is a removal coefficient estimated in a similar fashion to $\beta_{F,day}$.

Similarly the amount of faecal material cross-contaminated to adjacent pens is given by:

$$F_{xc}(j,l,t) = F(j,l,t-1) \cdot \beta_{xc,old}(j,l,t) \qquad (7.3)$$

where $\beta_{xc,old}$ is a removal coefficient estimated in a similar fashion to $\beta_{xc,day}$. Should $F_{old}(j,l,t)+F_{xc}(j,l,t) > F(j,l,t-1)$ then $F_{old}(j,l,t)+F_{xc}(j,l,t)$ is truncated such that $F_{old}(j,l,t)+F_{xc}(j,l,t) = F(j,l,t-1)$. (However, this is an extremely rare event with the current parameter estimation).

Therefore the amount of faecal material within a pen *j* at the end of time *t* is

$$F(j,l,t) = \begin{cases} F(j,l,t-1)+F_{pig}(j,l,t)[1-\beta_{Fday}(j,l,t)-\beta_{xcday}(j,l,t)]-F_{old}(j,l.t)-F_{xc}(j,l,t)+F_{xc}(j+1,l,t)/2 & \text{if } j=1 \\ F(j,l,t-1)+F_{pig}(j,l,t)[1-\beta_{Fday}(j,l,t)-\beta_{xcday}(j,l,t)]-F_{old}(j,l.t)-F_{xc}(j,l,t)+F_{xc}(j-1,l,t)/2+F_{xc}(j+1,l,t)/2 & \text{if } j=\{2,..\mathrm{n}_{\mathrm{pen}}-1\} \\ F(j,l,t-1)+F_{pig}(j,l,t)[1-\beta_{Fday}(j,l,t)-\beta_{xcday}(j,l,t)]-F_{old}(j,l.t)-F_{xc}(j,l,t)+F_{xc}(j-1,l,t)/2 & \text{if } j=\mathrm{n}_{\mathrm{pen}} \end{cases} \qquad (7.4)$$

We assume cleaning out of faecal material at this depopulation time is efficient, therefore $F(j,l,t) \sim 0$, for all rooms which are depopulated/re-populated, and $\mathbf{r(t')}=\{\mathbf{r_{far}},\mathbf{r_w},\mathbf{r_g},\mathbf{r_{fin}}\}$ represents the sets of pens depopulated (in farrowing, weaning, growing or finishing building) at times **t'** = {1,8,… 492}. In contrast, it is assumed that salmonella removal will not be 100% efficient (as salmonella may be released from the faecal material and reside in biofilms or hard-to-clean areas such as feeder tube nipples).

Infection status

Based on the literature review in Section 7.3, we assume that a slaughter pig *k* within pen *j* located in room/building *j* will be in any one of two states at time *t*

Susceptible ($\Omega(k,j,l,t = 0)$: Pig is not infected.

Lymph-node positive ($\Omega(k,j,l,t)= 1$): Pig is infected in ileo-caecal lymph-node and will excrete *Salmonella* intermittently. If it is shedding it sheds *Salmonella* at a concentration dependent on whether it was infected at a "low" ($<10^6$ CFUs) or "high" dose ($\geq 10^6$ CFUs) (based on the observed differences in shedding rates from Jensen *et al.* (2006). (More detail on how we model the shedding of *Salmonella* is given later).

Lymph-node positive status is used as it is an ideal characteristic at the point of slaughter for which to validate the model (given this was the primary sample type for the EFSA baseline slaughter pig survey). However, lymph-node positive does not equate to a status of infectious or excreting *Salmonella*. Rather, it is an indication of the fact the pig still has a *Salmonella* infection and can *potentially* shed *Salmonella*. We then loosely use this characteristic to determine whether the pig is shedding or not, and if so at what level. As no data were available, we assume pigs immediately return to the "Susceptible" state following recovery from being lymph-node positive. Recovery from the "infected" state takes t_{LN} days.

The number of susceptible and lymph-node positive pigs within a pen at time *t*, $S(i,j,t)$ and $I(i,j,t)$ respectively, are therefore defined as:

$$S(j,l,t) = n_{pig} - I(j,l,t)$$
$$I(j,l,t) = \sum_{k=1}^{n_{pig}} \Omega(k,j,l,t) \qquad (7.5a\text{-}b)$$

Sources of infection

Based on the EFSA Scientific Opinion on *Salmonella* in pigs (EFSA 2006) and the literature review carried out in Section 7.3, the following sources of infection are thought to be important: other infected pigs (including new stock/mixing of pigs/sows, but also the carry-over of *Salmonella* from previously infected batches), feed and wildlife. These three sources are modelled to varying degrees, depending on the data available.

Sows

The herd prevalence for *Salmonella* infection of breeding sows was estimated for each MS within the EU from the EFSA breeding survey, which was supplied directly via EFSA (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178662632875.htm).).
This survey provides the estimates for the herd prevalence of *Salmonella* infection in each case study MS, p_{herd}. The status of the breeding pig herd within the farm model is therefore given as:

$$\Omega_{binf} = \Re(B(1, p_{herd})) \qquad (7.6)$$

where $\Omega_{binf} = 1$ signifies that the breeding pig herd is infected with *Salmonella* and $\Omega_{binf} = 0$ signifies a *Salmonella*-negative breeding pig herd.

The within-herd prevalence of infection, p_w, will vary between farms, as well as MSs. The status of each individual sow entering a farrowing room is determined as follows:

$$\Omega_{sow}(j,l,t) = \begin{cases} 0 & \Omega_{\text{binf}} = 0 \\ \Re(B(1,p_w)) & \Omega_{\text{binf}} = 1 \end{cases} \qquad (7.7)$$

Where $\Omega_{sow}(j,l) = 1$ denotes that the sow in farrowing pen *j* in building *l* is *Salmonella* positive and 0 denotes a susceptible sow. As each group of piglets reach weaning age a new group of sows are placed into the farrowing house and Equation7.7 is used again to determine the status of each sow within that house (e.g. the first piglets in Farrowing House 2 will reach weaning age at day 8, and hence the sows are removed and replaced on this same day).

Each sow will produce $f_{sow}(j,l,t)$ faeces per day. If the sow is shedding it will shed salmonella into the environment at a rate $c_s(j,l,t)$ (CFUs per gram of faeces). Therefore over a daily period a sow will shed $f_{sow}(l,t)c_s(l,t)$ salmonellas (denoted as $\lambda(j,l,t)$). Note that sows are treated as a "static" source of infection within the model: they are not infected by either of the other sources considered below, or by the shedding of their neighbours.

Feed

For simplicity, we assume feed can be broken down into two major types: wet (*w*) and dry (*d*). We also assume that feed delivery type will be in bulk for large farms and bagged for small farms, where deliveries are made twice a week for bulk deliveries and once a month for bagged deliveries. We assume pigs will consume *g* grams of feed per day and that a pig is exposed to a single feedlot every 4 days on a large farm and every 28 days on a small farm.

We define the prevalence of feedlot contamination as p_{feed}, then the status of a particular feedlot, Ω_{feed}, can be determined using:

$\Omega_{feed} = \Re(B(1, p_{feed}))$

The concentration of *Salmonella* in feed (per gram) can therefore be given as:

$$c_f = \begin{cases} 0 & \text{if } \Omega_{feed} = 0 \\ c_f & \text{if } \Omega_{feed} = 1 \end{cases}$$

Estimation of c_f is described later.

External contamination (wildlife, boots etc)

There are no data to quantify the frequency and magnitude of any external contamination of the farm (and how these two factors will vary between farms). However, there are some data on wildlife incursion into farms, and the amount of *Salmonella* rodents or birds might contaminate the environment with defecation. We therefore chose to use wildlife (specifically rodents and birds) as a proxy for external contamination.

A study into the transmission of *Salmonella* between wildlife and meat-production animals (Skov *et al.* 2008) suggests that wildlife within the vicinity of farms are more commonly infected with *Salmonella* if the pigs themselves are infected. Therefore we assume that the status of the wildlife, $\Omega_w(t)$, is equivalent to the status of the farm, i.e. $\Omega_w(t)$ = 1 if or one or more slaughter pigs are in state lymph-node positive, i.e. $\sum_l \sum_j \sum_k \Omega(k, j, l, t) > 0$.

Rodents and birds are then assumed to contribute λ_e *Salmonella* organisms to the dose of each pig for each time step onwards (assuming, in the absence of any other data, each pig will ingest roughly 1g of rodent/bird faeces per day).

Studies have shown that prevalence within rodents/birds on an infected pig farm are fairly low, around 1-5% (Davies & Wray 1995; Skov *et al.* 2008). We therefore set a switch within the model such that pig ingestion of *Salmonella* through external contamination occurs relative to the prevalence within wildlife. The concentration of *Salmonella* within wildlife faeces appears to be similar to that within pigs (Davies & Wray 1995), hence, in the absence of rigorous quantitative data, we simply allocate a lognormal distribution for λ_e that on visual inspection gives a biologically plausible estimate.

Introduction of infection

From the above sources of infection pigs may be exposed at any time *t* to some *dose* from either sows, feed or external contamination, λ_s, λ_f and λ_e respectively. These are the daily doses (units of CFUs/day), hence we must scale up from the concentration in faeces, feed and external contamination to the amount of each ingested (for example, amount of faecal

material or feed ingested per day). Therefore the total dose each slaughter pig will ingest at time *t* before introduction of *Salmonella* into the *slaughter pigs*, is given by $\lambda_s + \lambda_f + \lambda_e$. The dose-response model described below is then used to determine whether one or more pigs become infected.

Transmission

Once infection of one or more pigs occurs, then transmission is driven by the shedding of contaminated faeces by slaughter pigs, as well as the sources of infection (sow, feed and external contamination). In order to model the required interventions, we have taken a more detailed approach than in other *Salmonella* in pigs transmission models such as that of Hill *et al.* (2008), which use an all-encompassing transmission parameter. Here we undertake the detailed modelling of faeces and the cross-contamination of this faecal material between pens, the removal of faecal material and its ingestion by pigs. A schematic diagram of the transmission model framework for one pen (relevant to all pens, buildings and stages of production) is given in Figure 7.4.

The amount of *Salmonella* shed into the pen environment each day, $\gamma(j,l,t)$ can be given by:

$$\gamma(j,l,t) = \sum_{k=1}^{k=n_{pig}} c_p(k,j,l,t)\cdot f(k,j,l,t)$$
$$\gamma_s(j,l,t) = \sum_{k=1}^{k=n_{pig}} c_p(k,j,l,t)\cdot f(k,j,l,t) + \lambda(j,l,t) \quad , \qquad (7.8)$$

where c_p is the amount of *Salmonella* shed by an infected pig, and is generated from a combination of a discrete general and uniform distribution, i.e. $\Re(U(10^{\Re(DG(a_\varepsilon,\ b_\varepsilon)-1}, 10^{\Re(DG(a_\varepsilon,\ b_\varepsilon)})$, where we choose the order of magnitude of shedding (e.g. 2, 3 log CFU per gram of faeces) based on published literature.

We can write very similar equations for the total amount of *Salmonella* in the pen environment as above for faecal material.

Therefore,

$$E_{day}(j,l,t) = \gamma(j,l,t)\cdot\beta_{f,day}(j,l,t)\cdot\beta_{xc,day}(j,l,t) \qquad (7.9)$$
$$E_{old}(j,l,t) = E(j,l,t-1)\cdot\beta_{f,old}(j,l,t) \qquad (7.10)$$
$$E_{xc}(j,l,t) = E(j,l,t-1)\cdot\beta_{xc,old}(j,l,t) \qquad (7.11)$$

where E_{old} and E_{xc} are amounts of *Salmonella* removed at t-1 and removed via cross-contamination respectively. As for faecal material, should $E_{old}(j,l,t)+E_{xc}(j,l,t) > E(j,l,t\text{-}1)$ then $E_{old}(j,l,t)+E_{xc}(j,l,t)$ is truncated such that $E_{old}(j,l,t)+e_{xc}(j,l,t) = E(j,l,t\text{-}1)$.

The total amount of *Salmonella* in pen *j* at the end of day *t* is given by

$$E(j,l,t) = \begin{cases} \left(10^{\log(E(j,l,t-1))-\delta}\right) + \gamma(j,l,t)[1-\beta_{Fday}(j,l,t)-\beta_{xcday}(j,l,t)] - E_{old}(j,l,t) - E_{xc}(j,l,t) + E_{xc}(j+1,l,t)/2 & \text{if } j=1 \\ \left(10^{\log(E(j,l,t-1))-\delta}\right) + \gamma(j,l,t)[1-\beta_{Fday}(j,l,t)-\beta_{xcday}(j,l,t)]E_{day}(j,l,t) - E_{old}(j,l,t) - E_{xc}(j,l,t) + E_{xc}(j-1,l,t)/2 + E_{xc}(j+1,l,t)/2 & \text{if } j=\{2,..n_{pen}-1\} \\ \left(10^{\log(E(j,l,t-1))-\delta}\right) + \gamma(j,l,t)[1-\beta_{Fday}(j,l,t)-\beta_{xcday}(j,l,t)] - E_{old}(j,l,t) - E_{xc}(j,l,t) + E_{xc}(j-1,l,t)/2 & \text{if } j=n_{pen} \end{cases} \qquad (7.12)$$

where δ is the decay rate of *Salmonella* per day.

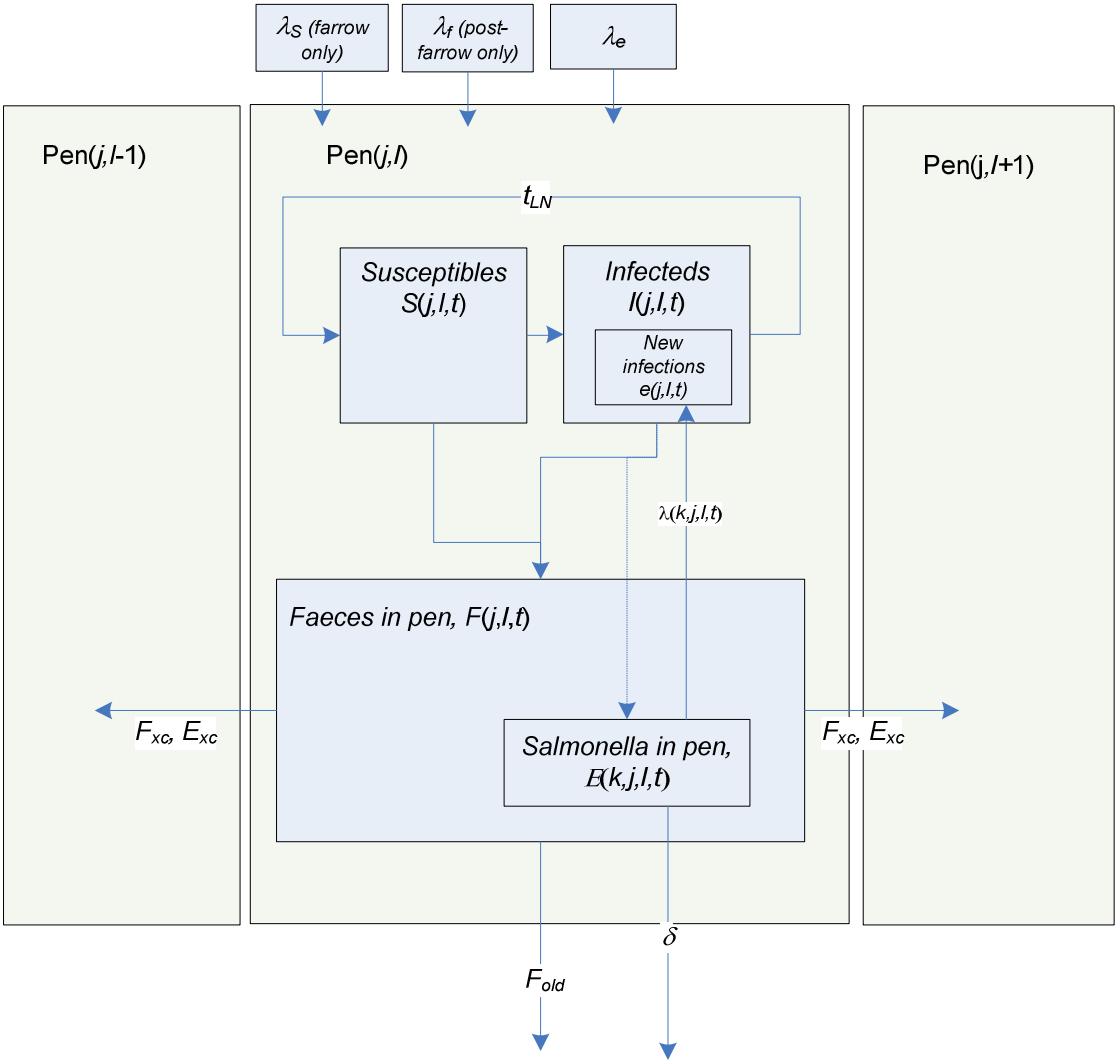


Figure 7.4: Schematic diagram of transmission model.
Only the interactions from pen(*j*,*l*) are shown. The total faecal material in the pen, *F*, is added to each day *t* by both Susceptibles (*S*) and Infecteds (*I*) and cross-contamination from other pens, F_{xc} (from either pen *j*+1 or *j*-1), or subtracted via cross-contamination or removal, f_{old}. This faecal material contains *E* salmonellas, added to each day from the infected group shedding in their faeces, and subtracted from via decay δ and cross-contamination E_{xc}. Susceptible pigs ingest λ organisms per day via the amount in the faeces, feed (λ_f) or environment (λ_e), which then produces *e* new infections according to the dose ingested and the dose-response relationship applied.

We assume there is imperfect removal of salmonella during cleaning and/or disinfection. Therefore, for rooms depopulated/repopulated **r**(**t'**), $E(j,l,t) = E(j,l,t)\cdot\beta_C$, where β_C ~ Beta(α_{β_c}, β_{β_c}) and is the percentage of salmonella remaining in the pen environment after cleaning and t_C is the time between depopulation and repopulation (7 days for farrowing, zero for other stages)[15].

For simplicity we assume that *Salmonella* is homogenously mixed within all faecal material within the pen. Therefore the average concentration of *Salmonella* within a gram of contaminated faecal material, *c*, can be given by

$$c(j,l,t) = \frac{E(j,l,t)}{F(j,l,t)}$$

We assume all (*Salmonella*-negative and positive) pigs ingest some faecal material each day. Therefore, each pig will ingest $\lambda_i(k,j,l,t)$ organisms through faecal ingestion on day *t* according to the following equation (shown in matrix form):

$$\begin{bmatrix} \lambda_i(1,1,l,t) & \cdots & \lambda_i(n_{pig},1,l,t) \\ \vdots & & \vdots \\ \lambda_i(1,n_{pen},l,t) & & \lambda_i(n_{pig}n_{pen},1,l,t) \end{bmatrix} = \begin{bmatrix} \mu(1,1,l,t)\cdot c(1,l,t) & \cdots & \mu(1,n_{pen},l,t)\cdot c(n_{pen},l,t) \\ \vdots & & \vdots \\ \mu(n_{pig},1,l,t)\cdot c(1,l,t) & & \mu(1,1,l,t)\cdot c(n_{pen},l,t) \end{bmatrix} \quad (7.13)$$

where $\mu(k,i,j,t) = \Re\left(N\left(m_{ing},\sigma_{ing}\right)\right)$ and m_{ing} is the mean mass of faeces ingested by pigs, and σ_{ing} is the associated standard deviation.

The number of *Salmonella* ingested through consumption of contaminated feed of type, $\lambda_f(k,j,l,t)$ is given by

$$\lambda_f(k,j,l,t) = \begin{cases} 0 & \text{if } \Omega_{feed} = 0 \\ g\cdot c_f & \text{if } \Omega_{feed} = 1 \end{cases}$$

where *g* is the amount of feed consumed per day. The total number of *Salmonella* ingested by each pig on day *t*, $\lambda(k,i,j,t)$ can therefore be given as:

$$\lambda(k,i,j,t) = \lambda_i(i,j,t) + \lambda_f(k,j,l,t) + \lambda_e(k,j,l,t) \quad (7.14)$$

[15] If $E(j,l,t) = E(j,l,t)\cdot\beta_C < 0$ then $E(j,l,t) = 0$..

The probability of a pig becoming infected because of ingesting $\lambda(k,i,j,t)$ organisms, $p_{inf,H,FT}(k,i,j,t)$, is assumed to follow a beta-binomial dose-response relationship, hence at an individual pig level:

$$p_{\inf}(k,i,j,t) = (1-\Omega)\cdot\left[1-\left((1-Beta(\alpha_{DR},\beta_{DR}))^{\lambda(k,j,l,t)}\right)\right] \qquad (7.15)$$

where α_{DR} and β_{DR} are the shape and scale parameters of the beta binomial dose response model, and are dependent on feed type (*wet* or *dry* – see parameter estimation).

The number of newly infected pigs in pen *j* and building *l*, $e(j,l,t)$, can therefore be defined as

$$e(j,l,t) = \sum_{k=1}^{n_{pig}} B(1, p_{\inf}(k,j,l,t)) \qquad (7.16)$$

Each of the newly infected pigs are assigned a duration for being lymph-node positive, t_{LN}. Hence, at time $t_{inf}+t_{LN}$ (time of infection + length of infection) a pig will return to "Susceptible" status (if it has not been transported to slaughter first). The number of recovered pigs within a pen at time *t*, $R(j,l,t)$, is simply the sum of infected pigs that have reached the end of their infection period.

Therefore the number of Susceptible ($S(j,l,t)$) and LN-positive ($I(j,l,t)$)) pigs within a pen at the end of day *t* is calculated as follows:

$$S(j,l,t) = S(j,l,t-1) - e(j,l,t) + R(j,l,t)$$

$$I(j,l,t) = I(j,l,t-1) + e(j,l,t) - R(j,l,t)$$

The prevalence of infection within each pen at time *t* is then simply $I(j,l,t)/n_{pig}$.

Output

The output of the model is the prevalence of infection (defined as lymph-node positive) within batches of pigs placed on transport to slaughter. This occurs weekly, i.e. one finishing room from one of the finishing buildings is emptied on each of the movement timesteps **t'** discussed above.

Therefore, the prevalence of lymph-node positive pigs at slaughter within a batch, r_{fin}, of pigs at time *t'*, $p_i(t')$, can be given by

$$p_i(t') = \frac{\sum_{i=1}^{4} I(r_{i,fin}, t')}{4 * n_{pig}}, \qquad (8)$$

where r_i is a member of the set $\mathbf{r}_{fin}$, the set of pens emptied at slaughter time t', and the denominator is multiplied by 4 as there are 4 pens in a room.

7.4.4 Parameter estimation

Management parameters

Management parameters are defined as those that determine the characteristics of a farm, or that of a MS's pig production structure, e.g. the number of pigs within a pen, or the proportion of farms within a MS that rear piglets outdoors. The estimates for each of the parameters specific to large and small farm management are shown in and Table 7.3 and Table 7.4.

The only published data we have been able to find from the case study MSs have been from MS2 sources. From the management data collected as part of the EFSA breeding survey it does not appear that there is a significant difference in the numbers associated with length of stage, numbers within in pens etc. As such, the estimates used are kept identical between the case study MSs, although they can be changed at a later date if desired.

We are aware that there is variability in the systems described above, such as when pigs will be weaned. However, such variability is difficult to include due to the complexity of describing the population/depopulation of different pens over time. We also realise that variability in weaning age etc might cause changes in the transmission of infection, but because of factors that we cannot capture in the model (e.g. stress, varying growth rate of pigs) investing time in including variability in weaning age etc was not considered efficient. A major factor in determining variation between MSs is the proportion of different farm types within that MS.

Little data were available to assess the number of pigs within a pen etc. Available books on the subject of pig housing and pig management tend to be older, although some information has been gleaned from these (Brent 1986; Sainsbury 1976). A 460-sow unit was chosen as a relatively large size for an EU pig farm. In addition, as an individual-based model the time it takes to run the large farm model is directly related to the number of pigs flowing through the farm, and hence a number was chosen which would represent a large farm but also restrict runtime of the model to a manageable level. Therefore, the framework described in Figure 7.2 and numbers chosen for the length of each stage and the size of buildings, rooms etc have been chosen to reflect expert opinion on the structure of a typical large commercial pig farm, and also to optimise the model.

Table 7.3: Estimates for large farm management parameters

Notation	Description	Stage*	Unit	Value	Comment/reference
n_{pig}	Number of pigs within a pen	Far W G Fin	-	11 40 40 40	Far - 1 sow, 10 piglets (Brent 1986; Sainsbury 1976) Pig Yearbook, 2008
n_{pen}	Number of pens within a room/building	Far W G Fin	-	16 AIAO 4 Cont 16 AIAO 4 Cont 24 AIAO 4 Cont 24	Assumed
n_{room}	Number of rooms within a building	Far W G Fin	-	1 4 6 6 (2 buildings)	Assumed
wa	Age at weaning		Day	28	
ga	Growing period		Day	42	(Brent 1986; Sainsbury 1976)
fa	Finishing period		Days	84	Pig Yearbook, 2008

The only published data we have been able to find from the case study MSs have been from MS2 sources. From the management data collected as part of the EFSA breeding survey it does not appear that there is a significant difference in the numbers associated with length of stage, numbers within in pens etc. As such, the estimates used are kept identical between the case study MSs, although they can be changed at a later date if desired.

We are aware that there is variability in the systems described above, such as when pigs will be weaned. However, such variability is difficult to include due to the complexity of describing the population/depopulation of different pens over time. We also realise that variability in weaning age etc might cause changes in the transmission of infection, but because of factors that we cannot capture in the model (e.g. stress, varying growth rate of pigs) investing time in including variability in weaning age etc was not considered efficient.

Table 7.4: Estimates for small farm management parameters.

Notation	Description	Stage*	Unit	Value	Comment/reference
n_{pig}	Number of pigs within a pen	Far W G Fin	-	11 10 10 10	Far - 1 sow, 10 piglets (i.e. # piglets from sow…assumed)
n_{pen}	Number of pens on farm	Far W G Fin	-	10 10 10 10	Assumed (i.e. equal to number of sows on farm)
wa	Age at weaning		Day	28	
ga	Growing period		Days	28	
fa	Finishing period		Days	63	

The farm type (whether it uses wet feed, keeps pigs outdoors or on slats etc) is likely to contribute more to the variability in *Salmonella* transmission than the number of pigs kept in a pen. This variability is reflected in the model, as each iteration picks one particular farm type to present that iteration's "farm". There are clear differences in the structure of each case study MSs' pig production (see Table 7.5) (from the EFSA breeding survey). This survey is also the most comprehensive and representative data we have, and so more resource has been invested in modelling the differences between MSs that can be quantified using this dataset (i.e. farm type).

Transmission parameters

Pig/*Salmonella* parameters for the transmission model are those physical characteristics that determine the transmission of *Salmonella* between pigs, e.g. the amount of faecal material produced by a weaner per day, or the concentration of *Salmonella* within that faecal material if the pig is infected. The parameter estimates for *Salmonella* and pig characteristics are shown in Table 7.6.

A full literature review was carried out to determine parameter estimates, however some parameters were not quantifiable. Where this occurred, we used expert opinion and for some rather abstract parameters (such as the amount of faecal material cross-contaminated between pens per day) the estimates were determined by graphing plausible estimates and assessing which looked more correct (via author's opinion). Clearly more data need to be collected for these parameters.

Sources of infection

Sow

The breeding pig herd prevalence of each case study MS was taken from the EFSA breeding pig survey, and assumed to be directly equivalent to p_{herd}. The within-herd prevalence of infection was available from additional work carried out for the EFSA breeding survey for MS2 and the MS4 (EFSA breeding survey data supplied by Michaela Hempen/Frank Boelaert).

The mass of faecal material produced by a sow is around 2kg (Brent 1986), which we have assumed a standard deviation of 100g. The concentration of *Salmonella* within a gram of contaminated faecal material, c_s, has been estimated previously by fitting a distribution to empirical enumeration data collected during a study of gilts (VLA 2009).

Table 7.5: Structure of case study MS pig populations reflected using the percentage of slaughtered head production that is reared through each farm type (EFSA breeding survey) (VLA, 2009). Only parameters within the model are used.

Farm type	Case study member state											
	MS2			MS1			MS4			MS3		
	Breeder-Finisher	Breeder-weaner	Finisher only$^{+\times}$	Breeder-Finisher	Breeder-weaner	Finisher only^{+*}	Breeder-Finisher	Breeder-weaner	Finisher only^{+*}	Breeder-Finisher	Breeder-weaner	Finisher only^{+*}
I - A - So - D	8.09%	4.94%	52.26%	0.00%	0.00%	0.00%	7.41%	4.48%	7.41%	4.84%	3.60%	4.84%
I - A - So - W	2.73%	0.21%	11.56%	3.30%	3.85%	3.30%	18.52%	23.88%	18.52%	25.81%	19.82%	25.81%
I - A - Sl - D	20.50%	15.05%	18.59%	3.30%	5.13%	3.30%	11.11%	14.18%	11.11%	9.68%	4.50%	9.68%
I - A - Sl – W	6.91%	0.63%	4.28%	20.88%	28.21%	20.88%	29.63%	41.04%	33.33%	9.68%	11.71%	9.68%
I – C - So - D	11.89%	3.86%	7.91%	0.00%	0.00%	0.00%	0.00%	0.75%	0.00%	1.61%	4.50%	1.61%
I – C - So - W	4.01%	0.16%	1.82%	10.99%	7.69%	10.99%	7.41%	2.99%	7.41%	29.03%	40.54%	32.25%
I – C - Sl - D	30.12%	11.77%	2.93%	1.10%	3.85%	1.10%	11.11%	3.73%	11.11%	0.00%	1.80%	0.00%
I – C - Sl - W	10.15%	0.49%	0.67%	45.05%	35.90%	60.43%	11.11%	5.22%	11.11%	16.13%	10.81%	16.13%
O - A - So - D	0.48%	8.37%	0%	0.00%	1.28%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%
O - A - So - W	0.16%	0.35%	0%	1.10%	0.00%	0%	3.70%	2.99%	0%	1.61%	0.90%	0%
O - A - Sl - D	1.22%	25.51%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%
O - A - Sl – W	0.41%	1.06%	0%	5.49%	3.85%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%
O – C - So - D	0.71%	6.55%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%
O – C - So - W	0.24%	0.27%	0%	4.40%	5.13%	0%	0.00%	0.00%	0%	1.61%	1.80%	0%
O – C - Sl - D	1.79%	19.96%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%	0.00%	0.00%	0%
O – C - Sl - W	0.60%	0.83%	0%	4.40%	5.13%	0%	0.00%	0.75%	0%	0.00%	0.00%	0%

Key: I – Inside, O- Outside, A – AIAO production, C – Continuous production, So – Solid floor, Sl – Slatted floor, D – Dry feed, W – Wet feed

$^{\times}$ Breeding survey does not include finisher-only farms; therefore for MS2 finisher-only farms we use management data collected from a VLA research project (VLA,2009)

* Breeding survey does not include finisher-only farms; for MS1, MS4 and MS3 we assume finisher-only farms have same proportions as breeder-finisher farms.

$^{+}$ Given negligible production from outside sources, for simplicity we assume only piglets reared outside; therefore outside production for finisher-only farms set to 0% (remainder added to most common type)

Table 7.6: Estimates for parameters relating to *Salmonella* infection.

Notation	Description	Units	Value/Distribution	Source
Source of infection				
p_{herd}	National prevalence of *Salmonella*-positive breeding pig herds	-	MS2: 0.44 MS4: 0.059 MS4: 0.084 MS3: 0.1386	EFSA breeding survey data
P_w	Prevalence of infection within a herd	-	MS2: 0.21 MS4: 0.21 (MS2) MS4: 0.21 (MS2) MS3: 0.21 (MS2)	EFSA breeding survey
P_f	Probability of feed lot contamination	-	0.10	Assumed from EFSA 2008b; VLA 2008
ϕ_{sow}	Mass of faeces defecated by sow per day	g	N(3000,150)	Brent 1986. S.D. assumed
g	Amount of feed consumed per day at stage H: Weaners (*H=wean*), Growers (*H=grow*), Finishers (*H=fin*)	g	Wean (~6 wks): 500 Grow (~12wks): 1620 Fin (~18wks): 3200	Carr 1998
c_s	Concentration of *Salmonella* in contaminated sow faeces	CFU/g	LogNormal(2.36,4.39)	VLA 2009
c_f	Concentration of *Salmonella* in contaminated pig feed	CFU/g	GPareto(0.001,0,1)	Sauli *et al.* 2005
c_e	Concentration of *Salmonella* in external environment	CFU/g	LogNormal(0.1,3)	Davies & Wray 1995
Transmission				
f	Mass of faeces defecated by piglet per day Mass of faeces per day; weaner Mass of faeces per day; grower Mass of faeces per day; finisher	g	N(100,10) N(753,50) N(1194,50) N(2580,50)	Carr, 1998 (assumed S.D.) (Leek 2005) assumed S.D.
c_p	Concentration of *Salmonella* in contaminated pig faeces	CFU/g	0-10^7 CFU/g (see text)	Jensen *et al.* 2006
β_F	Removal coefficient for fresh faeces on slatted flooring	-	Beta(40,10)	Assumed
β_{old}	Removal coefficient for old faeces on slatted flooring	-	Beta(2,10)	Assumed
β_C	Cleaning coefficient for solid flooring	-	Beta(3,2)	Assumed
β_C	Cleaning coefficient for slatted flooring	-	Beta(1,2)	Assumed
β_{xc}	Beta parameter of cross-contamination coefficient	-	Beta(1,10)	Assumed
δ	Decay constant	day^{-1}	0.04	Gray & Fedorka-Cray 2001; Tannock & Smith 1972
m_{ing}	Mass of faeces ingested by piglets per day Mass of faeces ingested by weaners/growers/finishers per day	g	U(0,21) U(0,100)	Sansom and Gleed 1981 Based on Kemme *et al.* 1997, expert opinion
α_{DR}, β_{DR}	Parameters of dose response model	-	0.1766; wet, 50235 dry, 20235	Loynachan & Harris 2005; Tenhagen *et al.* 2009
t_{LN}	Duration of intermittent shedding	days	Weibull(44.94,1.68)	Jensen *et al.* 2006

Feed

According to EFSA 2006 *Salmonella* contamination of feed will typically consist of small numbers of organisms scattered heterogeneously among the feedlot. Feed lots typically consist of 3-5 tonnes in large feed mills, which would typically last the large farm (minus sows) modelled here 2-3 days.

The prevalence of *Salmonella* contamination has been identified to be between 1-10% for samples from feed types commonly used for pigs (EFSA, 2006). We only have specific data for MS2 from the case study MSs (approximately 2% of pig feed contaminated, (VLA 2008)). However, there are many issues with sampling of feed in determining prevalence, as discussed by EFSA (2006). Of concern to us is the extremely small samples taken (e.g. the UK sampling procedure is to take two 25g aggregate samples from two weeks' worth of production), meaning that it is highly likely that positive batches are missed because of the heterogeneity of contamination. While home-mixing is quite common for pig feed production, industrial production of feed is presumably fairly standardised across the EU. Using this assumption, we therefore set a conservative estimate of p_{feed} at 10% for all case study MSs. This assumption is made purely on the opinion of the author based on discussions with experts on the subject of feed and *Salmonella*. This necessarily means it is an uncertain parameter and will be investigated within the uncertainty analysis.

Pigs consume approximately 4% of their bodyweight, and hence consume more feed as they gain weight. Therefore, assuming midpoints of each stage as 6 weeks old (weaner), 12 weeks old (grower) and 18 weeks old (finisher), then the average daily feed intakes (g) are 500, 1620 and 3200 g/day respectively (Carr 1998).

There have only been limited studies on the concentration of *Salmonella* within contaminated feed, but it appears to be present in concentrations between 1-1000 CFU/g (Thomas *et al.* 1981). Sauli *et al*. 2005 estimate the distribution of *Salmonella* concentration during finishing heat-treated pig feed production in a large Swiss feed mill. We have fitted a generialised Pareto distribution to the data from Sauli *et al*. to estimate c_f and in the absence of further data assume this is applicable across all EU-produced feed.

External contamination

As discussed above, we only consider contamination via birds/rodents, and treat this simply by adding an extra "dose" to individual pigs if the farm is already infected, λ_e. There are no data to suggest doses that pigs will be exposed to via external contamination. However, studies (Davies & Wray 1995; Skov *et al.* 2008) suggests that the prevalence of infection in rodents is low if infection is present (1-10%). We have fitted a lognormal distribution to the data provided on enumeration of rodent faeces in Davies & Wray (1995), assuming a pig will consume one whole rodent dropping per "external contamination" exposure. The lognormal fit to the data produces a distribution for λ_e as shown in Figure 7.5.

Removal/movement of faeces

Despite literature searches, no data exists to quantify the rate of removal of faecal material from either solid or slatted flooring, or the rate at which faecal material is cross-contaminated to adjacent pens or *Salmonella* cleaned out from the pen before repopulation. Hence, we have assumed that the percentage amount of faeces within a pen that is removed or cross-contaminated varies according to a beta distribution. Absolute values for the shape and scale parameters of the beta distributions have been chosen logically: i.e. more faecal material is removed from the pen on slats than on solid flooring, and the vast majority of

faecal material will remain within the pen where it originated from, instead of being cross-contaminated.

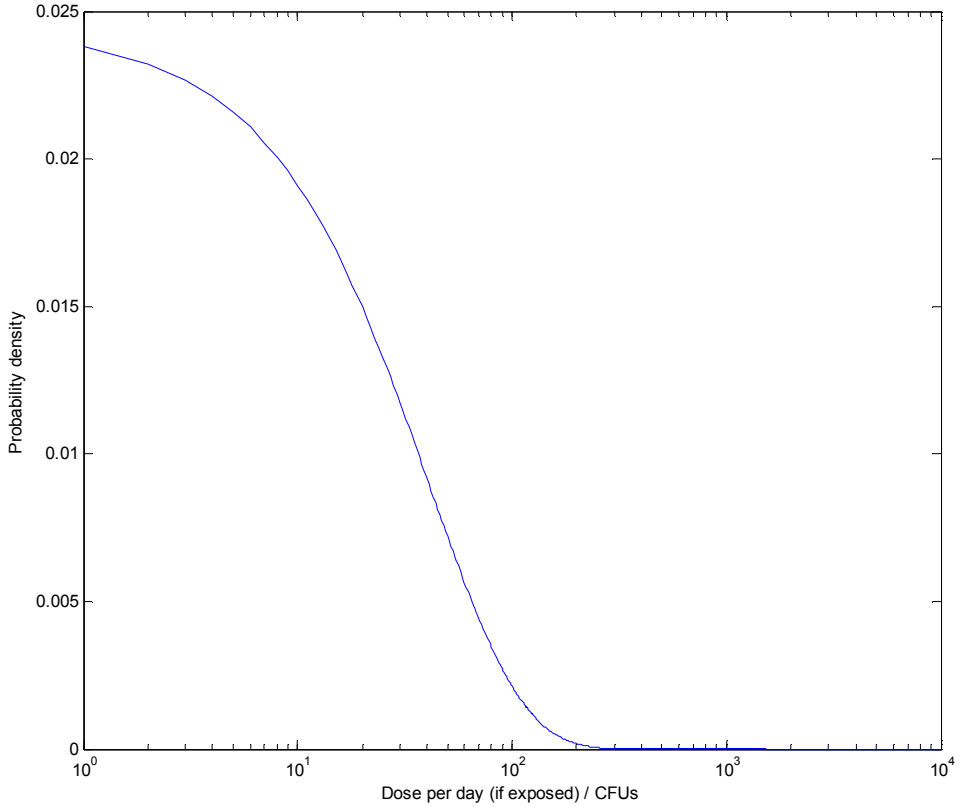


Figure 7.5: Distribution assigned to external contamination exposure events (on a per day basis). Fitted to data taken from Davies & Wray (1995).

Therefore, current estimates for the two distributions are given in Figure 7.6.

Duration of excretion and infection

Longitudinal data from two studies (Gray *et al.* 1996; Jensen *et al.* 2006) have been used to estimate the duration of excretion and lymph-node positivity. Carrying out survival analysis of the two datasets we produce the following distributions for length of excretion (faecal shedding) and carriage (LN-positive).

The two distributions are remarkably similar given they are derived from two different studies and were originally assumed to denote two different characteristics of infection. For this analysis we have assumed that the duration of excretion from Jensen *et al*. (2006) is equal to the total period from the first to last positive sample from a pig, therefore including sampling points when the pig provided a negative sample. These negative samples may be due to intermittent shedding or because of false negatives (sensitivity of faecal tests is considered to be poor, Rob Davies, VLA, personal communication). The results of Jensen *et al*. do concur with other similar longitudinal studies (Kranker *et al.* 2003; Osterberg *et al.* 2009; Scherer *et al.* 2008). The similarity of these two distributions tend to suggest that

intermittent shedding *could* occur over almost the whole time period in which an individual pig is infected. Therefore, we assume that there is no carrier phase as defined in previous models (such as Hill *et al*., 2008), and that excretion can occur over the whole period where a pig is lymph-node positive (albeit intermittently).

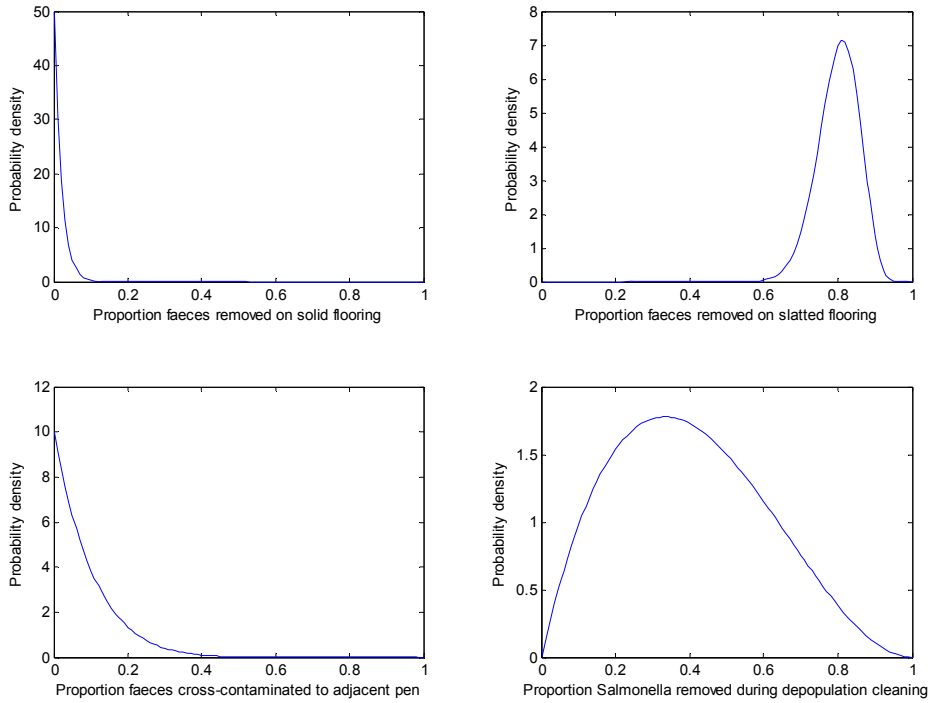


Figure 7.6: Current distributions for the removal and cross-contamination of faecal material.

Salmonella in pig faeces has not often been enumerated, and then mostly using semi-quantitative techniques. Therefore, we assume there is only one division between stages – the amount shed by sows and then the amount shed by slaughter pigs. However, one recent study by Jensen *et al*. (2006) did enumerate at an individual pig level for a longitudinal study of outdoor pigs. Two cohorts of pigs (one high and one low dose group) were seeded with experimentally infected pigs on outdoor paddocks, before these cohorts were removed and a new cohort placed on the vacated paddocks. There were significantly greater concentrations shed by the high dose group (0-10^6 CFU/g) than by the low dose group (0-100 CFU/g). Pigs from the second experiment cohorts were then infected quasi-naturally from the contaminated faecal material shed by the first cohorts. A summary of the data collected from the two second cohorts is shown in Figure 7.8 - these cohorts are assumed to be "naturally" infected.

The raw data from this study was obtained, giving us enumeration of samples taken at weekly intervals for all *Salmonella*-positive pigs from the second-experiment cohorts. From these data we were able to produce frequency tables for both low and high-dose groups, which were used to estimate the probability of shedding x log CFU/g on week t+1 if pig *k* was shedding *y* log CFUs at week *w*. The results of this analysis are shown in Table 7.7 and Table 7.8.

If pig *k* in pen *i* and room *j*, is shedding *x* log CFU during week *w* (according to one of the frequency matrices above) then during week *w*+1:

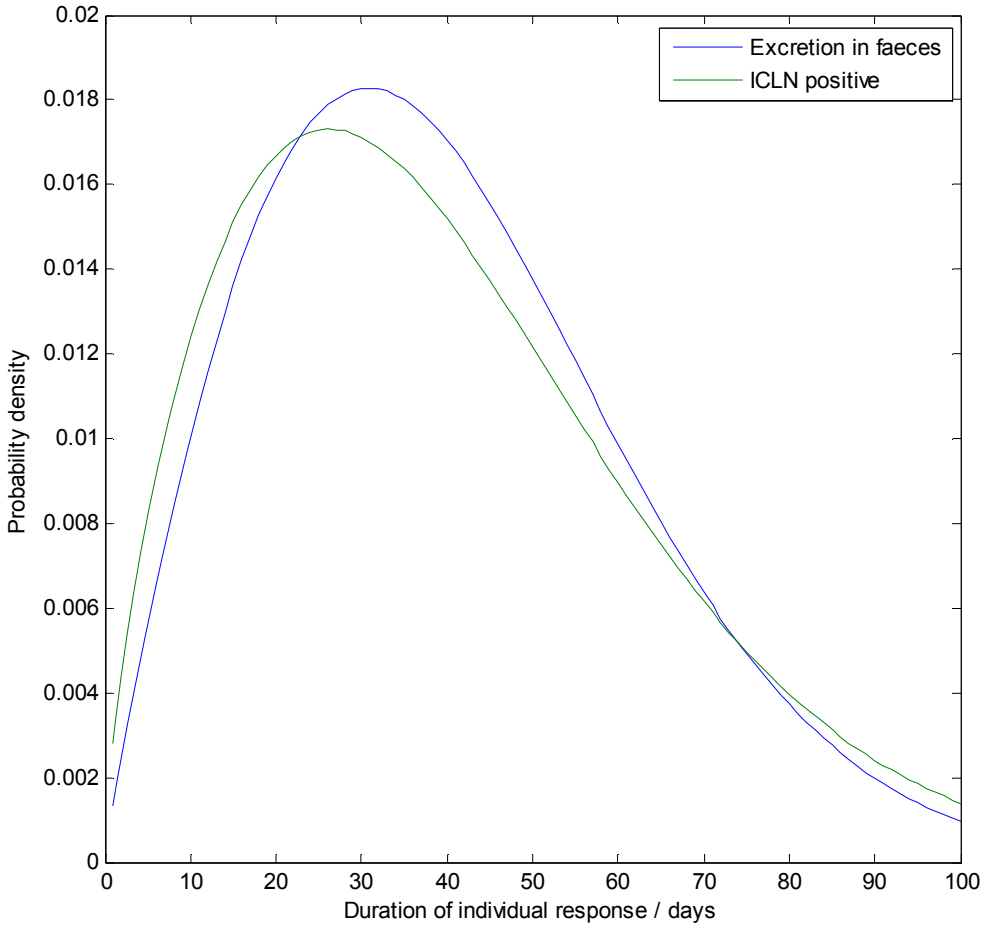


Figure 7.7: The duration of excretion and duration of positive ileo-caecal lymph-nodes (Gray *et al.* 1996; Jensen *et al.* 2006).

$$c_s = 10^{\Re(U(y-1,y))}$$

Once a pig is exposed to *Salmonella* the beta binomial dose response model is used to calculate the probability of infection, $p_{inf}(k,j,l,t)$. Experimental dose-response data (5 pigs each at 10^1, 10^3, 10^5 and 10^7 CFUs) was taken from a study by Loynachan & Harris (2005). *Salmonella* infection was reported in a number of different lymph-nodes. To ensure we compare the correct sample for validation at slaughter, we chose to define infection as a positive ileocaecal lymph-node sample, given that this lymph node is also the sample taken within the EFSA slaughter pig survey (EFSA 2008a). A Beta-Poisson model was then fitted to it by P. Teunis at RIVM using his own previously developed program. The alpha and beta

parameters from the beta-poisson model are also equivalent to the alpha and beta parameters of the beta-binomial model.

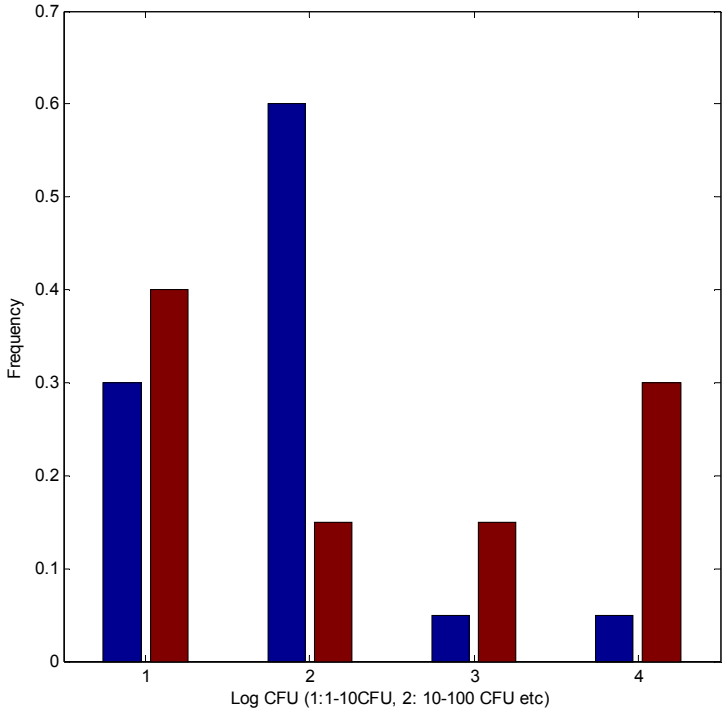


Figure 7.8: Quantitative enumeration of *Salmonella* in faeces applied to slaughter pigs (Jensen et al, 2006). Blue: distribution used for "low dose" (infected with < 10^6 CFU) pigs, red for "high dose" (> 10^6 CFU) pigs.

The resulting beta-binomial model is for pigs on dry feed. Pigs on wet feed will have a greater resistance to infection, due to the lowering of pH within the gut making it a more hostile environment for *Salmonella* (Wales *et al.* 2009). By a process of trial and error (i.e. changing the estimates until the same relative proportion of pigs on wet feed were infected with *Salmonella* compared to pigs on dry feed was observed as from a study by Tenhagen *et al.* 2009), we have developed the modified Beta-Poisson model of pigs on wet feed. The resulting baseline and modified PDFs are shown in Figure 7.9.

7.4.5 Sensitivity analysis

Standard risk assessment sensitivity analysis methods are hard to apply for farm transmission models such as this. Within the farm sensitivity analysis we identify the important parameters in determining the variation of the within-batch prevalence of infection for pigs at slaughter (i.e. $P_{LN}(b)$).

Using this method, we hope to be able to identify the important parameters for the farm model, whilst avoiding complex and lengthy simulations to link the farm model results to the rest of the model. (As previously described, the farm model produces a standalone database of results for $P_{LN}(b)$, known as the farm matrix (see Chapter 4), which is then sampled to provide the input for the rest of the risk assessment model. Linking the randomly selected results from the farm model to the rest of the risk assessment in order to conduct sensitivity analysis, apart from the direct output $P_{LN}(fr(t'))$, is a difficult procedure).

Table 7.7: Frequency matrix for shedding x log CFU at week t+1 – given infected at low dose (<10^6 CFU) (Jensen *et al*., 2006)

Magnitude of shedding at week *w*, *x* (log CFU)	Probability of shedding at magnitude *y* during week *w*+1			
	1	2	4	6
Newly infected	0	1	0	0
1	0.4444	0.5556	0	0
2	0.1389	0.6389	0.1944	0.0278
4	0	0.8333	0.1667	0
6	0	0	1	0

Table 7.8: Frequency matrix for shedding x log CFU at week t+1 – given infected at high dose (≥10^6 CFU) (Jensen *et al*., 2006)

Magnitude of shedding at week *w*, *x* (log CFU)	Probability of shedding at magnitude *y* during week *w*+1				
	1	2	4	6	7
Newly infected	0	0.6	0.39	0	0.01
1	0.3750	0.6250	0	0	0
2	0.1500	0.6000	0.2000	0.0500	0
4	0.0000	0.6667	0.2222	0.1111	0
6	0.0000	0.0000	0.3333	0.666	0
7	0	0	1	0	0

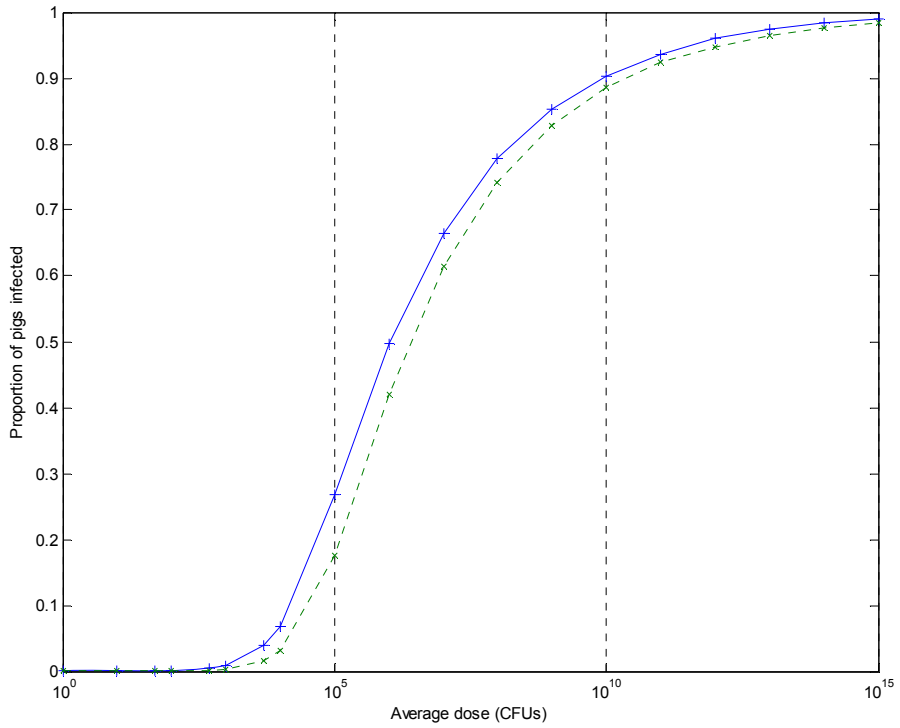


Figure 7.9: Probability of infection given dose for a) susceptible weaned pigs on dry feed (blue) and b) weaned pigs on wet feed (green).

Even just taking the unit of interest as the within-batch prevalence $P_{LN}(b)$, typical sensitivity analysis methods need modification. Based on trials conducted by Frey 2004 we have chosen to conduct an ANOVA method for our farm sensitivity analysis. More detail on this method is provided by Frey *et al*., but briefly the ANOVA method is a non-parametric method used to determine if values of the output vary in a statistically significant manner associated with variation in values for one or more inputs that have probability distributions assigned to them (see also Chapter 5 for a description of sensitivity analysis). If the output does not have a significant association with variation in the inputs, then the variation in the output is random. The F-test value typically associated with ANOVA can be used to represent the strength of association between input and output: that is, the larger the F-value, the stronger the association.

Complexity is added to the analysis as we are dealing with many batches within one iteration of the model, and also because the pigs are kept track of as they migrate through the stages of production (for example, so that we can relate the shedding of *Salmonella* within a farrowing pen to the prevalence of infection within a finishing batch at slaughter).

A major issue is that in order to run this ANOVA method, there must be only one sample from each probability distribution for each iteration, such that its value can be compared against the response variable value for that iteration. As is clear from the methodology described above there may be up to 29,000 individual samples of each distribution taken within a single batch calculation (i.e. 160 pigs over a 182 day lifespan ~ 29,000 samples of a probability distribution). Therefore a summary statistic must be used to describe the variance of these distributions against that of the response variable. We have chosen to

use the mean, and hence, for example, the comparison of the shedding of *Salmonella* by a sow against the prevalence of infection within her progeny at the point of slaughter is carried out by using the average concentration of *Salmonella* within the sow's faeces over the 28 days the piglets were suckling from their mother.

Because of this complexity we include only those parameters which are thought to be of obvious importance:

1. The average concentration of *Salmonella* shed by an infected sow
2. The average concentration of *Salmonella* shed by an infected piglet cohort (i.e. 10 piglets)
3. The average concentration of *Salmonella* shed by an infected weaner cohort (40 pigs)
4. The average concentration of *Salmonella* shed by an infected grower cohort (40 pigs)
5. The average concentration of *Salmonella* shed by an infected finisher cohort (40 pigs)
6. The average concentration of *Salmonella* in feed during weaning
7. The average concentration of *Salmonella* in feed during growing
8. The average concentration of *Salmonella* in feed during finishing
9. The average load of external contamination during farrowing.

Sensitivity analysis, as described here, allows us to investigate the effect of *naturally varying* parameters on the farm output, but does not allow us to investigate the effect of uncertainty. Uncertainty analysis of the farm model is discussed further in Chapter 12.

7.4.6 Major model assumptions and data gaps

Major assumptions

There are many assumptions made within the model, some due to a lack of data, but others due to simplifications required in order to produce a parsimonious model in the timeframe required. We state the major assumptions (and the reason for their inclusion) below:

1. *Breakpoint between small and large farms of 400 pigs slaughter per year*
   This was decided as the breakpoint at the Data Workshop in Copenhagen based on discussion with the experts present, as a suitable point to differentiate between those farms sufficiently large to invest in specific buildings and use more "biosecure" methods, and those small farms that probably wouldn't have the resources to invest in such methods.

2. *AIAO production by compartment/room.* That is, farmers will raise batches of pigs in the same building, but there will be compartments within the building to keep pigs (and faeces of those pigs) from separate batches apart.
   In discussion with experts few pig farmers practice true AIAO production (i.e. one batch, one building). We therefore assume that if AIAO production is specified in the EFSA breeding survey, it is AIAO by compartment, not building.

3. *Model only wet versus dry feed.*
   There is some evidence that pelleted vs non-pelleted feed also has a significant effect in reducing *Salmonella* prevalence on-farm. However, little quantitative data

was available for either moisture content or form of feed. We chose to reduce the complexity of the model by incorporating only wet vs. dry feed. The inclusion of wet vs. dry feed should be seen as an example of how feed can affect *Salmonella* prevalence.

4. *Homogenous mixing of faeces and salmonella within faeces.*
   This assumption was made in order to reduce complexity within the model. Homogenous mixing has the effect of exposing more pigs to infection, but at lower doses. We have not being able to quantify the uncertainty associated with this assumption, but it is likely to be a significant factor.

5. Assume proportional categorisation of breeding pig herd farm types can be applied to contract finishing farms.
   This is a major assumption that was necessary for MS1, MS3 and MS4 due to a lack of farm management data for contract finishing farms (despite information requests and assistance from experts within each of these MSs no relevant data could be identified).

6. Treat all salmonellas as equally infectious/zoonotic.
   This was made at the request of the EFSA ToRs. Evidence does suggest infection via multiple strains is likely, but we did not have the data or scope to consider these factors. The infectious dose, and shedding rate in faeces, especially for pig- or feed-adapted serotypes, is likely to be significantly different (Osterberg *et al.* 2009), and so there remains unquantified uncertainty within the model.

Major data gaps

During the paramterisation of the Farm model, the following data gaps were identified:

1. Allocation of contract finishing farm types
2. Dose response relationships for different strains of *Salmonella*
3. Shedding rates for different strains of *Salmonella*
4. Quantitative effect in modifying dose-response for different feed types
5. Prevalence and load of contamination within feed
6. Quantitative levels of exposure from external sources of infection (rodents, birds etc)
7. Faecal mass ingested by pigs
8. Cleaning and disinfection efficiency in removing *Salmonella*
9. Pen cross-contamination co-efficient
10. Rate of removal of faeces from pen
11. Frequency of cleaning

7.5 Results

7.5.1 Baseline results

Within-batch prevalence of lymph-node positive pigs at slaughter age

The within-batch prevalence at slaughter age is derived from the prevalence of infection within the batches of pigs being sent to slaughter (on a weekly basis in the large farm and every 22 weeks in the small farm). These batches can be *Salmonella* negative either because the farm was negative at the time of depopulation, or simply there were no infected pigs within that batch, despite there being infection present on the farm. The results are presented for the four case study MSs (MS1, MS2, MS3, MS4) in Figure 7.10.

It is clear most batches being sent to slaughter are either *Salmonella*-negative (each MS sends a similar proportion of completely *Salmonella*-negative batches – around 30-40%), or infected at a low prevalence. From Figure 7.10 it is clear that those MSs with a higher national pig prevalence (MS2, MS4) have a larger proportion of highly-infected batches from large farms. The within-batch prevalence in small farms appears to be much lower than for large farms.

7.5.2 Model validation/interrogation

Official model validation takes place at the point of slaughter (see Chapter 8). However, we can inspect the dynamics of infection for a range of scenarios/time points and compare them against published literature, to get an insight into how well the model compares against observed data.

Source of infection

We have investigated the relative importance of source of infection by simply turning off each source of infection within each MS model. The results are shown in Figure 7.11.

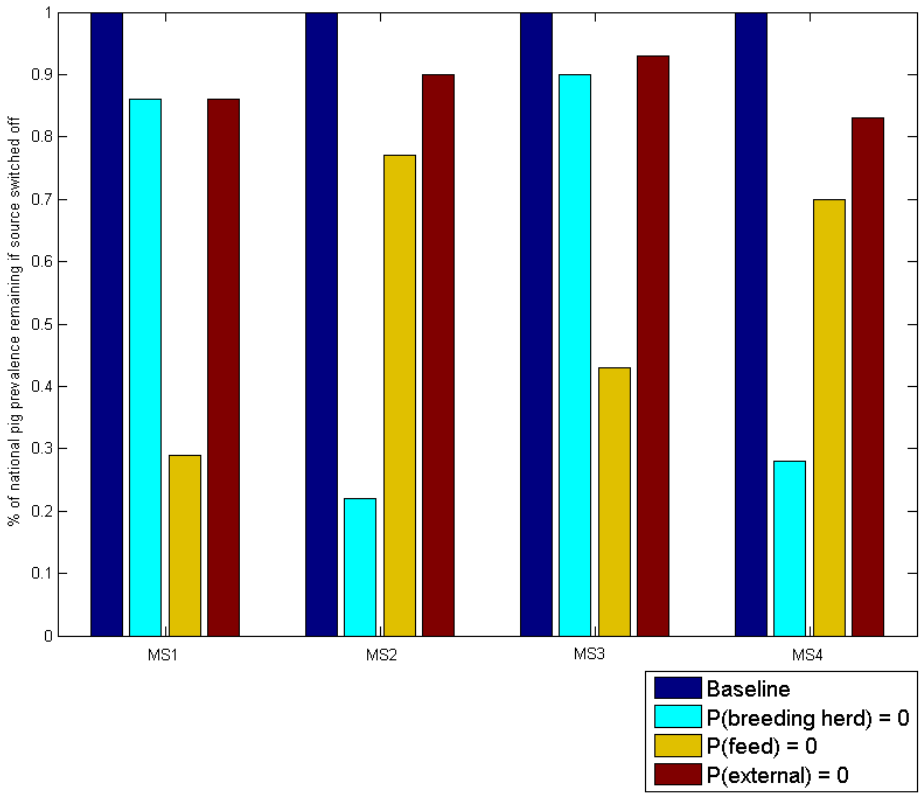
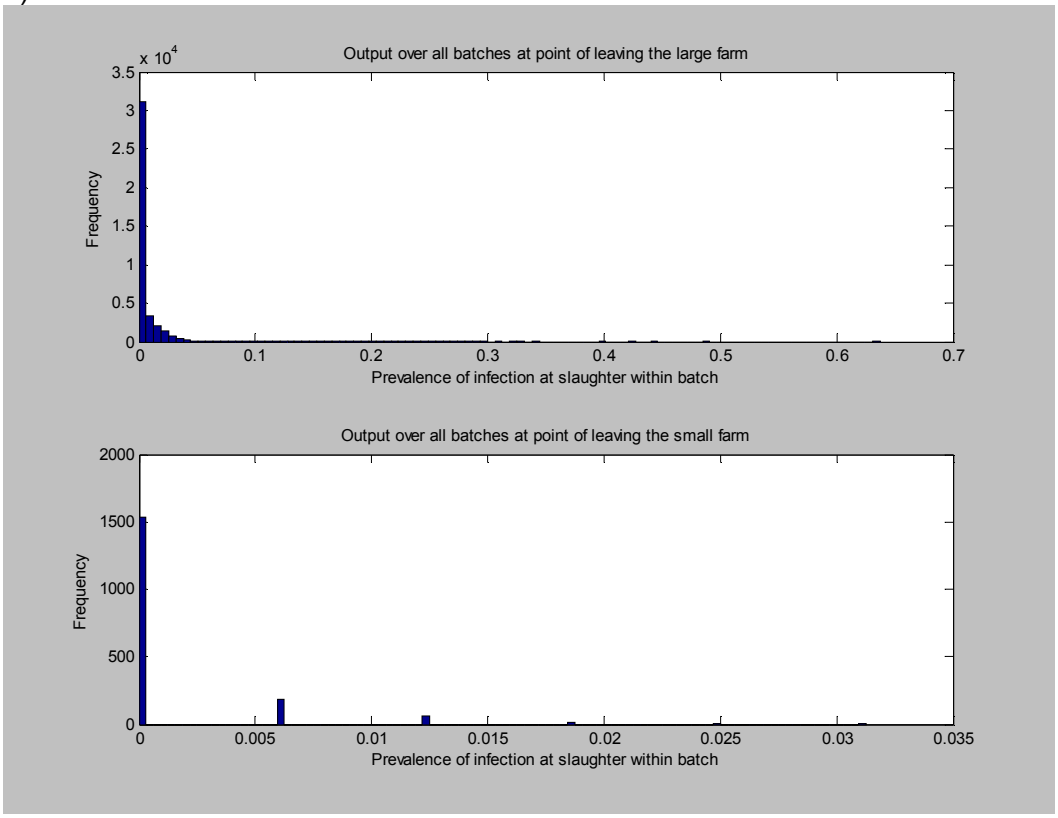


Figure 7.11.

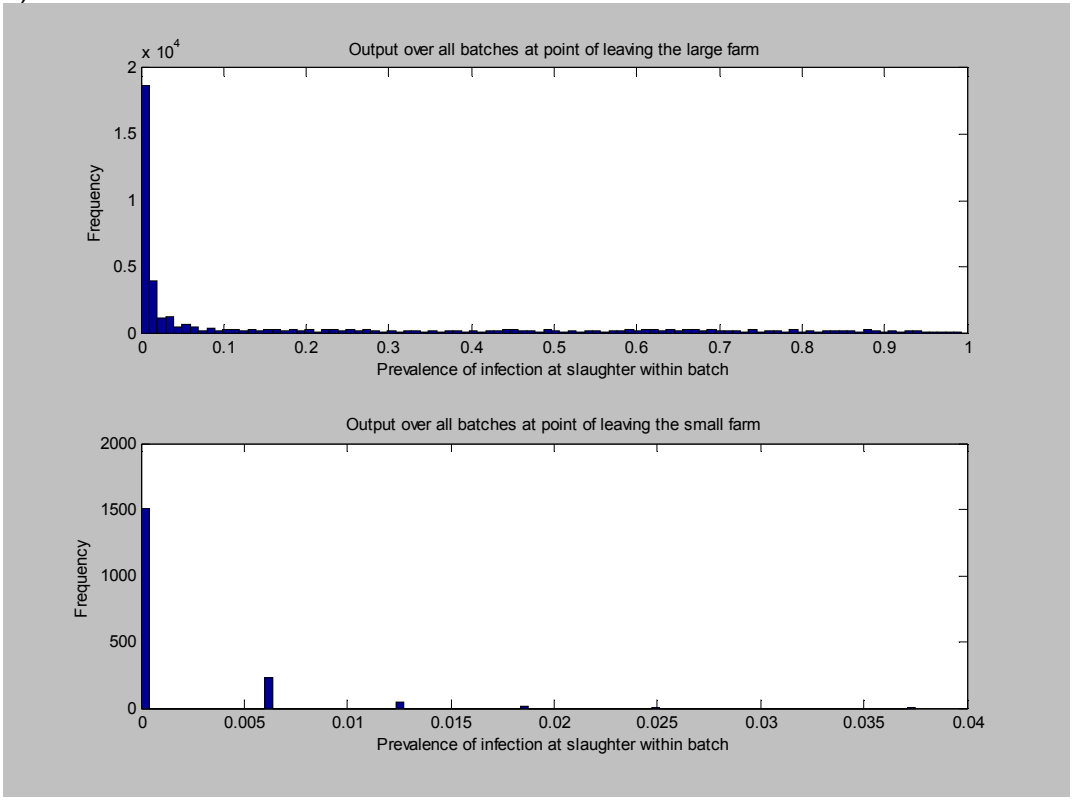
The effect is striking – for MSs with a higher breeding pig herd prevalence (MS2, MS4) switching breeding pig herd prevalence to zero removes the vast majority of infections at depopulation. Conversely, removing feed or external contamination from the model does little to change the national pig prevalence in MS2 and MS4. The reverse trend is true in MSs with low breeding pig herd prevalence (MS1, MS3) as feed contamination seems to be

the most important factor for the national pig prevalence in these MSs. This strongly indicates that breeding pig herd prevalence is a strong indicator of national pig prevalence – if a relatively low number of breeding pig herds are positive, national pig prevalence will be relatively lower than in other MSs with more infected breeding pig herds.

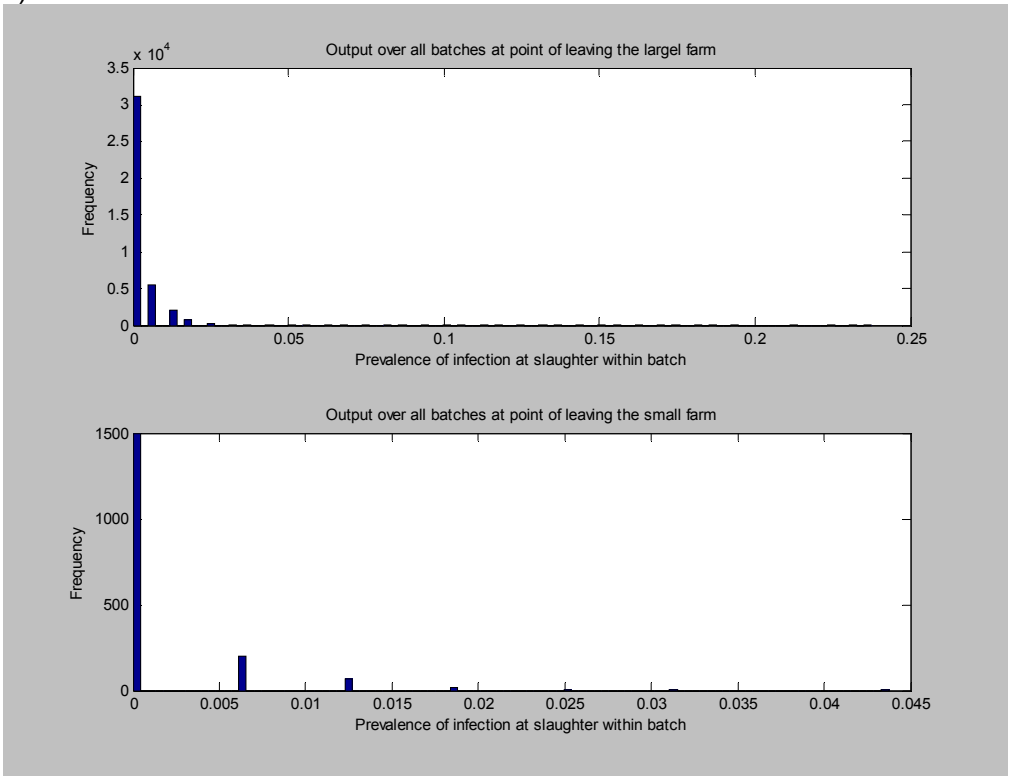
a) MS1



b) MS2



c) MS3



d) MS4

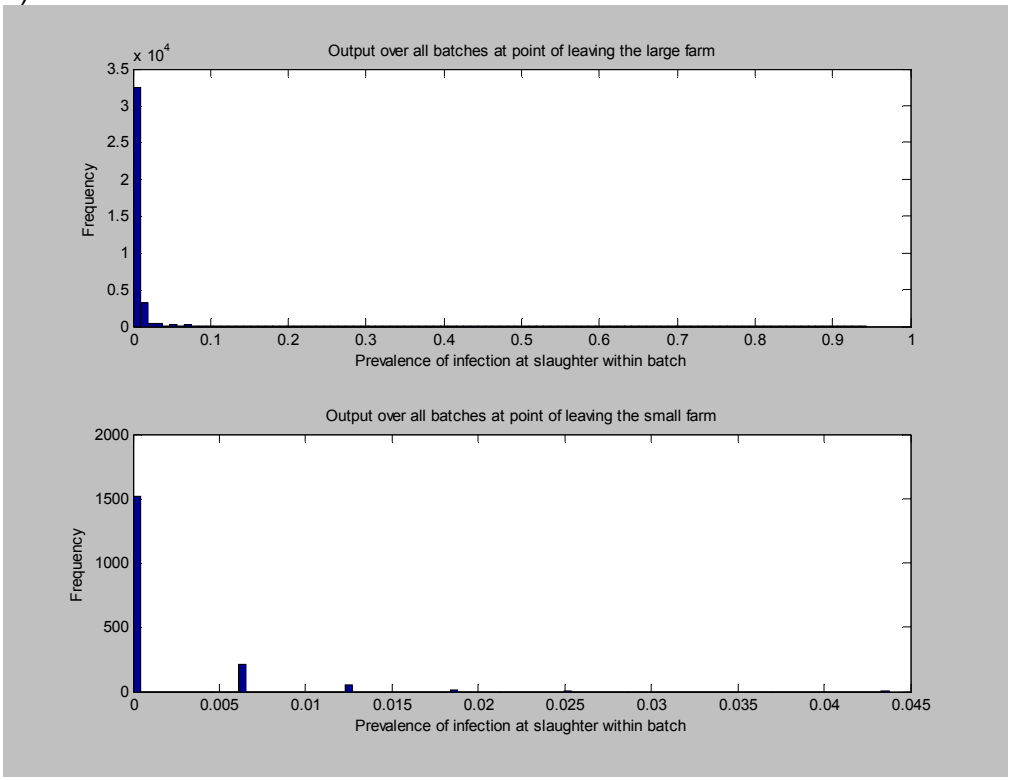


Figure 7.10: Distribution of within-batch prevalence for slaughter pigs. Charts are made from the 70,000+ batches of pigs run within each MS model. A) MS1, b) MS2, c) MS3 and d) MS4.

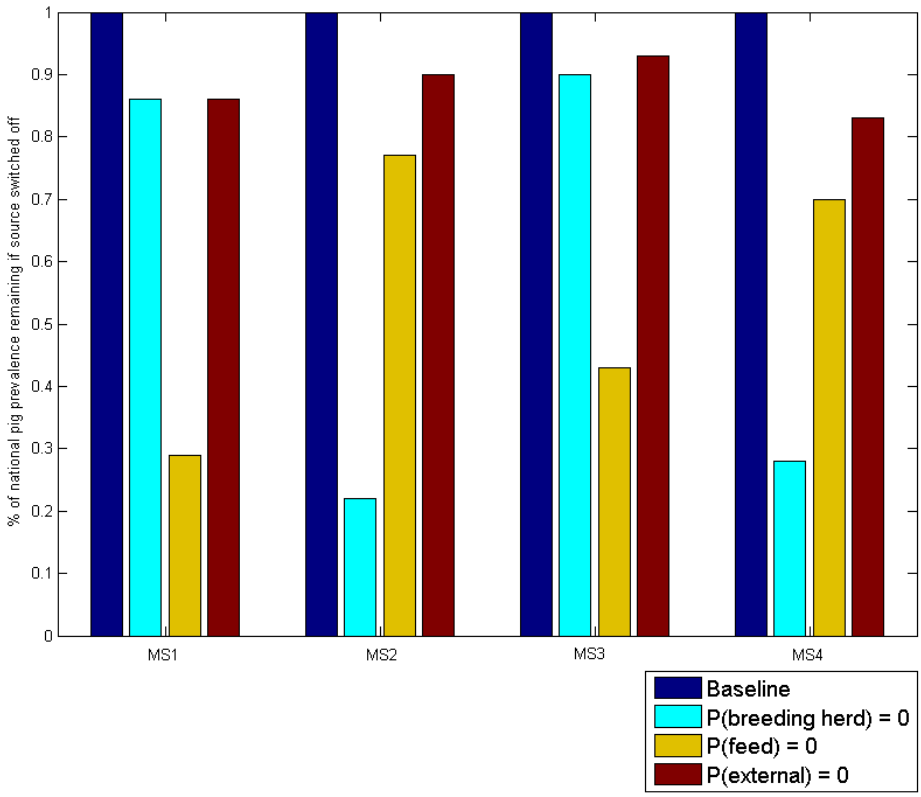


Figure 7.11: Relative impact on national pig prevalence for each MS if each source of infection is turned off.

It is hard to validate this type of result given the lack of quantitative data on the source of infection for pigs. However, contaminated feed and new pigs were identified as two common sources of infection by numerous other studies (EFSA 2006; Lo Fo Wong and Hald 2000), and there seems to be a correlation between breeding pig herd prevalence and slaughter pig prevalence (by lymph-node samples) comparing the two EFSA baseline surveys (Figure 7.12). Indeed, sampling of herds in several EU countries by Lo Fo Wong & Hald (2000) showed a rough correlation between the proportion of seropositive sows and the proportion of seropositive gilts. This would seem to indicate that an increased number of positive sows leads to increased probability of infection in gilts, which can then go onto infect more farms/pigs. However, a similar correlation between seropositivty of the sow and proportion of positive pen faecal samples was weak at best. Certainly there is evidence to suggest that infection passing from sow to offspring does occur, but equally there is evidence to suggest that the real-life situation is far more complex, with different serotypes colonising pigs depending on stage of production and the individual farm. The latter proliferation of serotypes is something not addressed within the farm model. In summary, we think the breeding pig herd can certainly be considered a significant source, but more research is needed before the strong correlation between breeding pig herd prevalence and slaughter pig prevalence can be validated fully.

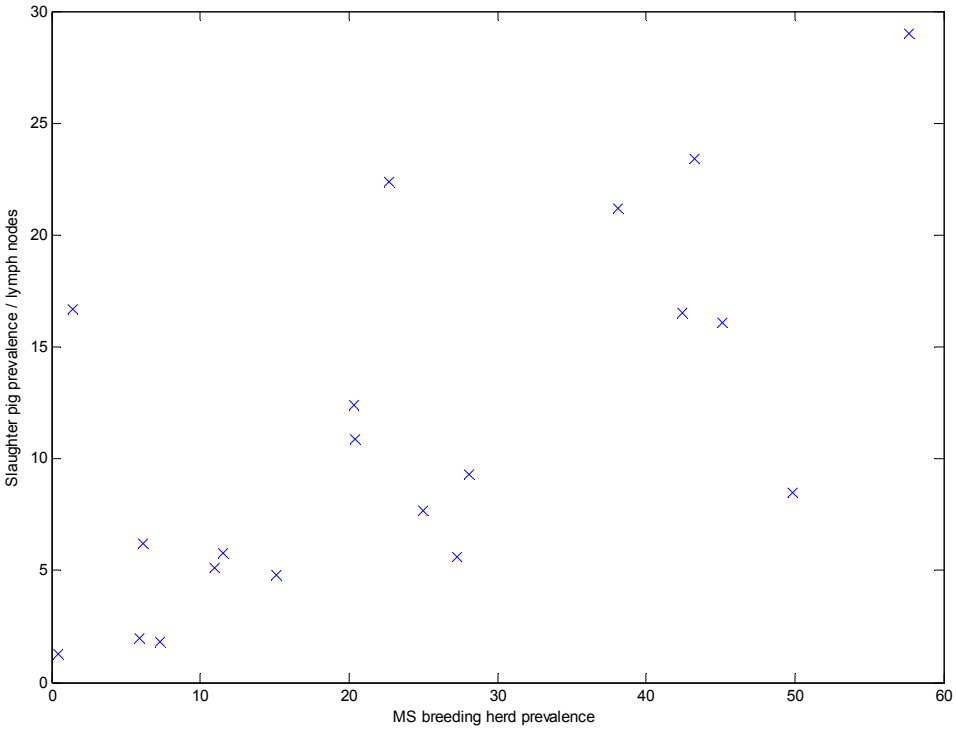


Figure 7.12: Plot of breeding pig herd prevalence within EU MSs (x-axis) vs slaughter pig prevalence. Correlation coefficient of 0.457.

Pen contamination

Pen contamination is highly variable, regardless of production stage (farrowing, weaning, growing or finishing), ranging between 0-10^9 CFUs within a farrowing pen on a single day, to 0 to 10^8 CFUs in weaning/growing/finishing. Examples of pen contamination for each stage of production are shown in Figure 7.13a-c.

The positive breeding pig herd has a large effect in increasing contamination rates within a pen. This is because sows shed proportionally more *Salmonella* than slaughter pigs if infected (as they shed more faeces). This means that more pigs are likely to be infected with "high" doses (> 10^6 CFUs), and hence these infected slaughter pigs are likely to shed more *Salmonella* than pigs infected at low doses.

Not only are slaughter pigs more likely to shed *Salmonella*, but relatively more pigs will become infected on positive breeding pig herds than those farms which only have feed and external contamination as a source. These two factors combine to give a much reduced contamination of pens for pigs produced from negative breeding pig herds/sows. Small farms, where the occurrence of high-shedding sow is less likely due to smaller numbers of sows on the farm, produce pens relatively low in contamination, even if the sow is positive. This results in most pens being negative for *Salmonella*, or contaminated at a much reduced level compared to larger farms (essentially because the tails of the distributions for concentrations in sows, feed and external contamination are not sampled due to small numbers). This lack of "highly-shedding" positive pigs results in a distinctly smaller prevalence of infection in pigs on small farms compared to large farms.

It is difficult to validate such results from the literature, primarily because enumeration of pen contamination is rarely done, and because the studies that are available are small, meaning that the rare high levels of contamination are probably missed. However, contamination levels of between 1.8-550 CFUs per 100cm^2 have been isolated from pens in lairage (Boughton *et al.* 2007). This is within the range commonly estimated by the model for given high shedding rates (which might be assumed for pigs during transport and lairage).

Doses ingested

Pigs will ingest up to 100g of faeces per day; from the section above contamination of these faeces may be significant. On top of the contamination via shedding by pigs, there is also the dose ingested via feed and/or external contamination (Equation 7.15). A sample of the distribution of doses ingested by pigs, for each production stage, is given in Figure 7.14a-b.

Combining all non-zero doses together, it is informative to compare the doses received by pigs against the dose-response model used within the model, see Figure 7.15. The vast majority of doses are zero, even on infected farms, so for clarity only non-zero doses are shown below. If a pig does ingest *Salmonella* (via either faeces, feed or external contamination) then the dose ingested is more likely to be at the lower end of the dose range. Within this range of doses, from 1-10^7 CFUs/day, infection is, on average, only more likely to occur than not occur for a very small proportion of exposure events (those above 10^6 CFUs). Hence, even with a heavily contaminated pen (>10^7 CFUs), it still requires continual exposure over a number of days to produce an epidemic-like transmission curve. This supports the results of Figures 7.10a-c, where the vast majority of batches sent to slaughter are infected at a very low prevalence, because infection within the finishing house is relatively rare given typical contamination rates within finishing pens.

7.5.3 Sensitivity analysis

The farm model sensitivity analysis has only been carried out on the large farm model. We have made a distinction between sensitivity analysis and uncertainty analysis within the risk assessment: hence this sensitivity analysis deals only with those parameters that have a variable distribution associated with them (e.g. Normal, Beta). Analysis of the effect of changing uncertain point value parameters is described in Chapter 12.

Given the varying parameter estimation for the different MSs we present a sensitivity analysis plot for each MS farm model in Figure 7.16a-d.

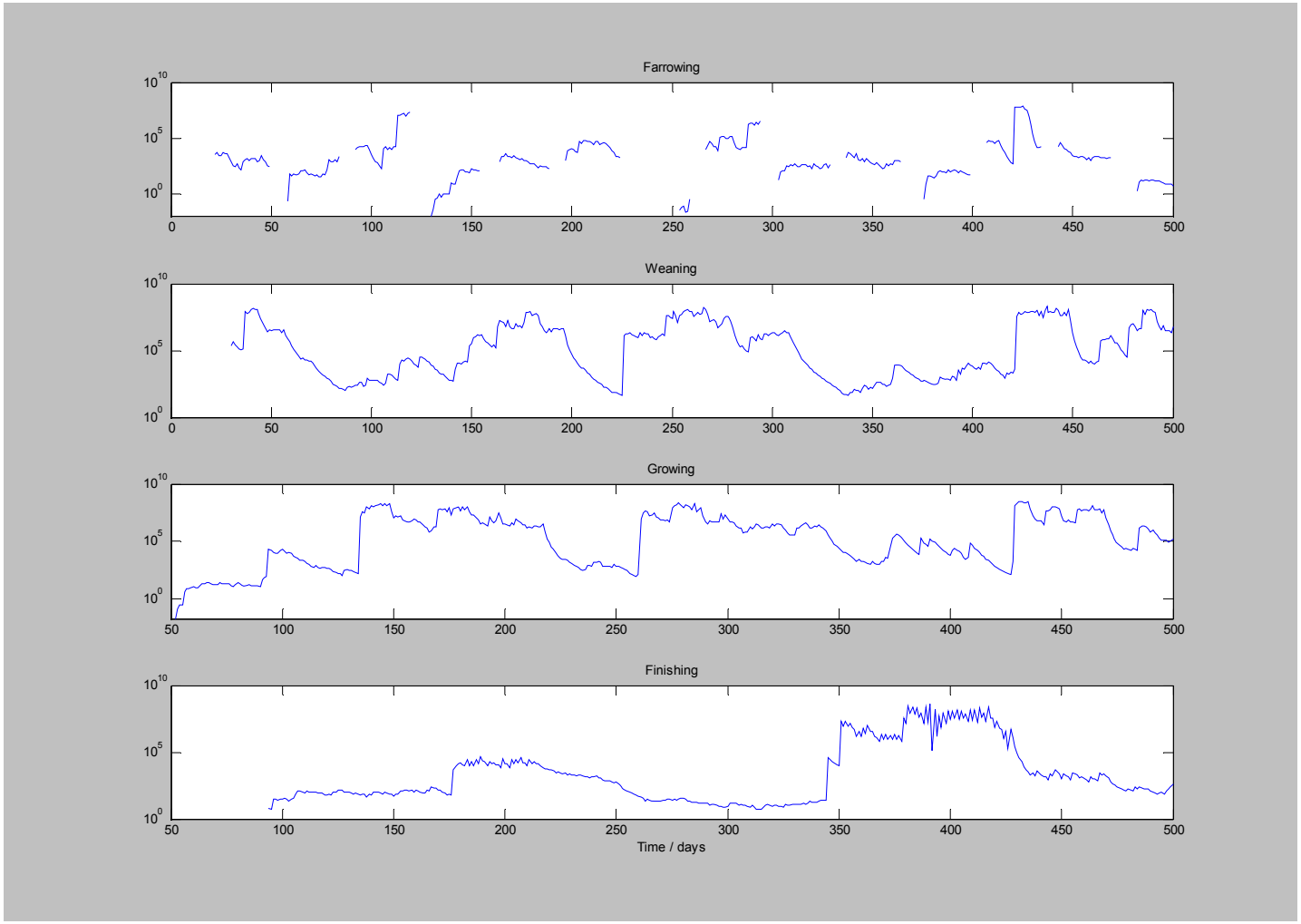


Figure 7.13a: Individual pen contamination given positive breeding pig herd profiles over time. Pens are picked to show examples of wide variation only, and are not representative over whole MS model.

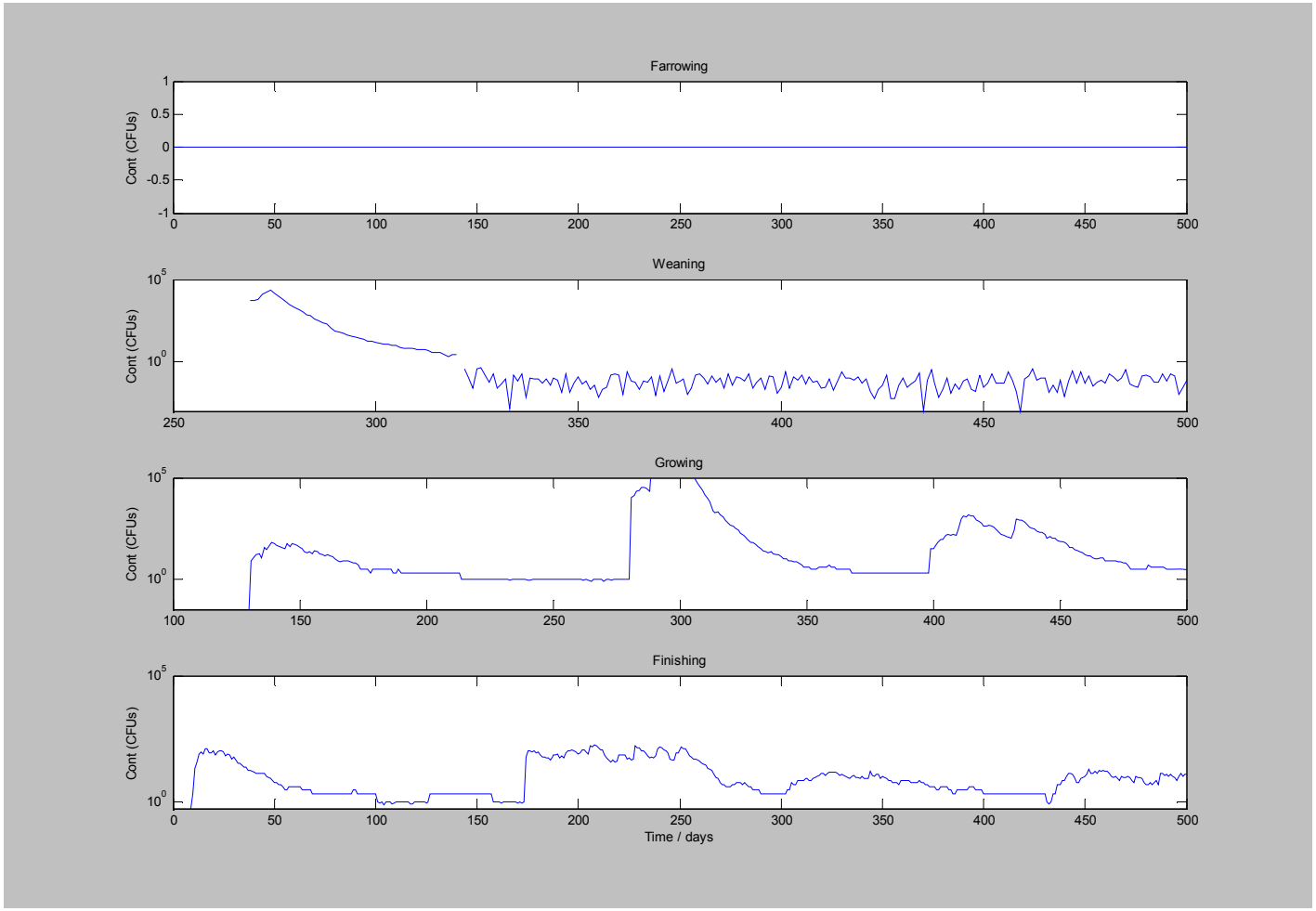


Figure 7.13b: Individual pen contamination given negative breeding pig herd profiles over time. Pens are picked to show examples of wide variation only, and are not representative over whole MS model.

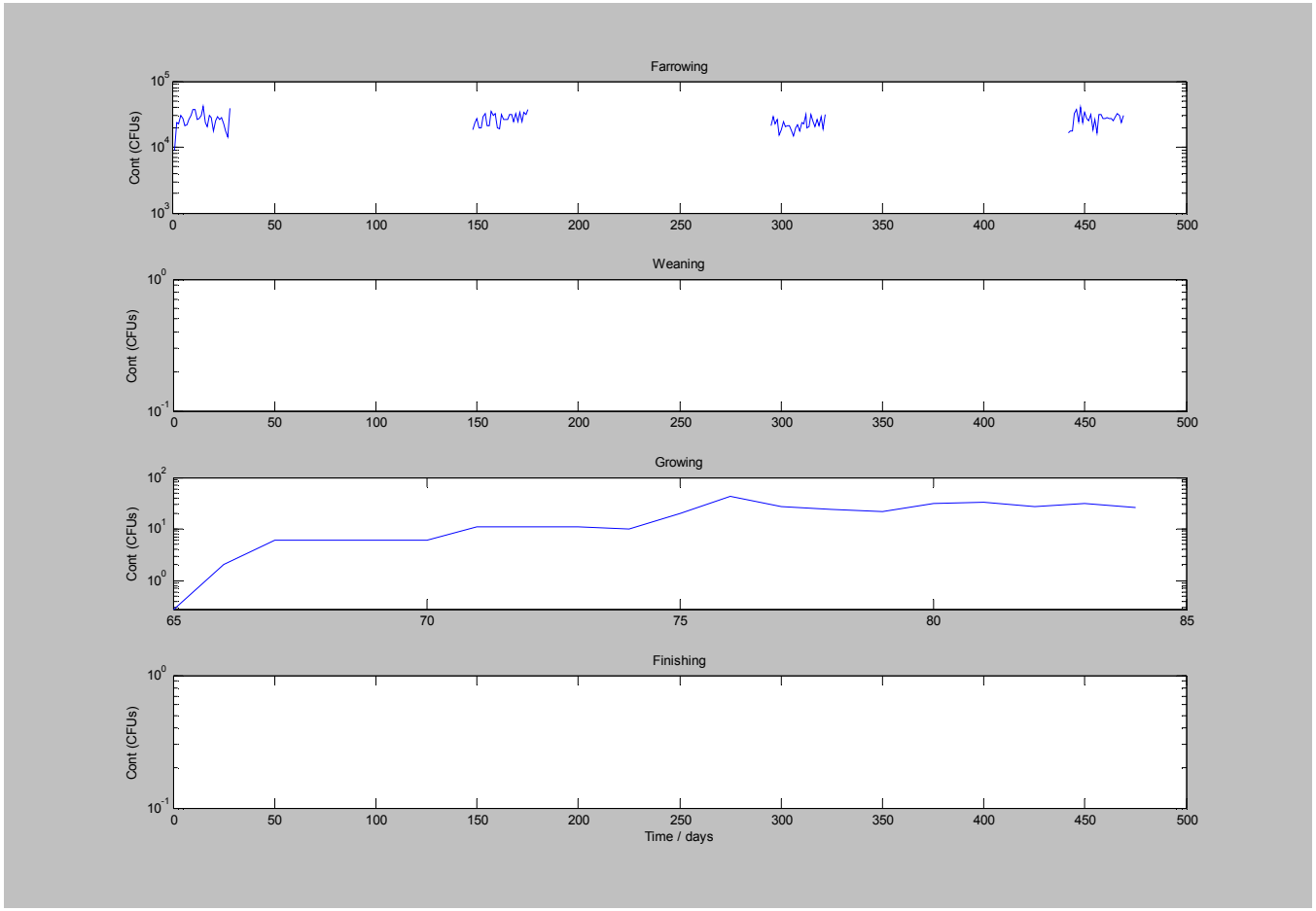


Figure 7.13c: Individual pen contamination profiles over time for small farm (breeding pig herd positive). Pens are picked to show typical examples, and are not representative over whole MS model.

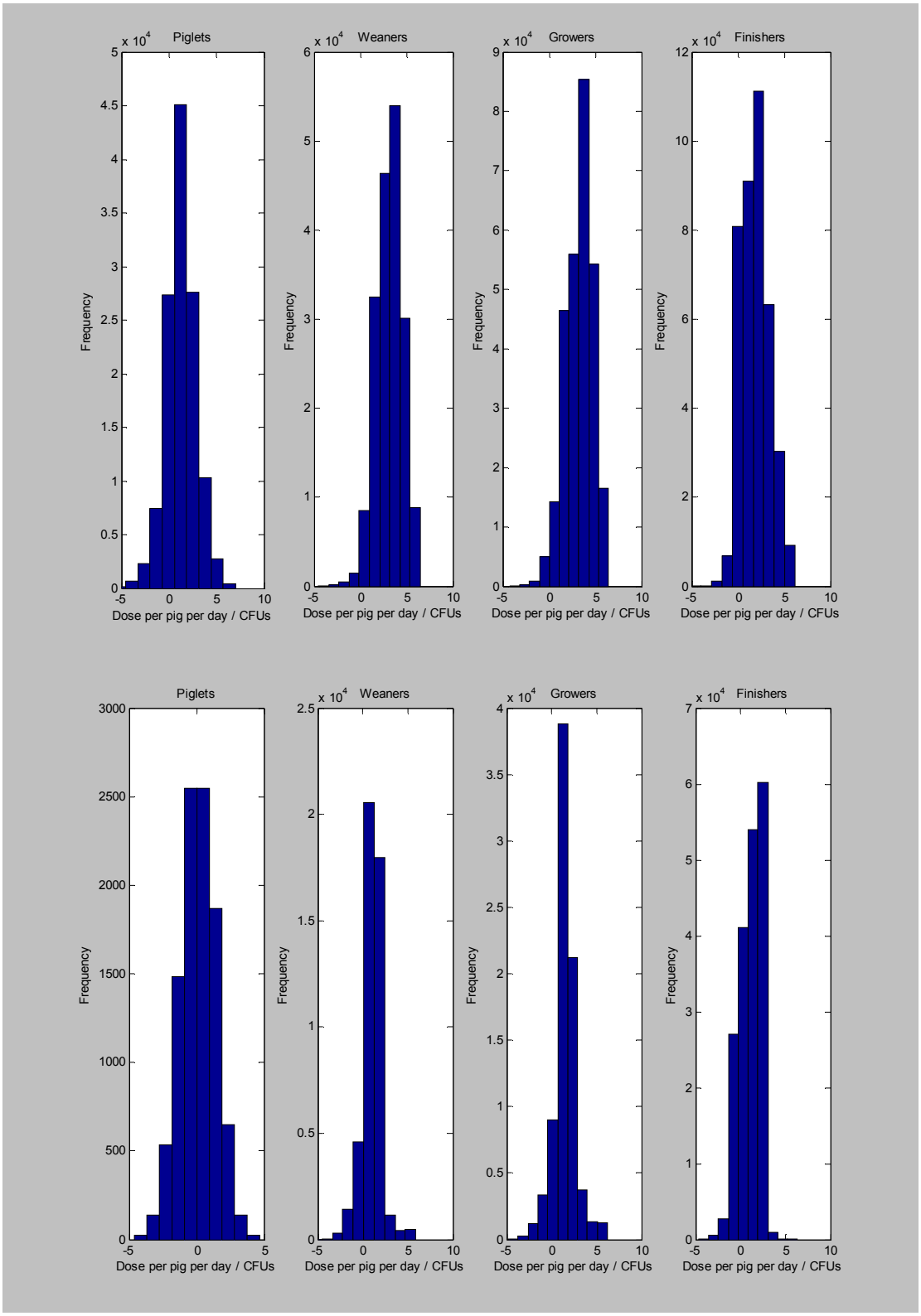


Figure 7.14a-b: Distribution of doses ingested by pigs during different stages of production for large farms producing pigs from a a) positive and b) negative breeding pig herd. The dose ingested is dependent on the contamination level within the pen, the number of infected pigs/sows, the amount of faecal material ingested. Only non-zero doses shown.

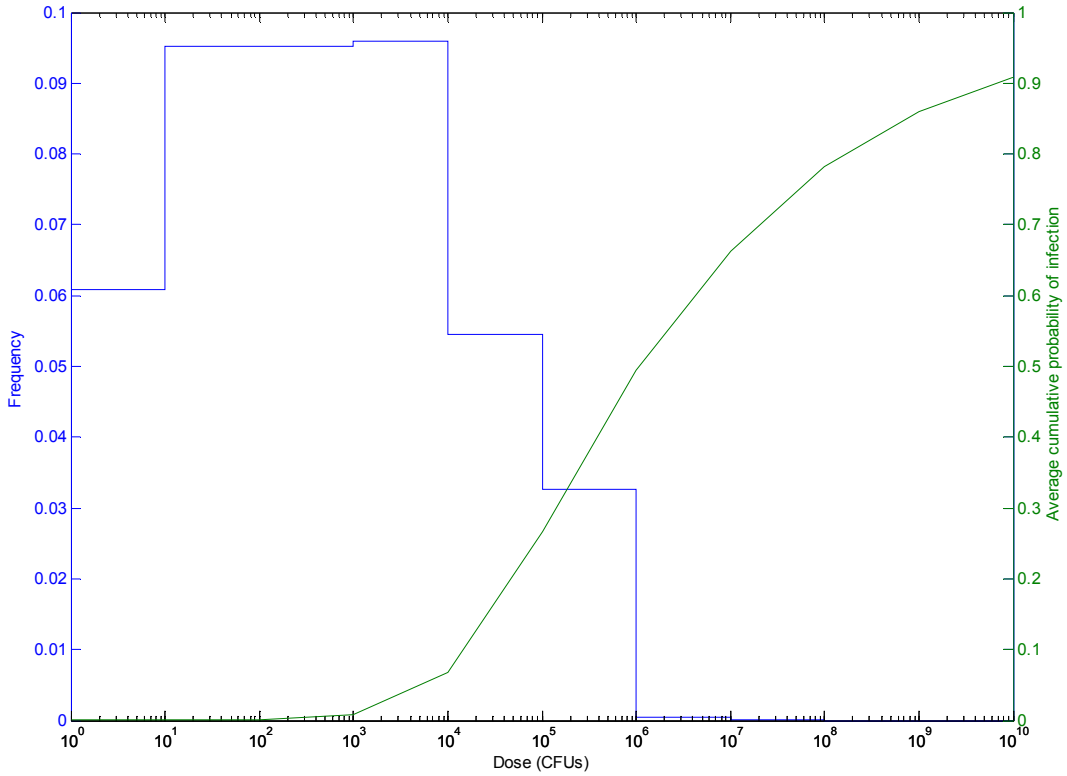
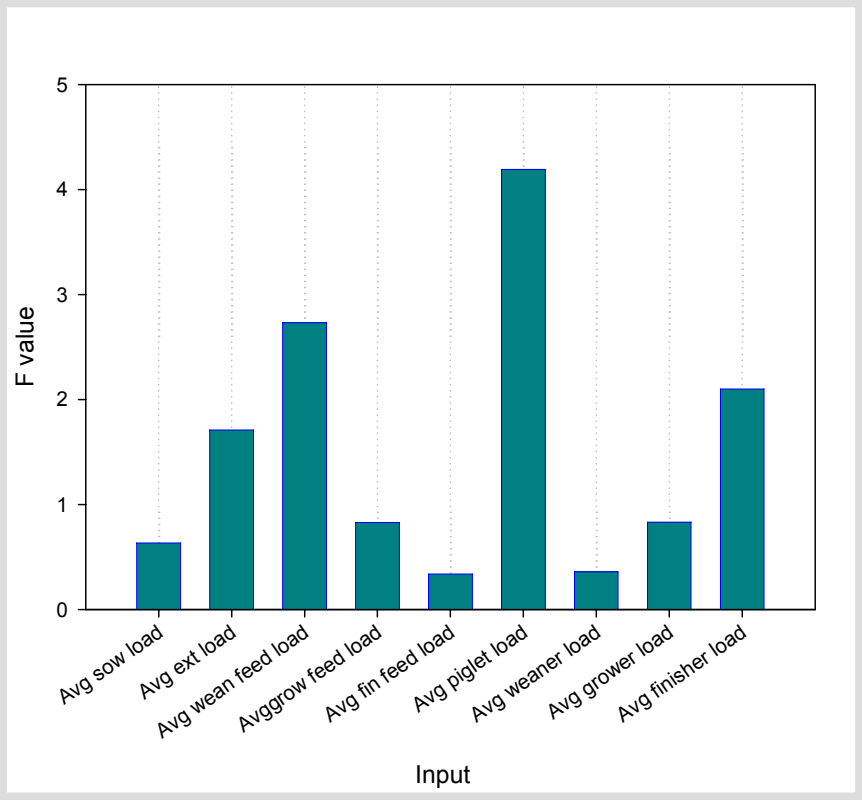


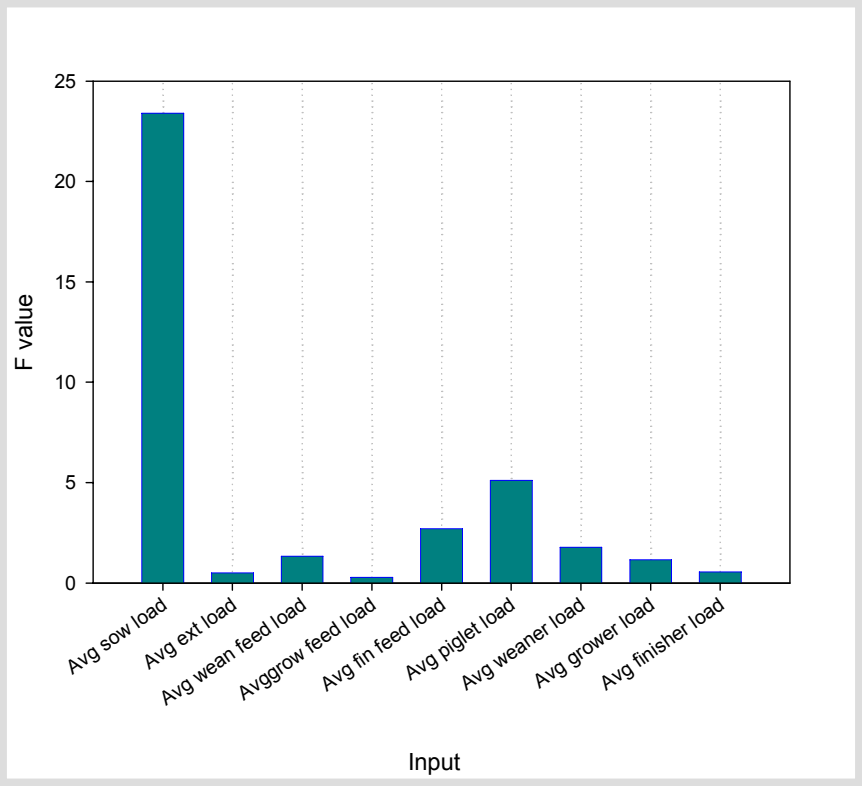
Figure 7.15: Comparison of doses ingested by pigs (from all stages of production) against the probability of infection (using only non-zero doses from the model). The majority of doses ingested by pigs (from faeces, feed and external contamination) are unlikely to result in infection at the average probability of infection. Note different scales of two y axes.

The sensitivity analysis clearly shows that if a MS has a relatively high breeding pig herd prevalence (MS2) then the average load of *Salmonella* shed by the sows is dominant (to the point where the other parameters make little difference). However, if breeding pig herd prevalence is low (MS1, MS3) then feed and external contamination parameters become relatively much more important, although ultimately the variability associated with the within-batch prevalence is driven by the average load shed by piglets and finishers within the batch. The MS4, with a slightly higher breeding pig herd prevalence, appears to be at the cusp of where the sow load becomes less important than feed. These results make intuitive sense – if there are a large number of sows shedding *Salmonella* then it becomes much more likely that one or more sows will shed at high enough levels to overcome any maternal immunity piglets have to *Salmonella* infection. If sows are not a common source of *Salmonella* on the farm, then feed and other sources of infection will become more important. Highlighting the amount piglets and finishers shed as important parameters also seems intuitive – weaning is a time of mixing within the model, thus if a piglet/s sheds high amounts of *Salmonella* at this time many pigs can be come infected. A high finishing load will increase infection near the point of slaughter, leading to a higher prevalence of infection than what would usually happen as pigs are tending to recover by this time.

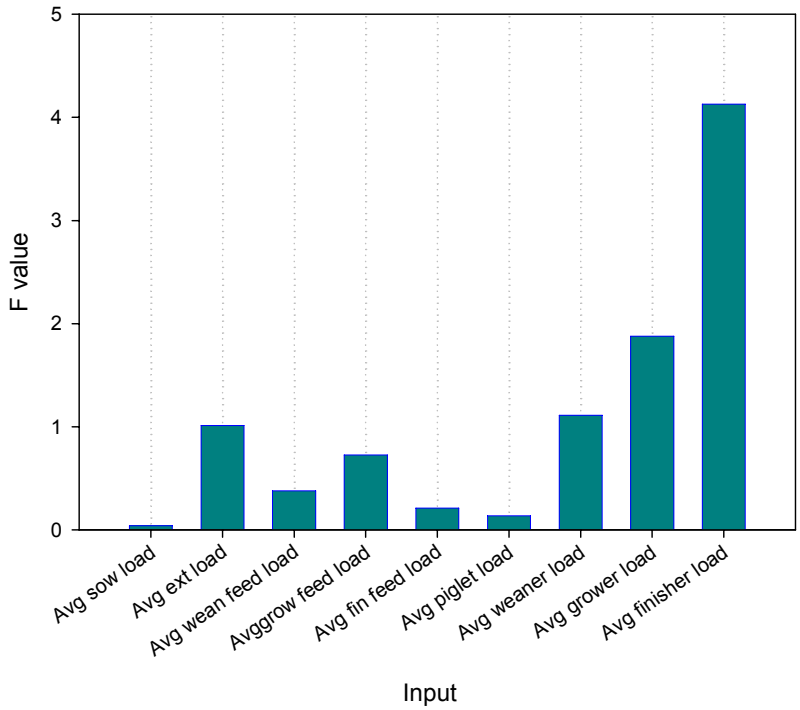
a) MS1



b) MS2



c) MS3



d) MS4

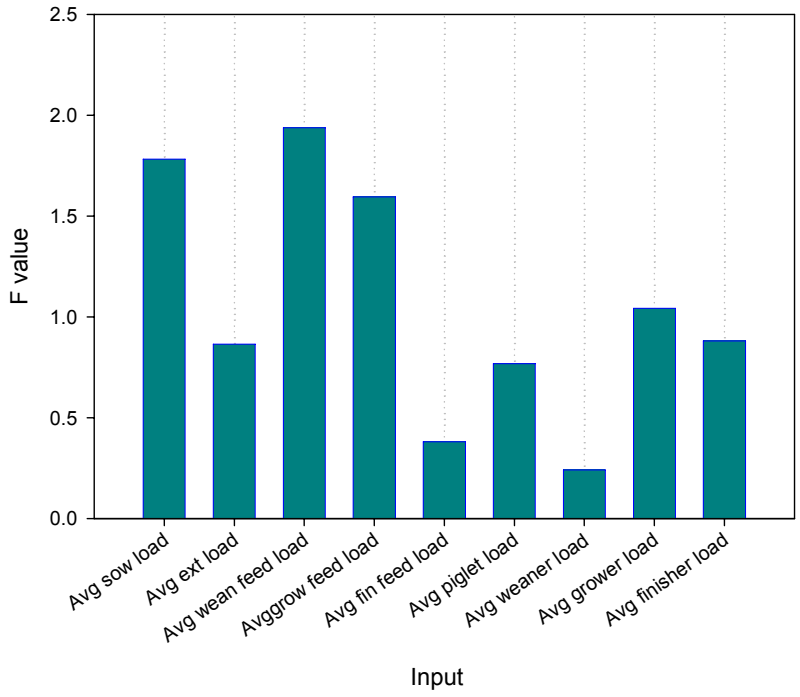


Figure 7.16a-d: Sensitivity analysis plots for each MS farm model, carried out using ANOVA for important large farm model parameters with variable distributions.

7.6 Discussion

The objective of this component of the QMRA was to describe the dynamics of *Salmonella* transmission in pigs in sufficient detail to a) estimate national pig prevalence; b) differentiate between MSs; c) investigate the sources of infection and the link, if any, between the breeding pig herd and infection at slaughter, and finally d) describe the effect of interventions in reducing slaughter prevalence. Each of these objectives have been met to varying degrees, but ultimately we think met sufficiently to provide enough insight for the EFSA scientific opinion which the risk assessment is designed to inform.

The model developed was designed to be generic, such that the model framework could be applied to all EU MSs. The parameterisation of the model then leads to a specific MS model, which has been done in this case for the four case study MSs.

The model was developed on the basis of a large literature review, information gained by talking to veterinary/microbiological and pig farming experts, plus a review of relevant transmission models. This review process highlighted the crucial fact that in order to a) differentiate between MSs and b) incorporate all interventions it was going to be necessary to include the pig environment within the farm model. The amount of *Salmonella* in the environment, to which pigs could be exposed to, is determined by a number of management factors. The factors included in this model were flooring, AIAO vs. continuous production, inside vs. outside production and feed. These were included on the basis that the literature provides evidence for these factors affecting *Salmonella* risk in individual pigs at the point of slaughter, and that we could model them sufficiently with the resources (time/data) available.

We have by no means captured all factors that describe variability in *Salmonella* risk in individual slaughter pigs between MSs and indeed between farms, and those that are captured are captured only to the extent which the data allows quantitative modelling. For example, we have split most management factors into two distinct, dichotomous options: wet/dry feed, solid/slatted flooring, AIAO/continuous production. However, in reality the options available for each factor are multiple and complex. Feed can be wet or dry, but also home-mixed or produced at a feed mill, pelleted or non-pelleted, acidified or non-acidified (and then acidified at different levels). Flooring can be fully or partially slatted, or concrete or straw bedded. Production can be strictly AIAO by building, or AIAO by compartment, or continuous for one stage but AIAO for the next. All of these different options could potentially affect *Salmonella* risk. Hence, the results produced must be viewed in conjunction with the simplifying assumptions made.

However, we believe this is the first real attempt to model the pig environment in such detail that enables differentiation between farm types. Lurette *et al*. (2008) model the pig environment as part of their transmission model for *Salmonella* in pigs, but they do not attempt to differentiate between farm types, and indeed it is not clear what farm type they presume from their parameter estimation. The transmission model is complex not only in the range of farm type it can incorporate, but also in mathematically describing transmission dynamics between pigs. The modelling of the pig environment enables us to quantitatively model the individual response of a pig to a variable daily exposure. Differentiating between farm types is fundamental in differentiating between MSs, and the current management factors included do mean that the results produced for each MS are very different according

to their particular parameter estimation. The differences between the MSs are discussed in more detail later in the discussion, but suffice to say we can be confident that we have captured a significant proportion of the variability between MSs with the current model. However, improvements can always be made, and one obvious example is differentiating between feed types in more detail. The data are available, and potentially support the differentiation between not only wet and dry feed, but also pelleted and non-pelleted feed (O'Connor *et al.* 2008).

Exposure to *Salmonella*, and the response to *Salmonella* infection in pigs, is incredibly variable, as evidenced by a wide range of observational and longitudinal studies (Jensen *et al.* 2006; Kranker *et al.* 2003; Lo Fo Wong & Hald 2000; Nollet *et al.* 2005). We estimate that contamination of the pen can vary between 10-10^9 organisms over short time periods; such large variation in contamination unsurprisingly leads to large variation in the amount of *Salmonella* ingested by a pig, and subsequently the incidence of *Salmonella* infection. It is difficult to validate the model at an individual farm level given this wide variation, but broadly speaking we see that the model results are highly variable, which at a very qualitative level is in line with observation (Lo Fo Wong & Hald 2000).

The validation of the farm model really takes place at the point of slaughter, where we compare the prevalence of lymph-node positive pigs at a MS level against the prevalence observed through the EFSA slaughter pig baseline results (EFSA 2008a). This is discussed in the next chapter, but some qualitative validation of trends has been done throughout the stages of the farm model.

Little evidence of infection has been found in piglets still suckling from their mother, although the evidence is mixed for whether (sero-) positive pigs make the progeny more or less likely to be infected at the point of weaning (Lo Fo Wong & Hald 2000; Nollet *et al.* 2005). Within these studies there is the indication that infection in piglets could be under-estimated because of a high likelihood of false negatives. The studies referenced were relatively small – there is certainly the probability they simply didn't sample any highly-infected piglet groups because these are relatively rare. However, the broad consensus from these studies is that it is not until weaning (when piglets are faced with the double stresses of being weaned and mixed with other unfamiliar pigs) that a significant proportion of pigs may become infected with *Salmonella*. Comparing against the model the broad trends are certainly the same as observed previously. Infection in piglets is rare and usually at a low incidence rate. However, the model does sometimes show a highly-infected batch of piglets if the sow is shedding relatively large amounts of *Salmonella* (over 10^6 CFU/g of faeces). While we do not explicitly model stress/feed change during weaning, we do mix pigs together. The larger amount of *Salmonella* shed by weaners relative to piglets, and the fact there are more pigs directly exposed to this *Salmonella*, means that within the model the peak prevalence of infection is usually observed during the weaning period (sometimes in the growing period). There is generally a diminishing prevalence of infection at the point of slaughter. This agrees with most current observational data (Kranker *et al.* 2003; VLA 2009).

If we capture the broad trends observed by observational studies, this does not mean further improvement cannot be made. There are many assumptions made within the farm model, some due to simplifying the complexity of the farm system or transmission dynamics, and some made simply due to a lack of data or evidence to be able to model either proven or anecdotal trends. A list of assumptions and data gaps is presented in Section 7.4.6, which we draw upon to highlight some crucial assumptions.

The following factors have not been included in the farm model: further differentiation between feed types, clustering of *Salmonella* in faeces, varying growth rate (such that pigs are held back in production), and transmission dynamics between sows. These are all potentially important factors for *Salmonella* infection in finishing pigs, but were not included because of the complexity of modelling such factors. Further differentiation between feed types would have been difficult to parameterise, but is certainly important. Clustering of *Salmonella* in faecal material has been modelled before (Arnold & Cook 2009), but would require a more complex model. The effect of clustering in faeces would be to vary (even more so) the daily exposure of pigs to *Salmonella*, where some pigs would ingest considerably more organisms, and some considerably less. Over the large number of pigs and timesteps (even within the small farm model) we would hope that the effect of this clustering averages out, but cannot be certain that this is the case. Another artefact of this assumption is that we assume if a pig is shedding *f* faeces per day and *y* organisms per gram of faeces, then the total amount of *Salmonella* shed per day is *fy*. This is probably an over-estimate as organisms are likely to be clustered and not at a constant concentration across the faeces. In reality, varying growth rate of individual pigs means pigs need to be kept back behind their cohort before reaching the correct weight to be moved into a different stage of production or sent to slaughter. We have not included this because of the difficulty in including any variation in pig group size (computationally pig cohorts are represented as matrices, and matrix manipulation is only possible with identical or compatible matrices). In addition, varying growth rates between farms will mean different ages of pigs being sent to slaughter, which has not been captured in the model, and so may well also alter the stage of infection of pigs. We judged the modelling of transmission dynamics in sows (apart from the shedding of the *Salmonella* in its faeces) to be unnecessary (despite the reference to it in the EFSA ToRs), primarily because we had good data on the proportion of herds with excreting sows from the EFSA breeding survey. Therefore, we simply assigned the distribution of shedding sows according to the survey results for each MS. A lack of data meant the within-herd prevalence was assumed to be the same within each MS (based on MS2 data), although data from the EFSA baseline survey does suggest that within-herd prevalence varies betweens MSs (and probably herds) varies just as much as the breeding pig herd prevalence does.

Important data gaps highlighted by the model development were the (variation in) dose-response of pigs to infection, the movement of faecal material and the amount of *Salmonella* that might be present in the environment due to feed or other external sources of contamination (rodents, birds etc). However, for all information gathered for this model, the trend was that regardless of the type of data needed, it was unlikely that current observational, experimental, longitudinal or survey data would be sufficient to be confident that all the variability had been accurately captured (e.g. the amount of *Salmonella* shed by a sow is based on one study that shows high variation between pigs – but did they capture the entire range of variation?). This is especially true when it comes to management data – this is as important, if not more important, than being able to describe *Salmonella* infection in the pig, as it determines the frequency and magnitude of exposure to *Salmonella*. Specific examples include categorisation of contract finishing farms – not captured in any of the EFSA surveys - and how individual farmers class AIAO production.

Having detailed many of the assumptions and uncertainties of the model, caution must be used when interpreting the results. However, some broad conclusions can be drawn from the current model results, and these are now described.

Sensitivity analysis of the model shows that the relative importance of parameters varies according to MS parameter estimation. The main example of this is the relative sensitivity of the model output (i.e. the variation in the within-batch prevalence of infection at slaughter age) to the burden of excretion by the sow if it is infected. In two MSs (MS2 and MS4) this is the foremost or second-most important parameter in describing the variability in the within-batch prevalence. It is no coincidence that these two MSs have relatively high breeding pig herd prevalence (44 and 13% respectively). For the other two MSs, with relatively low breeding pig herd prevalence, then the load shed by the sow is a relatively unimportant factor compared to the load being shed by the piglet, or that within the feed.

Further analysis of the model shows the reason for this dichotomy: if the sow is infected and shedding at high levels, then commonly (although not always) this will mean one or more piglets will become infected; when this occurs then the shedding of *Salmonella* by infected pigs, at the farrowing stage or later, dominates the risk. However, in MS1 and MS3 infection of the sow is relatively rare, and so the infections within the herd are generated by an initial infection of a piglet, weaner etc via either feed or external contamination. The sensitivity analysis also identifies another trend: that once a slaughter pig is infected, the subsequent shedding of *Salmonella* more than outweighs the contribution of contamination within the environment provided by feed and/or the external environment. In summary – breeding pig herd prevalence is a strong predictor of national pig prevalence, and while only simply modelled, scenario and sensitivity analysis suggest that mixing infected pigs with uninfected pigs at any stage of production will be an important source of infection. Finally, feed becomes an important source of infection once contamination of the environment by sows or other slaughter pigs is reduced to low levels.

It is difficult to validate the model result that seeding of infection into slaughter pigs is primarily governed by the status of the breeding pig herd. There is evidence in the literature for and against this conclusion (Berends *et al.* 1996; Lo Fo Wong & Hald 2000; Nollet *et al.* 2004), but certainly we have not fully captured the complexity of exposure via myriad sources and myriad serotypes. More (field) research into source of infection is needed to prove/disprove the result of the current model.

The comparison of dose-response versus dose ingested (shown in Figure 7.15) is enlightening and explains the dynamics described in the paragraphs above. For the vast majority of the time pigs are exposed to *Salmonella* levels that are extremely unlikely to cause infection. Therefore it takes large doses to trigger sufficient infection to cause large increases in prevalence. These large doses must come from a peak in pen contamination, which can either come from the shedding by sows or slaughter pigs, feed or external contamination. These peaks come from the tails of the distributions for concentrations of *Salmonella* in pig faeces, feed, rodent faeces etc, and are therefore only rarely sampled from. This explains the characteristic distribution for within-batch prevalence for each MS, where the majority of batches are either non-infected or have a very low prevalence, as it takes a rare, high-contamination event to cause a high prevalence of infection.

Finally, the farm model has been designed to provide the input to the Transport & Lairage model – a random sample of $P_{LN}(fr(t'))$ is taken, but also the paired output of the concentration within an infected pig's faeces at the time of transport to the slaughterhouse. The uncertainty and intervention analysis are discussed in Chapters 12 and 13 respectively.

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8 Transport & Lairage

8.1 Introduction

Transport and lairage are thought to be important stages for *Salmonell*a transmission in the pig farm-to-consumption chain. It has been reported that there are significant increases in the prevalence of pigs infected with *Salmonella* between the farm and the slaughterhouse (Davies *et al.* 1999, Berends *et al*. 1996, Hurd *et al.* 2002). Berends *et al.* (1996) report trials that showed *up to* 20% of uninfected pigs within a batch could become infected during transport and lairage. They also report that 2-6 hours of combined transport and lairage could cause the number of animals excreting *Salmonella* to more than double. While pigs are only in transport and lairage for a short period of time, research has shown that pigs from low risk herds are at risk of becoming infected with *Salmonella* when held in contaminated pens (Boes *et al.* 2001) and *Salmonella* can be isolated from the faeces of pigs exposed to a contaminated environment for as little as 2 hours (Boughton *et al.* 2007b, Hurd *et al.* 2001).

During transport it is believed that stress may play an important role, causing an increase in faecal shedding (Gronstal *et al.* 1974a) and also cause carrier animals to revert to excreting *Salmonella* in their faeces (Williams & Newell, 1970, Gronstal *et al.* 1974b). The study by Williams & Newell (1970), while small, showed that even though rectal swabs of pigs on the farm and swabs of the truck were all negative, 6 pigs were found to be excreting *Salmonella* after a 3 ¾ hours journey and all ten swabs of the truck also tested positive for the same strain (perhaps also suggesting that "carrier" pigs are initially shedding *Salmonella* beneath the limit of detection, hence the negative results from the swabs). Environmental contamination is also an important factor to consider. Many studies have shown *Salmonella* spp. to be present in trucks used to transport pigs (e.g. Rajowski *et al.* 1998, Rostagno *et al.* 2003), even after routine cleaning has been carried out (Mannion *et al.* 2008, Dorr *et al.* 2009). There are also numerous studies that have isolated *Salmonella* spp. in the lairage (Boughton *et al.* 2007a, Dorr *et al.* 2009, Rostagno *et al*. 2003, Swanenburg *et al.* 2001), where multiple batches of pigs can occupy the same living space in a short period (i.e. one day), with little or no cleaning between batches (expert opinion from MS2 suggests, between batches, the area would be hosed down with water, but thorough cleaning would only be done at the end of the day VLA (2009b)). A study by Rostagno *et al.* (2003) isolated *Salmonella* serovars from the caecum and ileo-caecal lymph nodes of pigs that were present in transport and lairage. Gebreyes *et al.* (2004) isolated serovars of *Salmonella* from the mesenteric lymph nodes and caecum of pigs that were not found on the farm, but were found in transport or lairage. These studies suggest that the transport and lairage environments should be considered important sources of infection.

In most previous pig *Salmonella* models there has been little development of the transport and lairage stages, with most relying on simple equations to model a proportion change in infection levels between farm and slaughterhouse (e.g. VLA, 2003). However, as already stated, it has been established that pigs can become infected with *Salmonella* very quickly and certainly in less time than the duration of transport or lairage. Also of concern is the fact that the skin of the pig could become contaminated with *Salmonella* once loaded into transport or lairage pens. It is therefore likely that there are many components of transport and lairage where interventions could take place to reduce the prevalence of infected pigs or

concentration of *Salmonella* on contaminated hides (e.g. more effective cleaning of trucks and lairage, separation of pigs, decontamination of hides). A mathematical model can be used to evaluate the effectiveness of these intervention strategies. These factors are the main driving forces behind this paper, where we propose a more in-depth framework to model the transmission of *Salmonella* during the transport and lairage of pigs.

8.2 Model Framework

8.2.1 Model overview

The Transport & Lairage module is designed to be a generic model for the EU, but in order to be adaptable to any EU country, many parameter estimates will differ between member states (e.g. the proportion of large to small farms, number of pigs slaughtered per day in a slaughterhouse). The model simulates the transmission of infection within batches of pigs, going to a specific slaughterhouse over the course of one day (thus each iteration of the model represents one day). For this model we define a batch to be a group of pigs that occupy the same 'living environment'. In transport this is a truck and in lairage a pen.

The model is stochastic, with the parameter values including the variability in the observed data. However, a decision was made not to include uncertainty in the model, the effects of this would be investigated in an uncertainty analysis (Chapter 12). The computational steps included in the model, for pigs from a large farm, are shown in Figure 8.1 Note that the steps for pigs from a small farm are the same except that the pigs go to a small slaughterhouse and so the number of pigs going to the large slaughterhouse, n_l, is replaced by the number of pigs going to the small slaughterhouse n_s.

8.2.2 Pig selection

For each iteration of the model, days worth of pigs to be slaughtered in a "large" and "small" slaughterhouse are selected, where size relates to the number of pigs slaughtered per day (we define a large slaughterhouse to be one that slaughters more than 100,000 pigs per year). We assume (due to lack of data to the contrary) that pigs from large farms will go to large slaughterhouses and pigs from small farms will go to small slaughterhouses. The model accounts for variation between slaughterhouses. For each iteration the model is first run for the small slaughterhouse (the results of which are inputs to the small slaughterhouse model (see Chapter 9) and then independently for the large slaughterhouse (the results of which are inputs to the large slaughterhouse model (see Chapter 9).

The selected slaughterhouse (large or small) is assigned a specified number (or 'capacity') of pigs to be slaughtered (denoted as n_l for large slaughterhouse capacity and n_s for small slaughterhouse capacity. This number is derived from data from member state slaughterhouse capacities). The model then randomly selects batches of pigs (with the appropriate large to small farm ratio) from the output of the farm module, until the capacity of the slaughterhouse is reached. These batches of pigs then enter the Transport & Lairage model, where the transmission of *Salmonella* within these batches are modelled on an individual pig basis.

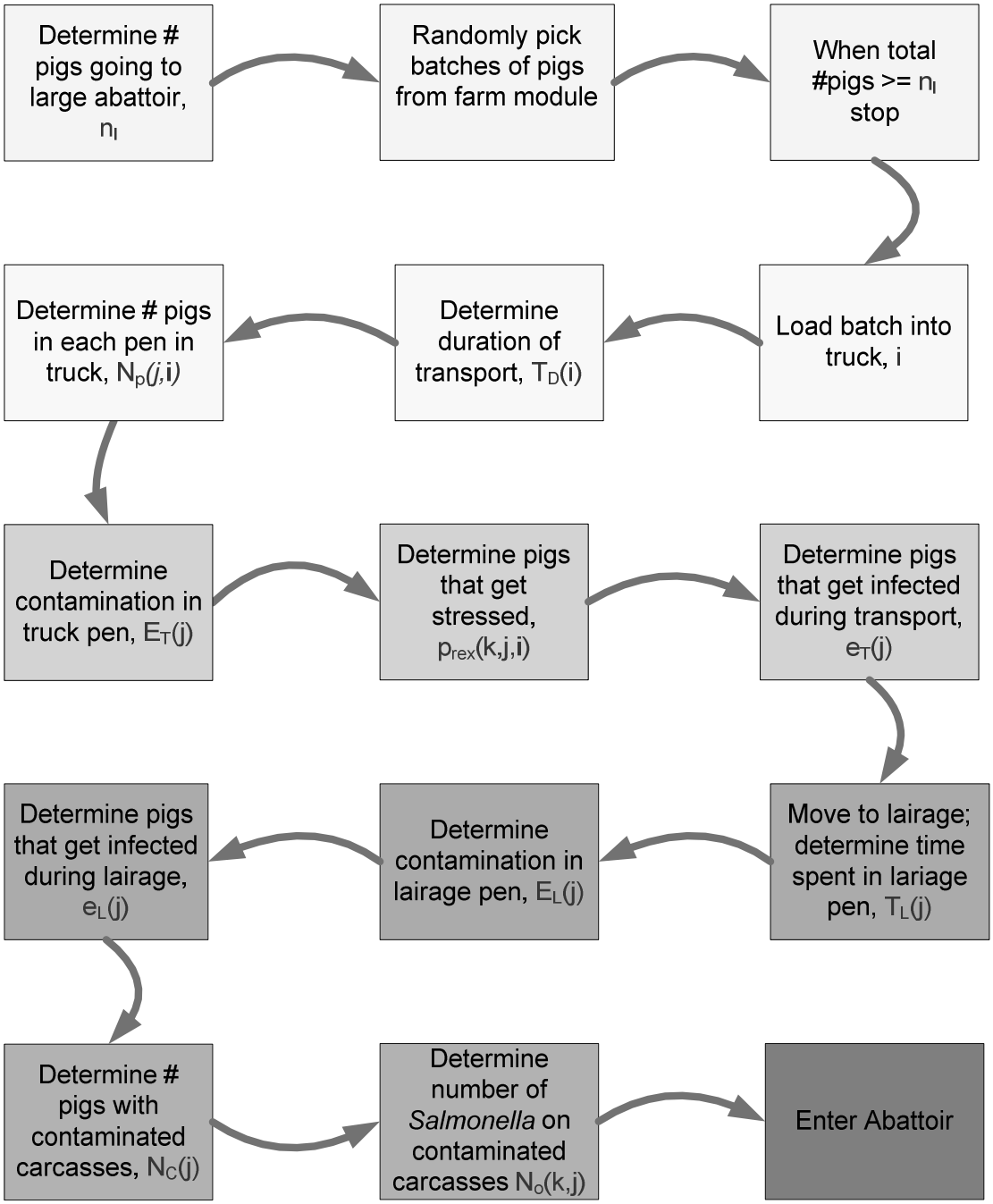

Figure 8.1: Computational steps in the Transport & Lairage simulation model (for pigs from a large farm)

8.2.3 Loading

Following batch selection, loading onto transport trucks is considered. Data and expert opinion collected from member states (EFSA, 2008a, Marier, 2009) suggest that it is very rare for a truck to pick up pigs from multiple farms in one journey (for the small farms, expert opinion suggested that many would transport their own pigs to slaughter), the main

exception being if two farms are owned by the same producer. Thus, for simplicity, we make the assumption in the model that trucks only pick up pigs from one farm. The effect of trucks picking up pigs from multiple farms is investigated as a scenario analysis (Chapter 13).

The next step is to determine duration of transport and the number of pigs in each 'pen', *j*, in the truck, *i*, $N_p(j,i)$. There are three main types of truck. The first is that it is just an empty space, with no divisions. In this case the whole batch of pigs would be in the same living space for the whole journey, and thus would be able to have direct contact with each other. In the second type, the truck is segregated into a number of 'pens', each containing a sub-set of the batch. The third type is a 'layered' truck, with multiple 'decks' and each deck housing a certain number of pigs. We assume that transport time is sufficiently short so that there will not be sufficient opportunity for between-pen cross-contamination. The differences between transport types are therefore negligible and each pen with $N_p(j,i)$ pigs can be treated as a closed population. General practice is for all pigs that are to be transported from a farm to be mixed together prior to loading, suggesting that any division of pigs on the farm would not necessarily carry through to transport. Therefore, in the model, the pigs in the batch are randomly 'shuffled' to account for mixing and then loaded onto the truck in this random order, (filling up the 'pens' and/or 'decks').

8.2.4 Lairage framework

The Lairage model simulates the transmission of *Salmonella* over the course of one day. Pigs that arrive late in the day may be held overnight, and slaughtered early the next day. To model this we assume that L_O number of lairage pens will house pigs overnight. Therefore, the first batches of pigs (as many as are needed to fill L_O pens) to arrive at lairage are considered to be housed overnight. This will affect the conditions of these pens and batches (e.g. a longer duration of stay in lairage, different effect of cleaning). We assume that the trucks arrive at the slaughterhouse over the course of the day during which time pigs that have arrived earlier will vacate the lairage pens to enter the processing stages. Pigs that arrive later in the day will enter the pens vacated by pigs that have gone to be slaughtered. We assume that during this short turnover the empty pen may undergo some cleaning (simple hosing down with water), but more thorough cleaning (such as use of disinfectant) will only be done at the end of the day (but not in the pens which contain pigs to be housed overnight).

8.3 Transmission of Infection during Transport & Lairage

8.3.1 Initial conditions – carryover

When pigs enter transport or lairage pens there is the possibility that these pens may be contaminated with *Salmonella* and/or faeces. We define this contamination as 'carryover' as it is what remains in the environment from pigs that have previously occupied the pen, but before the new batch of pigs enters.

For each truck and lairage pen, the model determines whether or not contamination has been carried over from the previous batch of pigs and if it has been carried over, the quantity is determined. We define the amount of faeces (g) left in pen *j*, after cleaning as $F_{carry,H}(j)$ and the amount of *Salmonella* (cfu) left in pen *j*, after cleaning as $E_{carry,H}(j)$ (where H={T,L}, to denote transport and lairage respectively).

Transport

For transport, it was not possible, due to lack of data, to consider the prior history of the truck (e.g. what animals were in the truck before? How many were there? Were they infected with *Salmonella*? Was the environment contaminated?). We estimate $F_{carry,T}(j)$ and $E_{carry,T}(j)$ from studies that record the frequency and degree of contamination of trucks before the pigs are loaded. Assuming independence between trucks

$$F_{carry,T}(j) = \Re\left(B(1,1-p^{T}{}_{FaecCarry}), j\right) * \Re\left(U(1, F_{TransMax}), j\right), \qquad (8.17)$$

where $p^{T}{}_{FaecCarry}$ is the probability that the truck has been successfully cleaned and all faecal contamination has been removed and $F_{TransMax}$ is the maximum amount of faeces carried over (note that $\Re\left(B\left(1,1-p^{T}{}_{FaecCarry}\right)\right)$ = {0, 1} with probability { $p^{T}{}_{FaecCarry}$,*1-* $p^{T}{}_{FaecCarry}$*}*, so if there is cleaning $F_{carry,T}(j) = 0$). Similarly

$$E_{carry,T}(j) = \Re\left(B(1,1-p^{T}{}_{EnvCarry}), j\right) * \Re\left(U(1, E_{TransMax}), j\right), \qquad (8.18)$$

where $p^{T}{}_{EnvCarry}$ is the probability that the truck has been cleaned and $E_{TransMax}$ is the maximum amount of *Salmonella* present in the truck when pigs enter

Lairage

Lairage is modelled throughout the day and thus the model provides an estimate of the prior history of the pens when new pigs are placed in them. However, we do not know the history of the pen for the first batch of the day. So here we use the same method as transport and estimate $E_{carry,L}(j)$ and $F_{carry,L}(j)$ from studies that record the frequency and degree of contamination of lairage pens. Assuming independence between pens $F_{carry,L}(j)$ and $E_{carry,L}(j)$ are given by

$$F_{carry,L}(j) = \begin{cases} \Re\left(B(1,1-p^{L}{}_{FaecCarry}), j\right) * \Re\left(U(1, F_{LairMax}), j\right) & L_B(j,t) = 0 \\ F_L^c(j) - F_L^c(j) * \Re\left(B(1, p^{L}{}_{clean}), j\right) * \chi_L^F(j) & L_B(j,t) > 0 \end{cases}, \qquad (8.19)$$

where $L_B(j,t)$ is the number of batches of pigs that have previously occupied pen *j* during the day, $F_L^c(j)$ is the amount of faeces left in the pen after previous occupation, $\chi_L^F(j)$ is the proportion reduction of faeces due to cleaning and $p^{L}{}_{clean}$ is the probability that the pen is cleaned (note that $\Re\left(B\left(1, p^{L}{}_{clean}\right)\right)$ = {0, 1} with probability {*1-* $p^{L}{}_{clean}$*,* $p^{L}{}_{clean}$*}*, so if there is no cleaning $F_{carry,L}(j) = F_L^c(j)$). Treatment of *Salmonella* in a lairage pen is given by

$$E_{carry,L}(j) = \begin{cases} \Re\left(B(1,1-p^{L}{}_{EnvCarry}), j\right) * \Re\left(U(1, E_{LairMax}), j\right) & L_B(t) = 0 \\ E_L^c(j) - E_L^c(j) * \Re\left(B(1, p^{L}{}_{clean}), j\right) * \chi_L^E(j) & L_B(t) > 0 \end{cases}, \qquad (8.20)$$

where $E_L^c(j)$ is the load of *Salmonella* left in the pen after previous occupation and $\chi_L^E(j)$ is the proportion reduction of *Salmonella* due to cleaning.

We must also take account of the fact that the type of cleaning employed at the end of the day is also often more rigorous and so the proportion reduction in cleaning is considered to be more effective (FSA, 2006)

8.3.2 Transmission of infection

During transport and lairage, we assume that a pig can be in one of two states at any time: susceptible (0) or infected (1). Thus, during transport, the infection status of pig *k*, in pen *j* of truck *i* is denoted by $\Omega_T(k, j, i)$ where $\Omega_T(k, j, i) \in \{0,1\}$. During lairage the infection status of pig *k*, in pen *j* from truck *i* is denoted by $\Omega_L(k, j, i)$. We define the variables $S_H(j)$ and $I_H(j)$ to be the total number of susceptible and infected pigs respectively, from pen *j* after stage *H*.

As in the farm model (Chapter 7) we define the infected state to mean that a pig is infected in the ileo-caecal lymph-node and will intermittently excrete *Salmonella* in the faeces, of varying concentrations ranging from 0 to 7 log cfu/g, as suggested by Jensen *et al*., (2006). During transport and lairage there are events that can cause either a change of state (e.g. susceptible pigs becoming infected) or a change in the concentration of *Salmonella* excreted by infected pigs.

<u>Increased shedding of infected animals due to transport stress</u>

During transport it has been observed that the prevalence of shedding will increase (Davies *et al.* 1999, Berends *et al*. 1996, Hurd *et al.* 2002). There are many possible causes for this, but one of the most important is thought to be stress during transport (Berends et al, 1996), this includes stress caused prior to transport when pigs may be held in lairage overnight or mixed with unfamiliar pigs.

To account for the effect of stress we assume that there is a fixed probability, p_{rex}, that lymph-node positive pigs will become stressed during transport (note, there is little evidence to suggest that stress is such an important factor during lairage and in fact longer lairage times are beneficial in reducing the previous stress of transport (Warris *et al.* 1998)).

A US study (Callaway *et al.,*2006) looked at the effect of mixing (social) stress on populations of *Salmonella* Typhimurium in segregated early weaning pigs. After 5 days they found that the incidence of faecal *Salmonella* shedding was higher in mixed contact pigs. They concluded that social stress of weaned pigs may increase susceptibility to and/or faecal shedding of *Salmonella*. This study is not directly related to transport stress, but it does suggest the effect that stress will have on pigs infected with *Salmonella.* Therefore, in the absence of other relevant data, we assume that the concentration of *Salmonella* excreted in their faeces in stressed pigs will be increased. To model this, we change the distribution for concentration of *Salmonella* excreted in the faeces, so that higher concentrations are more likely and consequently, under stress, more infected pigs will be excreting *Salmonella*. There is little data to determine exactly how we should change this distribution. As discussed in the Chapter 7, there is an observable difference in excretion levels between pigs infected with a low dose of *Salmonella* and those infected with a high dose (Jensen *et al*., 2006). Given the lack of data, we assume that the effect of stress is equivalent to the difference between excretion levels of low dose and high dose pigs. Thus if a pig becomes stressed during transport, the amount of *Salmonella* they shed is increased by between 1-3 log cfu/g (determined by a random sample from a U(1,3) distribution), but

with a maximum of 6 log cfu/g (so a pig that was already shedding 5 log cfu/g would not increase to any more than 6 log cfu/g)).

Environmental infection

During transport and lairage pigs are kept in confined spaces and in close contact. Riches *et al.*, 1996 reported a mean stocking density of 239 kg/m^2 for full truck loads in winter (standard deviation of 38). This high stocking density means that there is a high risk of exposure to *Salmonella* contaminated faeces. This risk is further heightened by the likelihood of carry over from previous batches of pigs, as while trucks may be cleaned between journeys it is reported that this cleaning will not remove all of the *Salmonella* from a contaminated vehicle (e.g. Mannion *et al.* 2008, Rajkowski *et al.* 1998). However, different methods of cleaning have different effects (Small *et al.* 2007).

The way in which we model environmental infection is shown in Figure 8.2. This framework is applicable to both transport and lairage, but the parameter estimates will differ. We determine how much *Salmonella* each pig will ingest and use the dose-response relationship derived for the farm model (Chapter 7) to determine whether or not it becomes infected. We estimate the amount of *Salmonella* ingested by calculating the amount of faeces ingested and the concentration of *Salmonella* in the faeces. To do this we use the methodology adopted in the farm model (Chapter 7). As for the farm model, we assume that *Salmonella* and faeces will be homogenously spread throughout the pen (cleaning and movement of pigs in a confined space is likely to spread *Salmonella* over the whole pen (VLA, 2009b).

Amount of faeces in a pen

To estimate the amount of faeces in pen *j* after stage *H*, $F_H(j)$, we sum the environmental carryover, $F_{carry,H}(j)$, and the faeces excreted by pigs in pen *j*, $F_{pig,H}(j)$

$$F_H(j) = F_{carry,H}(j) + F_{pig,H}(j). \quad (8.21)$$

The amount of new faeces excreted in pen *j*, is estimated by summing up the amount of faeces excreted by all pigs currently in pen *j*

$$F_{pig,H}(j) = \sum_{k=1}^{P_H(j)} f_{pig}(k, j), \quad (8.22)$$

where $P_H(j)$ is the total number of pigs in pen *j*. The amount of faeces excreted by pig *k* in pen *j* is estimated as

$$f_{pig}(k, j) = \bar{f}(k, j) * \Re\left(B\left(T_H^D(j), P^D\right), j\right), \quad (8.23)$$

where $\bar{f}(k, j)$ is the amount of faeces excreted by pig *k* in pen *j*, per defecation, P^D is the probability of a defecation per hour and $T_H^D(j)$ is the duration of time the pigs spend in pen *j* at stage *H.*

Amount of *Salmonella* in a pen

To estimate the load of *Salmonella* in pen *j* after stage *H*, $E_H(j)$, we sum the number of *Salmonella* in the environmental carryover $E_{carry,H}(j)$ and the *Salmonella* excreted by infected pigs, $E_{pig,H}(j)$

$$E_H(k) = E_{carry,H}(j) + E_{pig,H}(j). \quad (8.24)$$

The *Salmonella* excreted by infected pigs is given by the formula

$$E_{pig,H}(j) = \sum_{k=1}^{P_H(j)} f_{pig,H}(k, j) * \varepsilon(k, j), \quad (8.25)$$

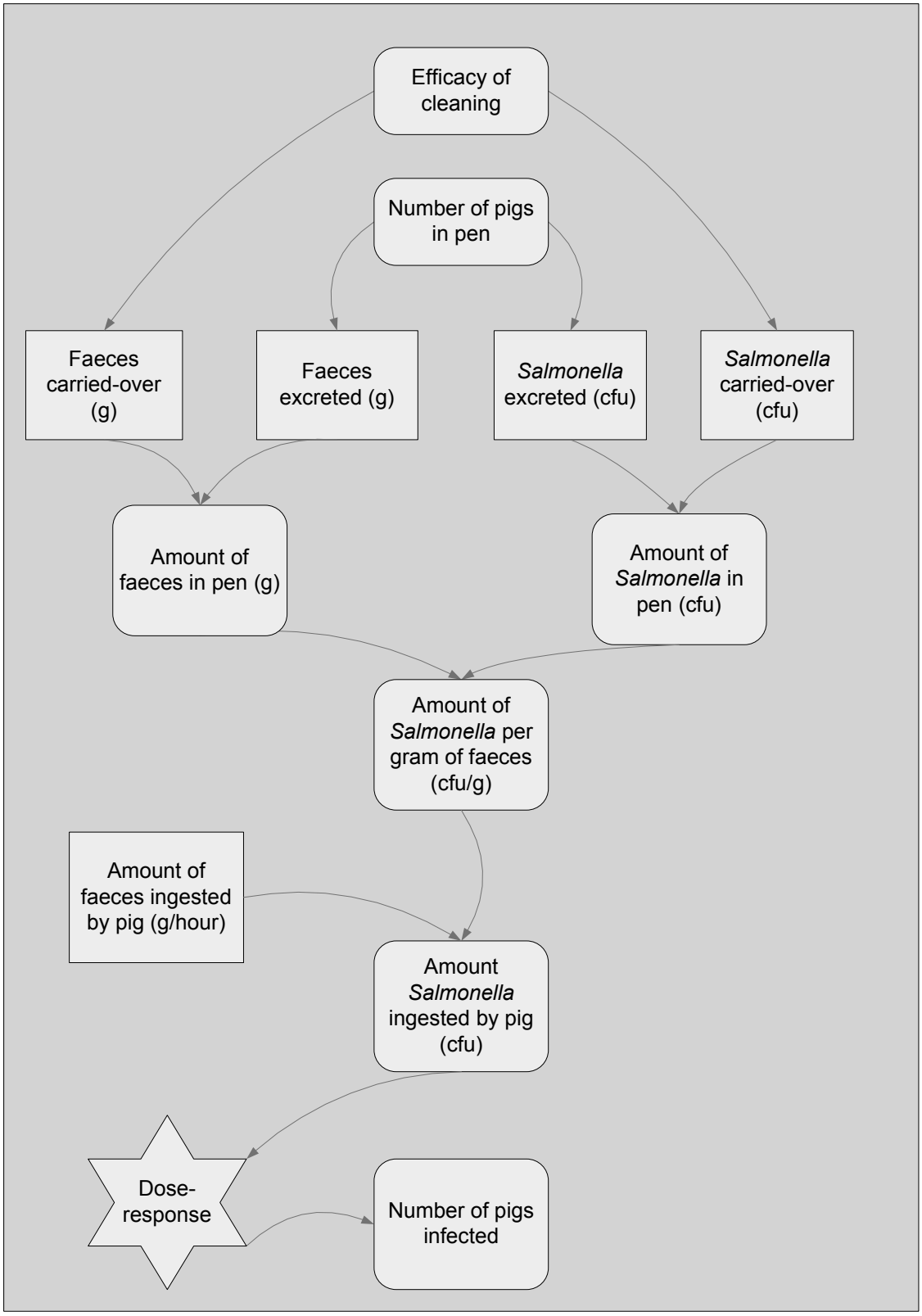

Figure 8.2: Computational steps for environmental infection of pigs

where $\varepsilon(k, j)$ is the concentration of *Salmonella* (cfu/g) excreted in the faeces by pig *k,* which is an output from the farm module (see Chapter 7).

Given the amount of *Salmonella* in the environment, $E_H(j)$, and the amount of faeces in the environment, $F_H(j)$, we estimate the concentration of *Salmonella* in the environmental faeces.

$$c_H(j) = \frac{E_H(j)}{F_H(j)} \qquad (8.26)$$

Amount of *Salmonella* ingested

We calculate the amount of *Salmonella* ingested by pig *k* during its stay in pen *j*, $\lambda_H(k, j)$ by multiplying the amount of faeces (in grams) ingested by pig *k,* $m_{ing}(k, j)$ by the concentration of *Salmonella* in the ingested faeces

$$\lambda_H(k, j) = \Re\left(Po\left(c_H(j)\right)\right) * m_{ing,H}(k, j) \qquad (8.27)$$

Having determined the ingested dose we then use the beta-binomial dose-response model, used for finishing pigs in the farm model (Chapter 7), to determine if any susceptible pigs become infected during their stay in the pen.

8.3.3 Skin contamination

When pigs enter the slaughter process, the characteristic of interest changes from the infection status to the proportion and load of contaminated hides and guts. Slaughter pigs that carry *Salmonella* in their gut are known to be a considerable risk for contamination of the carcass, and consequently the meat product (Berends *et al.* 1997; Botteldoorn *et al.* 2003). There is a lack of data concerning the prevalence of contaminated hides immediately after lairage. There are many studies that report the prevalence of carcass contamination during the slaughterhouse process (e.g. EFSA 2008b), but very few actually record the prevalence at the start of processing (i.e. immediately post-lairage).

Within groups of slaughter pigs, there is a correlation between the proportion of animals carrying *Salmonella* in the faeces and the proportion of contaminated carcasses. Data from a study by Davies *et al*. 1999 shows the results of samples taken from pigs during eight visits to one slaughterhouse (with a total of 2,205 samples). Isolations from the large intestine and the carcass are recorded. We assume that the large intestine results represent the number of infected animals and the carcass swabs the number of contaminated carcasses. We then calculate the ratio of these values, R_{CONTAM}. The proportion change between infected pigs and contaminated carcasses, $F_{CARCASS}$, is derived by fitting an empirical distribution to the values of R_{CONTAM} (see Figure 8.3), where if $F_{CARCASS}$ <1 there are more infected pigs than contaminated carcass and if $F_{CARCASS}$ >1 there are more contaminated carcasses than infected pigs. The number of pigs from each visit with positive caecal contents is assumed to be the sum of pigs from the infected class from the end of the lairage, $I^L(j)$. The mean proportion change from these data is 0.62 with a standard deviation of 0.31 and a 95% confidence interval of [0.41-0.84].

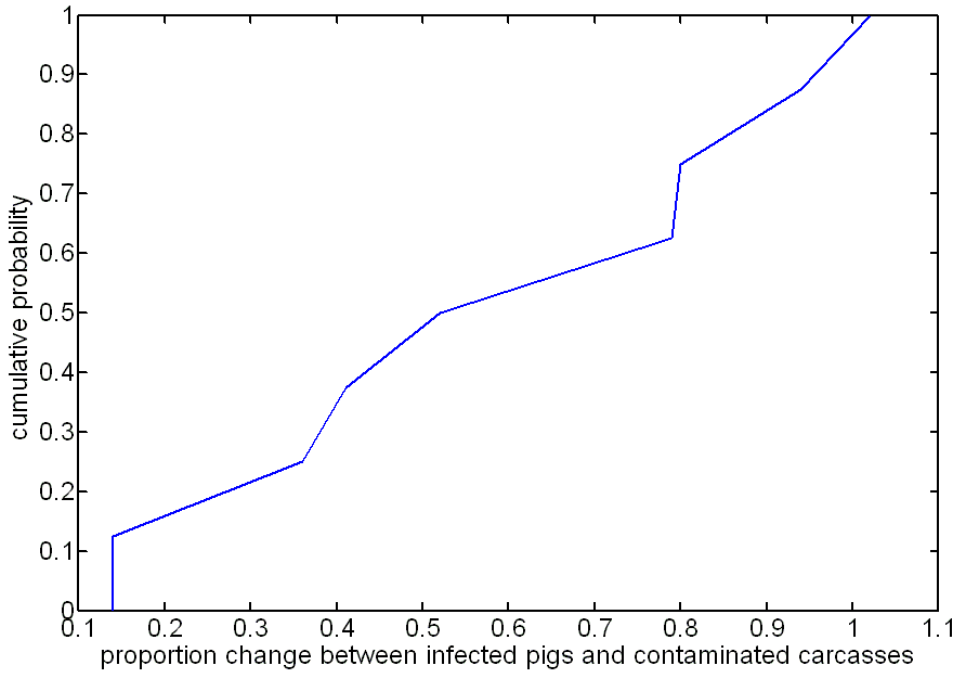

Figure 8.3: Cumulative empirical distribution for proportion change between infected pigs and contaminated carcasses

Thus, the number of pigs from lairage pen *j,* with contaminated hides, $N_{car,L}(j)$ is

$$N_{car,L}(j) = F_{CARCASS}(j) * I_L(j) \quad (8.28)$$

8.3.4 Number of *Salmonella* on the skin

There is very little information on *Salmonella* counts on hides at the slaughterhouse as most studies look at total aerobic count (TAC) or enterobacteriacae counts. However, data from Davies, 1999 show that post-bleed (which we assume to be equivalent to the end of lairage as the bleeding stage is not modelled in this risk assessment) the mean log *Salmonella* score per 0.1m^2 carcass is 1.9, with a maximum of 3. On this scale a score of 0 relates to 0 organisms, 1 relates to 1-10 organisms, 2 to 10-10^2 organisms and 3 to 10^2 - 10^3 organisms. This mean score is obtained from 10 contaminated carcasses. To achieve a mean score of 1.9 from 10 observations, we estimate that 30% of contaminated carcasses had score 1, 50% had score 2 and 20% had score 3. Therefore, the likely score of a contaminated carcass is ascertained as shown in Table 8.1.

Table 8.1: Postulated probabilities of *Salmonella* score of a contaminated pig carcass post-bleeding (based on Davies *et al.* (1999).

Score	Probability of occurrence	$h_c(i,j)$, cfu/cm^2
1	0.3	Uniform(1, 10)/ 1000[a]
2	0.5	Uniform(10,10^2)/1000
3	0.2	Uniform(10^2, 10^3)/1000

[a] we divide by 1000 to convert the data from 0.1m^2 to cm^2.

We then calculate the total number of *Salmonella* on the skin of pig *k* in pen *j,* $N_0(k,,j)$

$$N_0(k, j) = h_c(k, j) * O(k, j) \quad (8.29)$$

where *O(k,j)* is the surface area of the skin and is derived from a relationship between body mass and surface area (Kelly *et al.* 1973)

$$O(k, j) = 734 * m(k, j)^{0.656} \quad (8.30)$$

8.3.5 Differences between small/large farms and small/large slaughterhouses

Little data exist to describe small farm transport within any of the case study MSs. Expert opinion resulted in contradictory approaches (e.g. whether a haulier would stop at multiple farms or not), suggesting that knowledge of this area is limited. Given that small farmers are unlikely to be able to change their transport methods as a viable *Salmonella* intervention we do not consider that there will be any differences in the modelling of transport between small/large farms and slaughterhouses other than the number of pigs being transported. Similarly, we consider there to be little difference in the modelling of the lairage setup. The capacity and number of pens will differ for a small slaughterhouse, but these are parameter inputs rather than modelling assumptions.

8.3.6 Output to Slaughter & Processing module

The outputs of the Transport & Lairage module relevant to the Slaughter & Processing module are

- Total number of pigs to go through the slaughter line of the large slaughterhouse, n_l and small slaughterhouse, n_s.
- Concentration of *Salmonella* (cfu/g) in the faeces of the pig at the end of lairage, $\varepsilon_L(k, j)$
- Number of *Salmonella* (cfu) on the skin of the pig at the end of lairage, $N_0(k, j)$

8.4 Parameter Estimation

8.4.1 Overview

In this section we outline how the parameters for the Transport & Lairage module were estimated. The estimates for the parameters that vary between MSs are discussed in the

following Sections. All parameter estimates are shown in Table 8.2, Table 8.3 and Table 8.4. Where applicable, the different MS estimates are given.

The Transport & Lairage module parameters can be divided into two distinct categories: parameters governing the management and logistics of transport and lairage, and those governing the transmission of *Salmonella* between pigs during these stages. Further information relating to the parameter estimates is given below.

Table 8.2: Global parameter estimates

Parameters	Description	Value used in simulations	Reference (if applicable)
$S_L(q)$	Number of pigs to be slaughtered at large slaughterhouse	MS1: $\Re(U(4000,5000))$ MS2: $\Re(General([1,5000,\ 10000,15000],\ [16,\ 5,\ 1]/22))$ MS3: $\Re(U(4000,5000))$ MS4: 680	Chapter 9
$S_s(q)$	Number of pigs to be slaughtered at small slaughterhouse	MS1: $\Re(U(1,400))$ MS2: $\Re\left(General\left(\begin{matrix}[1,\ 4,\ 20,\ 40,\ 80,\ 120,\ 400],\\ [0.26,\ 0.33,\ 0.11,\ 0.09,\ 0.06,\ 0.06]/0.91\end{matrix}\right)\right)$ MS3: $\Re(U(1,400))$ MS4: 3	Chapter 9
$\overline{f}$	Average amount of faeces shed by pig per defecation	$\bar{f}(i,k)=\frac{\Re\left(\Gamma\left(\frac{2580}{50^2},\frac{2580^2}{50^2}\right)\right)}{3.1}$	Chapter 7
	Mean number of defecations per hour	$\frac{3.1}{12}$ defecations / hour	Aarnik *et al.* (2005)
$\varepsilon_H(i,j,k)$	Concentration of *Salmonella* (cfu/g) shed by pig *i*	$E_{pig,H}(j)=\sum_{k=1}^{P_H(j)} f_{pig,H}(k,j)*\varepsilon(k,j)$ (8.25) ,	Chapter 7
α_{pigD}	Alpha parameter for pig dose response	0.3781	Chapter 7
β_{pigD}	Beta parameter for pig dose response	57878.9616	Chapter 7
F_{eatMax}	Maximum amount of faeces eaten by pig	$\frac{100}{12}$ g/hour	Cook (2009) expert opinion

Table 8.3: Transport parameter estimates

Parameters	Description	Value used in simulations (MS2 values)	Reference (if applicable)
p_{rex}	Probability of pig becoming stressed during transport	0.2	Assumed by author based on Davies (2008), Cook (2008), expert opinion on reversion to excretion at farm
$\tau_{cap}(j)$	Number of pigs in pen in transport	MS1: $\Re(BP(10,12.5,15))$ MS2 & MS4: $\Re(U(14,20))$ MS3: $\Re(BP(5,15,32))$	Much (2009) Guise *et al.* (1996) Mizgier (2009)
$T_{cap}(j)$	Number of pens in truck	Equal to batch size	Assumed by author
$P^{T}_{EnvCarry}$	Probability of environmental carry over in truck	5/18	Mannion *et al.* (2008) & VLA (2009a)
$P^{T}_{FaecCarry}$	Probability of faeces carry over on truck	1/9	Mannion *et al.* 2008
$F_{transMax}$	Maximum faeces carry over in transport (g per truck).	990g	Serrano-garcia (2008)
$E_{transMax}$	Maximum *Salmonella* carried over in transport	$\Re(U(0,0.11))$ cfu/cm^2	Mannion *et al.* (2008)
$F_T^c(k)$	Faeces left in truck pen *k,* before pigs enter	$\Re(U(1,\mathrm{F}_{\mathrm{TransMax}}))$	-
$E_T^c(k)$	*Salmonella* in truck pen *k,* before pigs enter	$\Re(U(1,\mathrm{E}_{\mathrm{TransMax}}))$	-
$\chi^{E_T}(k,j)$	Proportion reduction of *Salmonella* due to cleaning	0.621	VLA (2009a)
$\chi^{F_T}(k,j)$	Proportion reduction of faeces due to cleaning	0.621	VLA (2009a)
$T_D(j)$	Duration of transport	MS1: $\Re(BP(0.5,1,8))$ MS2 & MS4: Figur MS3: $\Re(BP(0.7,3.1,10))$	Much (2009) AMLS (2005) Mizgier (2009)

Table 8.4 Lairage parameter estimates

Parameters	*Description*	*Value used in simulations (MS2 values)*	*Reference (if applicable)*
L_{cap}	Lairage capacity as proportion of throughput	MS1, MS2 & MS4: $\Re(U(0.2, 0.7))$ MS3: $\Re(BP(1.17,1.34,2.21))$	Defra (2004) Mizgier (2009)
L_{pencap}	Number of pigs in a pen in lairage	50	Boughton *et al.* (2007)
L_{stock}	Stocking density of pigs (pigs/cm^2)	$\Re(U(0.42/10000, 0.83/10000))$	Defra (2004)
$L_{timeDay}$	Time (hrs) spent in lairage during day	MS1, MS2 & MS4: $\Re(\Gamma(2.8,7.84))$ MS3: $\Re(BP(1.53,5.13,21.5))$	FSA (2006) Mizgier (2009)
$L_{timeNight}$	Time (hrs) spent in lairage if kept overnight	MS1, MS2 & MS4: $\Re(\Gamma(3.83,58.52))$ MS3: $\Re(BP(8.06,12.71,27.22))$	FSA (2006) Mizgier (2009)
$P_{overnight}$	Number of pens used for overnight stay	MS1, MS2 & MS4: [0pens 1pen 2pens]= [0.2 0.7 0.1] MS3: $\Re(BP(0.3,0.45,0.99))$ * L_{pencap}	FSA (2006) Mizgier (2009)
$P^{L}_{envLair}$	Probability environmental carryover in lairage	51/150	Davies *et al.* (1999); Boughton *et al.,* (2007) & VLA, (2009a)
$Max_{envLair}$	Max *Salmonella* carry over in lairage	550/100	Boughton *et al.* (2007)
P^{L}_{clean}	Probability pen is cleaned between batches	0.25	FSA (2006)
$X^{E}_{L}(k,j)$	Reduction in *Salmonella* due to cleaning	Equation (8.32)	Small *et al.* (2007)
$P^{L}_{FaecCarry}$	Probability carry over of faeces	8/10	
$X^{F}_{L}(k,j)$	Reduction in faeces due to cleaning	0.019	VLA (2009a)
$F_{CARCASS,L}$	Proportion increase/decrease between caecal infection and skin contamination	Figure 8.3	Davies *et al.* (1999)
h_c	Probability of concentration of *Salmonella* on skin	Figure 8.3	Davies *et al.* (1999)

8.4.2 Management parameters

Number of pigs per pen in transport vehicle, $N_p(j)$

The capacity of the truck is governed by the number of pigs in a batch from the farm module. We assume that each batch of pigs uses a different truck. Data from the Animal Movements Licensing scheme (AMLS, 2008) shows the number of pigs per animal movement, from an agricultural holding to a slaughterhouse, in MS2. These data show that there is rarely, if ever, more than one movement record per day for a given farm. Thus we assume that multiple trucks are not used to transport one batch of pigs. There was no data available to confirm if this would be different for other member states.

A MS2 study (Guise *et al.* 1996) found that the number of pigs per pen in trucks varied between 12 and 16 for single deck trucks and on one sampled double-deck truck there were 19 pigs per pen. There was no data available to confirm if this would be different for other member states.

Probability of transport stress, P_{rex}

No data are available to estimate this parameter from published data. Expert opinion (Cook, 2008, Davies, 2008) suggests that pigs would revert to shedding from a carrier status (defined as infected but not excreting *Salmonella*) around 10% of the time. We assume the carrier status is analogous to the infected animals in the current model that are either not shedding *Salmonella* or shedding at a low-level (<2 log cfu/g) and that the increase in shedding observed during transport is simply these low-level shedders excreting enough to test positive again (appearing as carriers reverting to excretion). As stress during transport is assumed to increase this rate, and in the absence of any other data, we double this estimate to p_{rex}= 20%.

Probability of environmental carry over in truck, $p^T_{EnvCarry}$

To estimate this parameter we combine data from two studies (Mannion *et al.* 2008, VLA, 2009a) that sampled trucks before pigs were loaded. Mannion *et al.* 2008 took samples from 9 trucks pre-loading and found *Salmonella* in 3 of them. VLA, 2009a sampled 9 trucks and found *Salmonella* in 2 of them. Combining these gives us s_{TE}=5 out of n_{TE}=18 trucks testing positive for *Salmonella*. Thus, the probability of a truck being contaminated with *Salmonella* prior to the loading of the pigs, $p^T_{EnvCarry}$ is estimated by

$$p^T_{EnvCarry} = \frac{s_{TE}}{n_{TE}}$$

Probability of faecal carry over in truck, $p^T_{FaecCarry}$

The study by Mannion *et al.* 2008 reported that s_{TF}= 1 truck out of n_{TF}= 9 was visually contaminated on arrival at the farm. Thus, we estimate the probability of faecal carry over as

$$p^T_{FaecCarry} = \frac{s_{TF}}{n_{TF}}$$

Effectiveness of cleaning in trucks, χ^E_T

Testing trucks before loading pigs, VLA, 2009a found that 5 out of 45 trucks tested positive for *Salmonella*. At the lairage 54 out of 97 samples tested positive. From this we can estimate that the probability of contamination before loading is p_F =0.1111 and the probability after is p_A= 0.5556. Thus we estimate that the effectiveness of cleaning is (p_A-p_F)/ p_A=(0.5556-0.1111)/0.5556 = 0.8 and so there is an 80% reduction in contamination due to cleaning. The way this study was set up, the trucks were sampled at the farm first and at the lairage last. If the study recorded data from a truck at lairage first and then from the same truck on arrival at the farm, we could use this matched individual truck data to get an estimate of effectiveness of cleaning. However, these type of data were not available.

Maximum amount of faeces, $F_{TransMax}$, *and Salmonella,* $E_{TransMax}$, in truck before pigs enter

If faeces carry over is determined to occur ($p^T_{EnvCarry}$) then the amount of faeces carried over is determined from a uniform distribution with a maximum of $F_{TransMax}$= 990g (based on data from Serrano-garcia, 2008). If *Salmonella* carry over is determined to occur ($p^T_{EnvCarry}$) then the amount carried over is determined from a uniform distribution with a maximum of $E_{TransMax}$=0.11 cfu/g (based on data from Mannion *et al.*, 2008).

8.4.3 Parameters relating to *Salmonella* infection

Amount of faeces excreted, $\bar{f}(k,j)$

To calculate the amount of faeces shed we estimate the number of defecations while in the pen and the amount of faeces excreted in each defecation. Data from Aarnik *et al.* 2005 records the number of times pigs excrete per day by weight class. As we are modelling finishing pigs we use the 105kg weight class (the largest weight), which were found to excrete an average 3.1 times per day.

Data collected for the farm module (Chapter 7) suggests that the amount of faeces shed by a finisher pig per day has a mean of 2580g and a standard deviation of 50g. We fit a gamma distribution to these values (as the amount of faeces shed per day can not be negative). To determine the amount shed by a particular pig, *k,* in pen *j*, per excretion, $\bar{f}(k,j)$, we sample from this distribution for each individual pig and then divide the answer by 3.1 (the average number of times finisher pigs excrete per day).

$$\bar{f}(k,j) = \frac{\Re\left(\Gamma\left(\frac{2580}{50^2}, \frac{2580^2}{50^2}\right), (k,j)\right)}{3.1}$$

To estimate the probability of an excretion per hour we divide 3.1 by the number of hours a day a pig is active (and thus able to excrete). We assume this to be 12 hours and so estimate the probability of an excretion per hour to be P^D = 3.1/12=0.2583.

Probability of environmental carry over in lairage, $p^L_{EnvCarry}$

To estimate this parameter we combine data from 2 studies (Boughton *et al.* 2008, VLA, 2009a). Boughton *et al.* 2007 took 120 samples over 2 days from lairage and 33 tested positive for *Salmonella.* VLA, 2009a took 90 samples and isolated *Salmonella* from 49. Combining these, and therefore assuming that the studies are equivalent, gives us s_{LE}=82

out of n_{LE}=210 pens testing positive for *Salmonella*. Thus, the probability of a pen in lairage being contaminated with *Salmonella* before pigs enter (at any time), $p_{carry,L}$ is estimated by

$$p^{L}{}_{EnvCarry} = \frac{s_{LE}}{n_{LE}}$$

Probability of lairage pen being cleaned during the day, $P^{L}clean$

Data from a study by the UK Food Standards Agency (FSA, 2006) found that pens were only washed out between each group in 25% of cases. Thus we assume that P^{L}_{clean}=0.25. As before, we assume that this applies to both large and small slaughterhouses.

Effectiveness of cleaning in lairage, χ^{E}_{L}

There are many different types of cleaning that could be implemented to clean out lairage pens (e.g. pressure washing, steam washing, use of sanitiser). Qualitative data from the UK (FSA, 2006 suggests that most premises use pressure washing or steam-cleaning. A paper by Small *et al.* 2007 shows results of a laboratory study on the log reduction of *Escherichia coli* (*E.coli*) counts using different cleaning methods on either a visually clean or visually dirty concrete slab. Log10 reductions were recorded immediately after cleaning and again one hour after. We assume that the immediate reduction is applicable to cleaning out between batches of pigs during the day and the reduction after an hour is applicable to overnight cleaning. The study found that pressure washing gave an immediate 2.5 log10 reduction (standard deviation 0.7) on a visually dirty slab. One hour after cleaning the overall reduction was 4.1 (standard deviation 1.7). For steam cleaning there was an immediate 0.9 log10 reduction (standard deviation 0.7). After one hour there was a 1.7 log10 reduction (standard deviation of 1.6). Reductions are also given for mains pressure water, sanitizer, steam, mains pressure followed by steam and pressure wash followed by steam. The sanitizer showed the largest reduction (4.5 standard deviation of 0.9). We assume that all premises will use either pressure washing or steam cleaning with equal probability and estimate the log reduction in contamination due to cleaning during the day:

$$\chi^{E}{}_{L}(j) = \begin{cases} \Re\left(N(2.5,0.7), j\right), & y < 0.5 \\ \Re\left(N(0.9,0.7), j\right), & y > 0.5 \end{cases} \quad (8.31)$$

and overnight:

$$\chi^{E}{}_{L}(j) = \begin{cases} \Re\left(N(4.1,1.7), j\right), & y < 0.5 \\ \Re\left(N(1.7,1.6), j\right), & y > 0.5' \end{cases} \quad (8.32)$$

where *y* is a random number generated from a uniform distribution between 0 and 1. With appropriate data on frequency of use of other cleaning practices we can incorporate all types of cleaning.

Note that this estimation assumes that reduction in *E.coli* counts is equivalent to reduction in *Salmonella* counts. It may be useful to conduct experiments to determine the relationship between reduction in counts of *E.coli* and *Salmonella* so that an appropriate conversion factor could be used if appropriate (e.g. derive a relationship to say that a 1 log reduction in *E.coli* is equivalent to a *x* log reduction in *Salmonella*).

Salmonella in lairage pens, *F(j)*

An FSA study for MS2 (FSA, 2006) found that routine cleaning practices in lairage did not remove all microbiological contamination, with up to 2.8 $\log_{10}$ cfu cm^2 of *E.coli* remaining at some sampling sites. A study by Boughton *et al.* (2007a) found an average of between 1.8-11.5 MPN/cm^2 *Salmonella* in lairage pens.

8.4.4 Parameter estimation - MS2

Duration of transport, *T(i)*

The duration of transport is estimated by fitting an empirical distribution to data from AMLS, 2005 (see Figure 8.20). These data contained the duration, in minutes, of transport times for 14,088 movement records from agricultural holdings to slaughterhouses during 2005. The mean duration time was 60.71 minutes with 5th and 95th percentiles of 6 and 204 minutes.

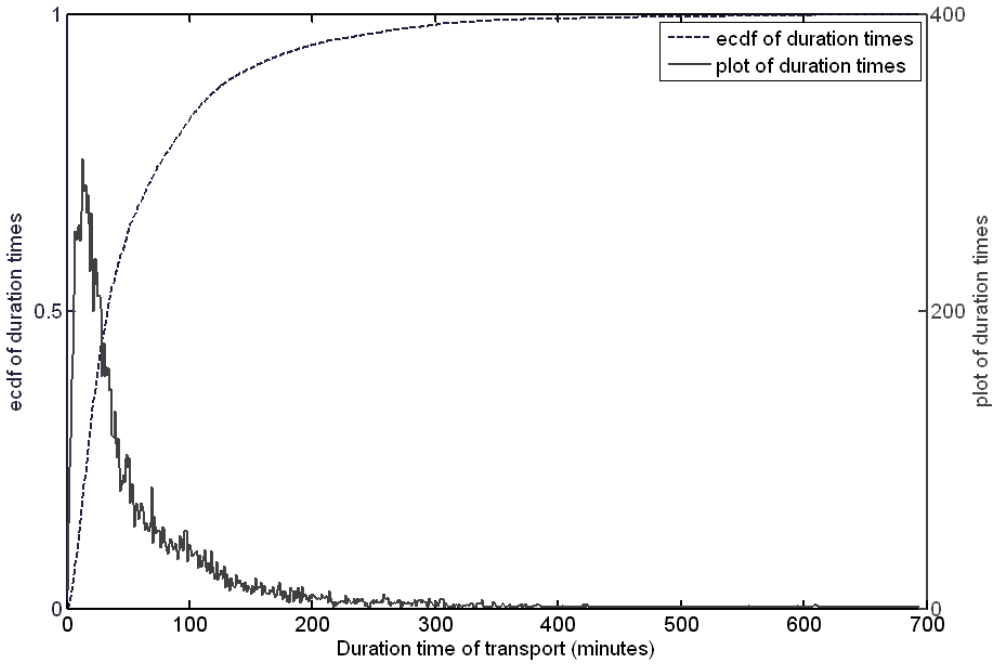

Figure 8.4: Plot of MS2 transport duration times and empirical cumulative distribution function for duration of transport time (minutes).

Lairage throughput, n_l, n_s

The lairage capacities for large, n_l, and small, n_s, slaughterhouses are discussed in the Slaughter & Processing module (Chapter 9).

Number of pigs in pen in lairage, L_{pencap}

Data from Defra, 2004 reported that group sizes were generally between 21-60 pigs in 12 MS2 slaughterhouses. For simplicity and ease of calculation purposes we assume a group size of 50 pigs (as lairage capacity is variable we wish to avoid the cases where we would get a large number of pigs in lairage coupled with a small number of pigs in a group).

Duration of lairage, $L_{TimeDay}$, $L_{TimeNight}$

The duration of time that pigs spend in lairage is dependent on whether they are kept overnight or not. Data from FSA, 2006 records the number of pens that house pigs overnight on specific days. They found that the number of pens housing pigs were [0, 1, 2], with probabilities $p_{overnight}$=[0.2, 0.7, 0.1]. Thus each iteration of the model we determine the number of overnight pens from these probabilities using the multinomial distribution, $N_{overnight}=\Re(Mn(L_{pens}, p_{overnight}))$, where L_{pens} is the number of lairage pens in the slaughterhouse, which is derived by dividing the slaughterhouse capacity by the pen size (n_l/L_{pencap}).

The FSA, 2006 study also provided data for the mean time that pigs are kept in lairage when held overnight, was 15.3 hours, with a range of 10-20 hours. From this we assume a standard deviation of 2 and thus we estimate the parameters of a gamma ($\Gamma(\alpha,\beta)$) distribution as $\alpha=15.3/2^2$ and $\beta=15.3^2/2^2$. Therefore, we estimate that $L_{TimeNight} = \Re(\Gamma(3.83,58.52))$. If pigs are not kept overnight then the study found that the mean time was 2.8 hours with a range of 0-6 hours. From this we can assume a standard deviation of 1, and thus we estimate the parameters of a $\Gamma(\alpha,\beta)$ distribution as α=2.8/1 and β=2.8^2/1. Therefore, $L_{TimeDay}=\Re(\Gamma(2.8,7.84))$. (N.B. we use a gamma distribution to avoid negative duration times). Using this distribution 99.47% of duration times are less than 6 hours.

Lairage capacity, L_{cap}

As part of a larger study Defra, 2004 conducted 12 visits to pig slaughterhouses. This study confirms that the lairage capacity is often smaller than the throughput of animals per day. Among the larger slaughterhouses (>2000 pigs per day) lairage capacity could be as small as 22% of throughput.

We estimate the capacity of lairage as a proportion of the throughput of pigs for the day. Data from Defra, 2004, recorded the throughput of pigs on the sampling day and the lairage capacity of 12 pig slaughterhouses. From these data we estimate that the lairage capacity of the larger slaughterhouses (throughput >1000 pigs per day) is generally between 20 and 70% of the throughput of pigs. Therefore, we assume the lairage capacity to be uniformly distributed between 0.2 and 0.7.

A general study on cattle, sheep and pig lairages found that up to 25 groups of animals could pass through each holding pen in one day (FSA, 2006, pg65). However, they report that the mode was 2 groups.

The throughput of the smaller slaughterhouses (<1000 pigs per day) generally seemed not to exceed the lairage capacity. Thus, we assume that the lairage of a small slaughterhouse is sufficient to hold all pigs for the day.

Stocking density, L_{stock}

Data from a lairage study (Defra, 2004) found that the stocking rates of pigs (m^2 per animal) ranged from 0.42-0.83. Thus, we assume that the stocking rates follow a U(0.42,0.83) distribution.

8.4.5 Parameter estimation – MS1

Data from MS1 (Much, 2009 pers. comm.) gives the minimum, maximum and most likely values for member state specific parameters. Using this information we can fit beta pert (BP) distributions to these data (see Appendix 8.1 for fitting method). The estimates are shown in Table 8.5.

Table 8.5: BP(α, β) parameter estimates for MS1 specific Transport & Lairage parameter values

Parameter	Minimum	Most Likely	Maximum	Estimate for α	Estimate for β
duration of transport (hrs)	0.5	1	8	1.05	3.94
# pigs in a pen in truck	10	12.5*	15	4	4

*No most likely value was given so it was assumed to be the median value between the minimum and maximum

Data were not available for every parameter. In the absence of such data we use the UK data. If further data for MS1 becomes available in the future the appropriate parameter estimates can be added to the model.

8.4.6 Parameter estimation – MS3

Data from MS3 (Mizgier, 2009) gives the minimum, maximum and most likely values for the member state specific parameters. Using this information we can fit beta pert distributions to these data (see Appendix 8.1, for fitting method). The estimates are shown in Table 8.6.

Data was not available for every parameter. In the absence of such data we use the UK data. If further data for MS3 becomes available in the future the appropriate parameter estimates can be added to the model.

8.4.7 Parameter estimation – MS4

There was no transport or lairage data available for MS4. Therefore we use the UK data as a proxy, because the data available for the parameter estimation is deemed to be of the best quality and thus the parameter estimation could be considered to be more accurate.

If data for MS4 becomes available in the future the appropriate parameter estimates can be added to the model.

Table 8.6: BP(α, β) parameter estimates for MS3 specific Transport & Lairage parameter values

Parameter	Minimum	Most Likely	Maximum	Estimate for *α*	Estimate for *β*
Duration of transport (hrs)	0.70	3.10	10	2.46	4.67
If separated within truck, number of pigs within a group	5	15	32	2.59	4.67
Ratio of lairage capacity to throughput of slaughterhouse (e.g. lairage capacity of 100 pigs; 200 pigs slaughtered in a day; ratio 1:2 or 0.5)	1.17	1.34	2.21	1.74	4.50
Ratio of number of pigs to lairage capacity that are kept overnight (e.g 50 pigs; lairage capacity of 100; ratio 1:2 or 0.5)	0.30	0.45	0.99	2.09	4.63
Time spent in lairage during day(hr):	1.53	5.13	21.50	1.82	4.54
Time spent in lairage overnight (hr):	8.06	12.71	27.22	2.28	4.66

8.5 Data Gaps

- *Skin contamination.* It was not possible to explicitly model the change in skin contamination during the Transport & Lairage phases as there was simply not enough data that would have allowed us to accurately estimate this. In order to model this quantitative data would be needed at transport and lairage on contact rates of pigs with surfaces, faeces and other pigs as well as transfer rates of faeces from surfaces to skin (how much is transferred and how often).
- *Prevalence of skin contamination at the start of the slaughter line.* This estimate is based on only a small study as most studies that report the prevalence of carcass contamination do so at a stage further down the slaughter line (e.g. evisceration). These studies are not a reliable estimate for the prevalence at the beginning of the slaughter line as most processes during slaughter will increase or decrease the prevalence. Indeed this is one of the main effects modelled in the slaughter model. Therefore a reliable estimate of the prevalence at the start of the slaughter line is very important.
- *Concentration of Salmonella on hides at the start of the slaughter line*. Similar to the prevalence, this parameter is also based on a small study and therefore further studies to quantify this parameter would be useful. This parameter is equally as important as the prevalence as higher concentrations on contaminated hides will likely lead to high concentrations in products and thus higher risks of illness.

- *Amount of Salmonella carried over.* While there is reasonable data on whether *Salmonella* was isolated from a pen/truck before pigs enter it, the data on how much is present is limited.
- *Amount of faeces carried over*. Similar to the *Salmonella* carry over, there is little data on exactly how much faeces is likely to be present in a pen before pigs enter.
- *Probability of pigs being stressed and the effect of stress on the pigs.* While it is well established that pigs get stressed during transport, there is little or no quantitative data on how likely it is that a pig will become stressed. There is also little quantitative data available on what effect stress will have on the pig, in relation to transmission of *Salmonella*.

8.6 Major assumptions

The following assumptions were included in the Transport & Lairage model.

- Pigs from small farms go to small slaughterhouses and pigs from large farms go to large slaughterhouses (due to lack of data)
- Trucks do not pick up pigs from multiple farms (expert opinion suggests this is generally the case and further modelling assumptions would be needed to implement multiple farm pickups)
- Pigs in the batch are allowed to mix during loading.
- When data for a model parameter for a particular member could not be obtained data from another member state was used. The author used his best guess as to which data would be most appropriate (based on quality of data and similarity of member states).
- Cross-contamination of faeces and *Salmonella between* pens will not occur due to the relatively short time pigs spend in transport and lairage pens.
- The amount of *Salmonella* shed in faeces will be increased by 1-3 log10 cfu/g if the infected pig becomes stressed during transport.
- Farm model dose response relationship assumed applicable to both transport and lairage
- Homogenous mixing of *Salmonella* within faeces, within a pen.
- *E.coli* is an adequate surrogate organism for *Salmonella,* for modelling reduction in microbial load due to cleaning in lairage.

8.7 Results

To show results from the Transport & Lairage model we define the total number of pigs in a batch $N^{SL}(j)$ and the number of excreting pigs in a batch, $I^{SL}(j)$ and the prevalence of excretion (i.e. lymph node positive)

$$I_{prev}(j) = \frac{I^{SL}(j)}{N^{SL}(j)},$$

Figure 8.5 to Figure 8.8 show the results of the batch prevalence of lymph node positive pigs at different stages of transport and lairage for the four case study member states. It can be seen that there is an increase in the number of lymph node positive pigs after both transport and lairage for all member states.

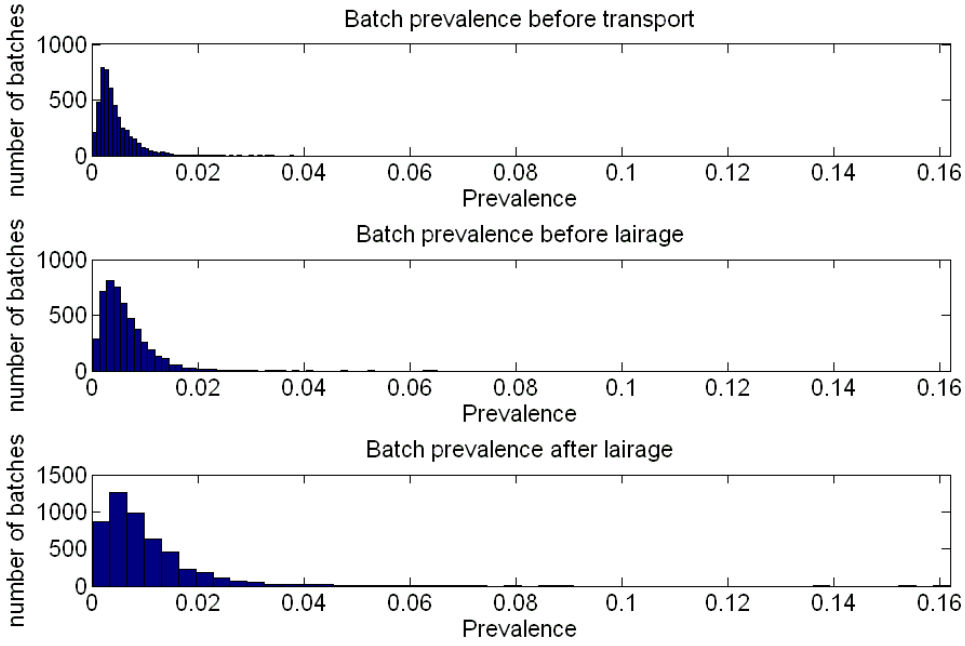

Figure 8.5: Distribution of batch prevalence at different stages of transport and lairage (MS1)

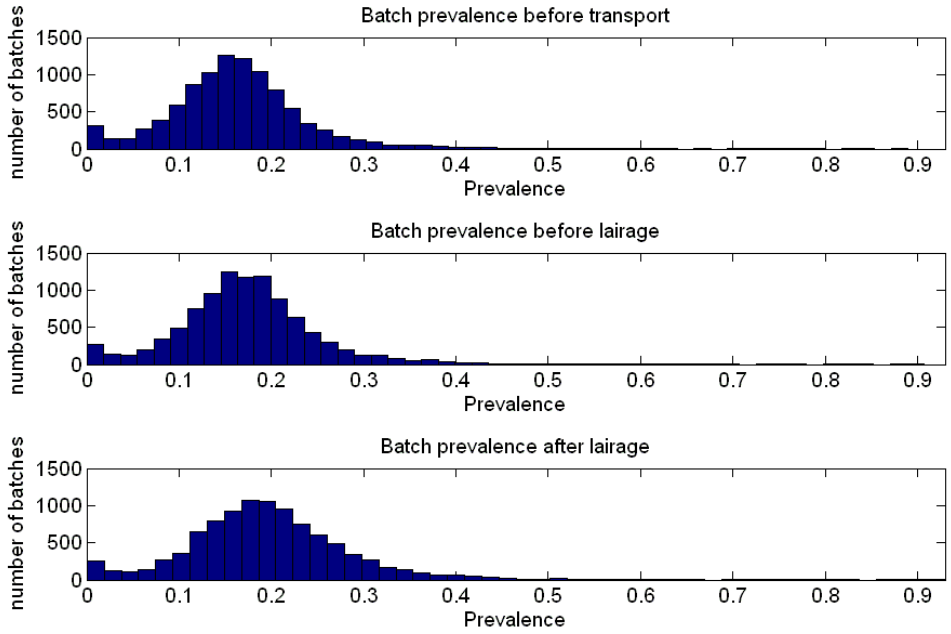

Figure 8.6: Distribution of batch prevalence at different stages of transport and lairage (MS2)

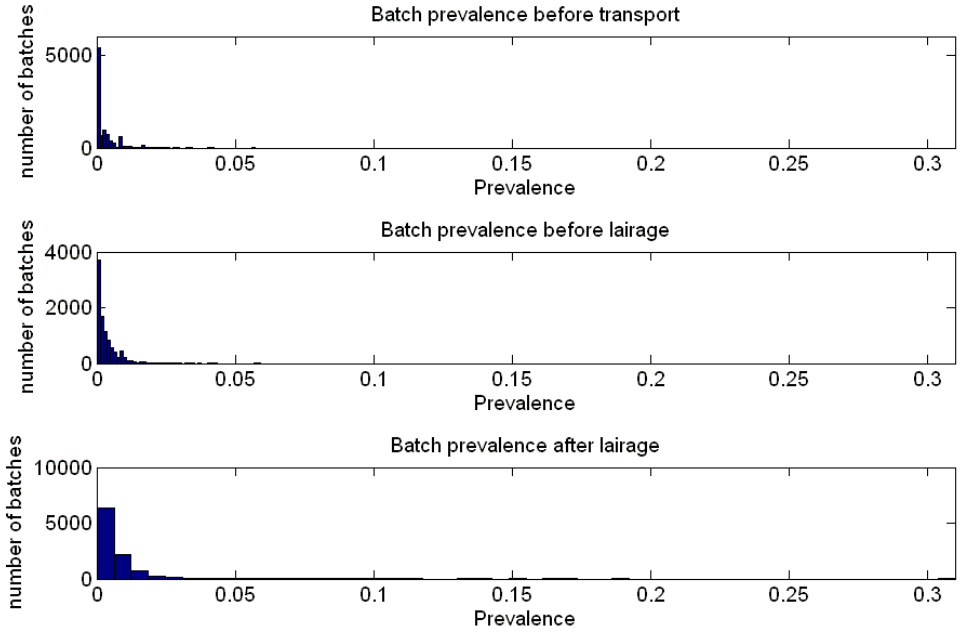

Figure 8.7: Distribution of batch prevalence at different stages of transport and lairage (MS3)

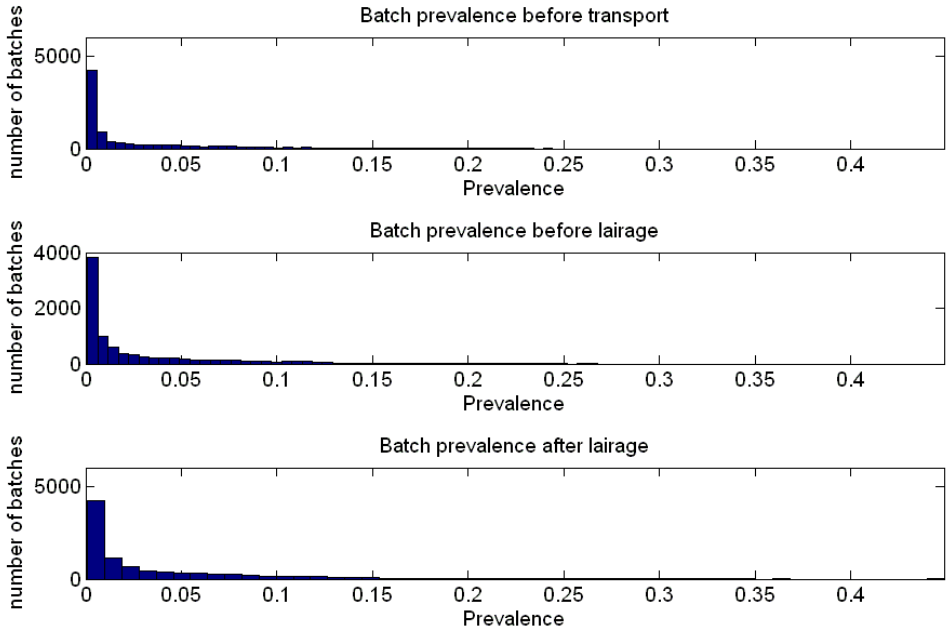

Figure 8.8: Distribution of batch prevalence at different stages of transport and lairage (MS4)

Table 8.7 shows the average lymph node positive prevalence over the whole simulation for each member state, before transport, after transport and after lairage. It can be seen that MS2 has the highest prevalence at each stage, with an average prevalence of 20% at the end of lairage. MS3 has the lowest prevalence of 0.67%, with MS1 1% and MS4 3.5%. It can be seen that for every member state the average prevalence increases between transport and lairage. The 5th and 95th percentiles show that there is a large degree of variation between days, with the average prevalence for some days reaching almost 20% for MS4 and 50% for MS2. It can be seen that the distributions are skewed for all MSs with the mean much closer to the 5th percentile than the 95th. This shows that it is a rare occurrence that you get the batches with particularly high prevalence (i.e. close to the 95th percentile).

Table 8.7: Mean, 5th and 95th percentiles of lymph node positive batch prevalence before transport, after transport and after lairage for each member state.

	Mean, (5th, 95th percentiles) of prevalence (%)		
Member state	**Before transport**	**After transport**	**After lairage**
MS1	0.43 (0.35, 1.46)	0.62 (0.5, 2)	1 (0.8, 3.7)
MS2	16.5 (13.4, 45.5)	17.6 (13.5, 47.8)	20 (15.1, 55.4)
MS3	0.26 (0.25, 1.16)	0.33 (0.32, 1.4)	0.67 (0.64, 2.82)
MS4	2.35 (2.35, 13)	2.69 (2.69, 14.1)	3.53 (3.53, 17.5)

8.8 Validation

We can validate the results of the model at this stage by comparing the average lymph-node positive prevalence at the end of lairage for each member state with the lymph-node positive prevalences given in the EFSA slaughter pig baseline survey (EFSA, 2008b). This is done in Table 8.16, where it can be seen that the results match the EFSA survey quite well, particularly for MS1, MS2 and MS4. The model seems to underestimate the lymph-node positive prevalence for MS3 as it predicts a lower average prevalence than MS1 (while the EFSA baseline results are higher) and the predicted prevalence is also quite a bit lower than the 5th percentile of the baseline prevalence for MS3 (3.7%), although still only one order of magnitude out. Investigation of the model suggests that this discrepancy likely comes from the model not capturing a specific aspect of PO at the farm, as the prevalence at the end of the farm stage is lower than that of MS1. This could be due to MS3 having a much larger proportion of small farms than other member states. We should note that the EFSA baseline results are from tests done part way through the slaughter line, rather than immediately after lairage. However, given that the pigs are killed almost immediately after leaving lairage, it is reasonable to assume that the change in lymph-node positive prevalence will be negligible.

8.9 Sensitivity Analysis

The sensitivity analysis methodology is described in Chapter 5. For the Transport & Lairage module we conduct two sensitivity analyses, one for transport and one for lairage. For the transport sensitivity analysis we use the lymph-node positive prevalence at the end of transport as the response variable and for the lairage sensitivity analysis we use the lymph-node positive prevalence at the end of lairage as the response variable (note that due to this choice it does not make sense to include parameters that relate to skin contamination in this analysis, as this is effectively just an input to the slaughterhouse model).

Table 8.8: Comparison of post lairage lymph-node positive prevalence with EFSA slaughter pig baseline survey (EFSA, 2008b)

Member State	EFSA Baseline results: LN+ve prevalence (%) (mean, [5th 95th] percentiles)	Model LN+ve prevalence (%) (mean)
MS1	2, [1.1 – 3.6]	1
MS2	21.2, [17.8 – 25]	20
MS3	5.1, [3.7 – 6.9]	0.7
MS4	5.8, [3.8 – 8.9]	3.5

Figure 8.9 to Figure 8.12 show the results of the transport sensitivity analysis in the form of bar graphs. Note that we plot the F value, so that the bigger the bar the more significant the variation in the parameter is on the lymph-node positive prevalence at the end of transport (although factors with bars of similar height should be considered equally significant). From these graphs it is clear that stress is the most important factor in our model. Stocking density is also relatively important for MS1, MS3 and MS4. Note that the initial batch prevalence is not included as a factor as it is not a parameter input of the model, rather an output of the

Farm module. However if it is included it is by far the most important factor. This suggests that the within batch prevalence is more influential on the lymph-node positive batch prevalence at the end of slaughter that the distributions used in the transport module.

Figure 8.13 to Figure 8.16 show the results for the lairage sensitivity analysis. Interestingly the significance of the parameters differ between member states. For MS1 and MS4 it is the number of pigs kept overnight that is most important while for MS3 and MS2 it is the load of *Salmonella* carried over in the pens between batches. It is clear that many of the parameters have similar significance on the prevalence at the end of lairage and that it is not just one parameter that overwhelms everything else (as stress seems to during transport). Again we do not include the batch prevalence at the beginning of lairage as a parameter. When it is included it is much more significant than the other parameter, as the farm prevalence is in transport, thus again suggesting that the within batch prevalence is highly influential.

8.10 Discussion

The Transport & Lairage module has been developed to incorporate factors that are thought to influence the prevalence of *Salmonella* in slaughter-age pigs, including stress during transport, contamination of the environment and cleaning of the environment. These factors were included with the aim of assessing the effect of various interventions implemented at the transport and lairage stages.

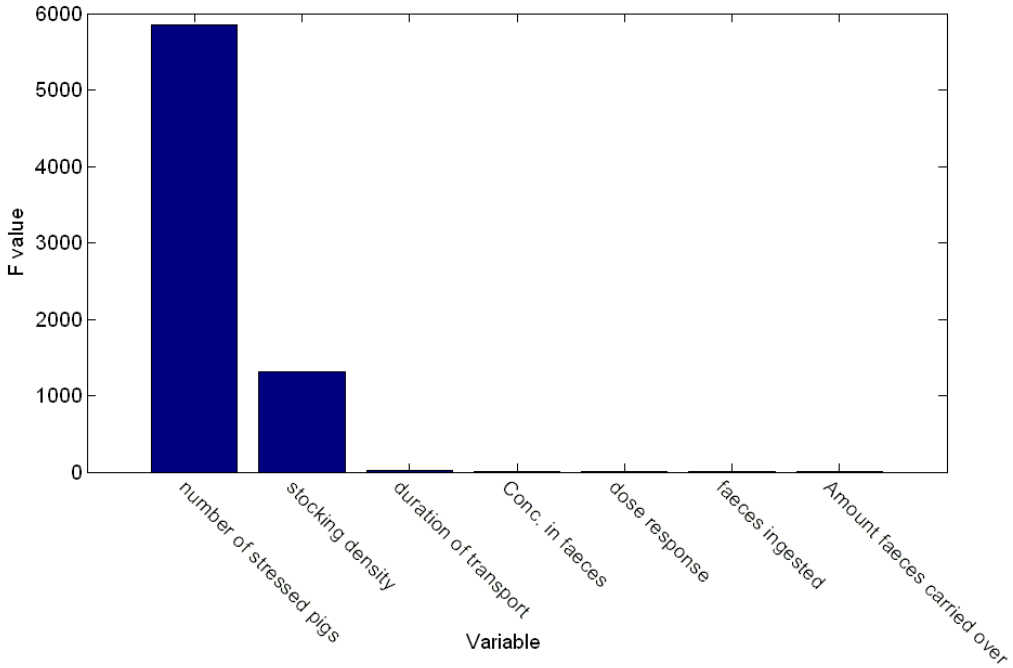

Figure 8.9: Transport sensitivity analysis for MS1

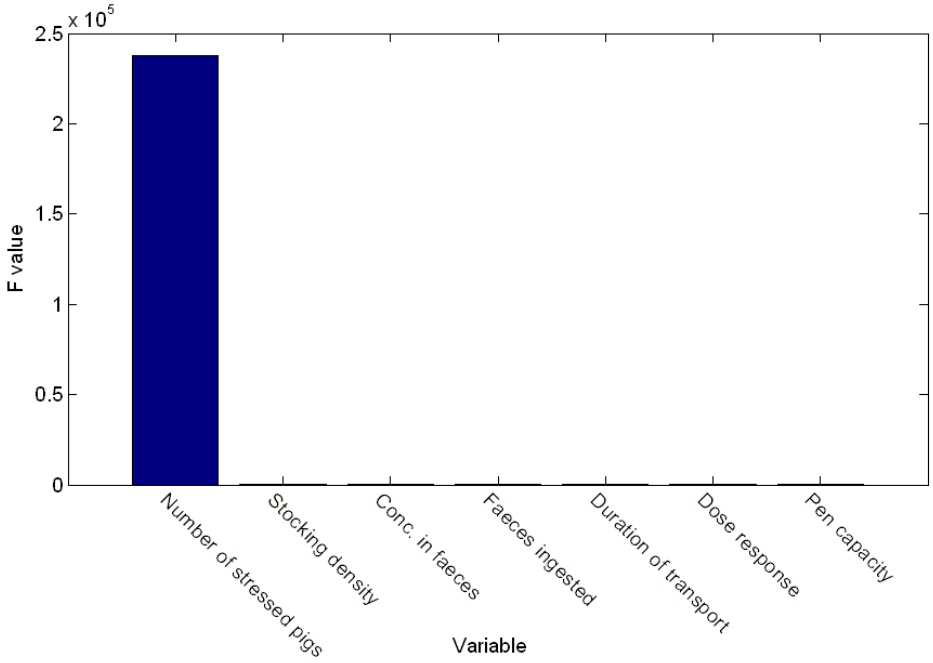

Figure 8.10: Transport sensitivity analysis for and MS2

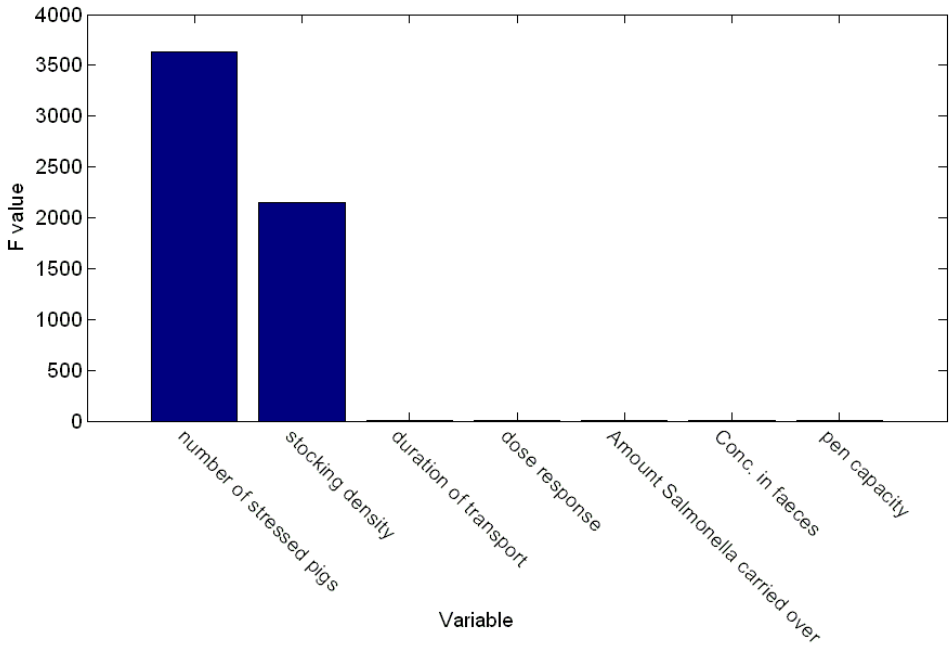

Figure 8.11: Transport sensitivity analysis for MS3.

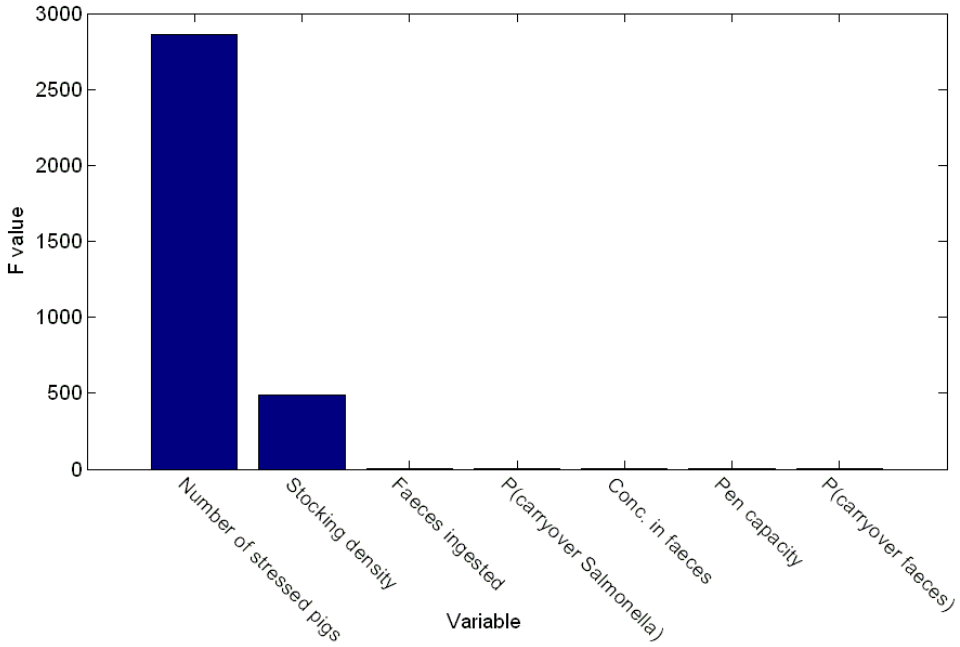

Figure 8.12: Transport sensitivity analysis for MS4

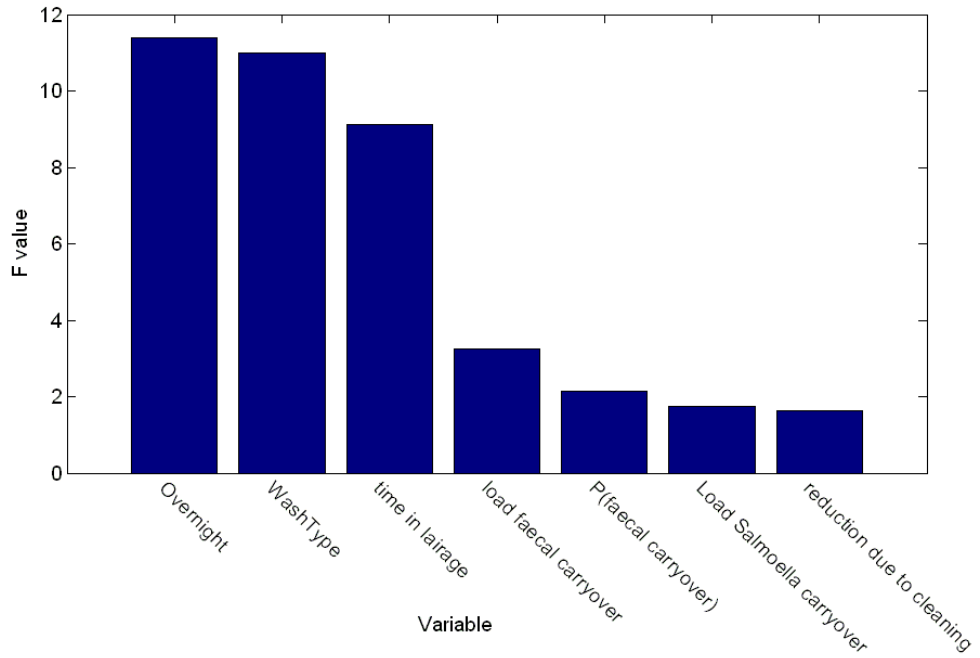

Figure 8.13: Lairage sensitivity analysis for MS1

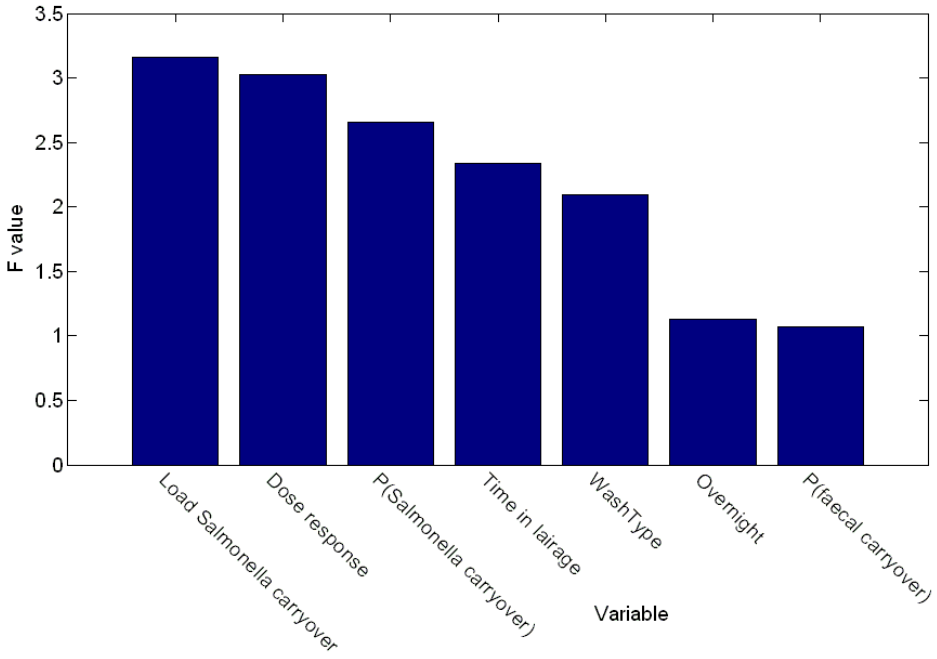

Figure 8.14: Lairage sensitivity analysis for MS2

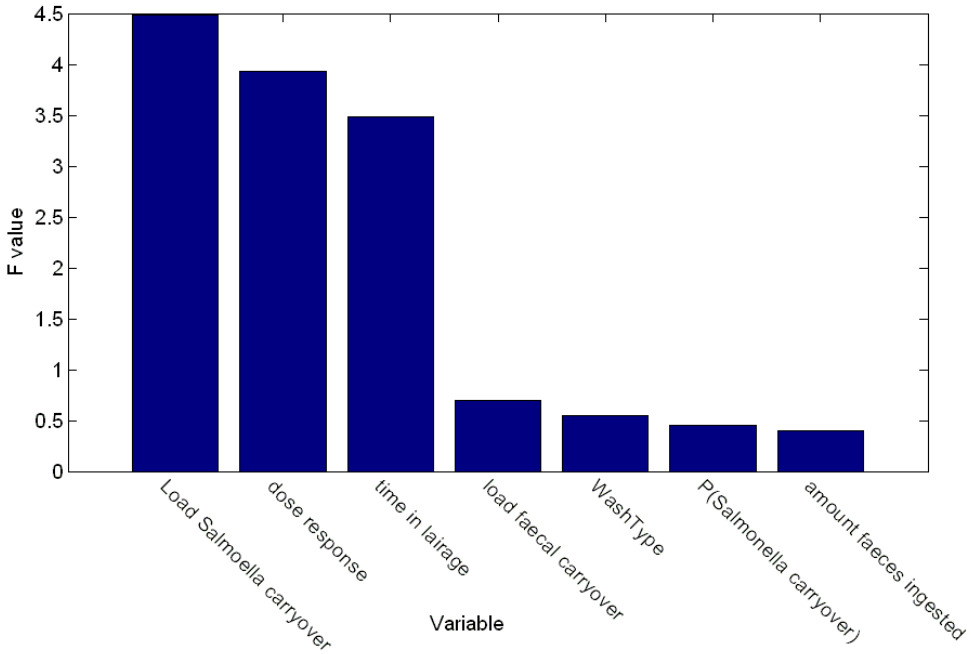

Figure 8.15: Lairage sensitivity analysis for MS3

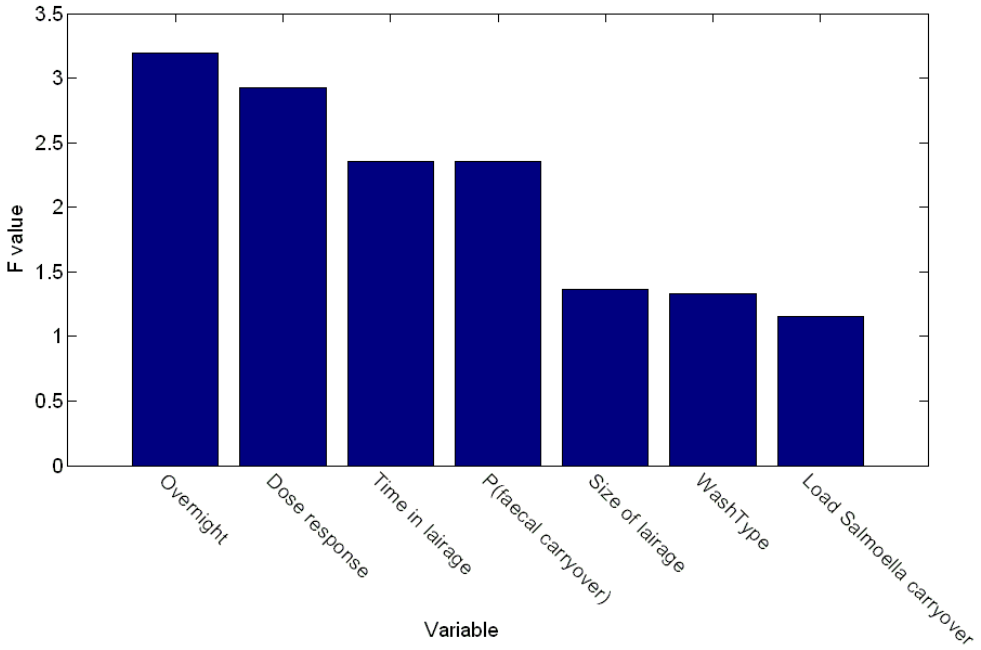

Figure 8.16: Lairage sensitivity analysis MS4

The results from this stage show that the prevalence does increase, both during transport and lairage. The average batch prevalence for each of the four member states compares favourably with the findings of the EFSA slaughter pig baseline survey (EFSA, 2008b), albeit with a few deviations (particularly the lower prevalence for MS3 predicted by the model) suggesting, as would be expected, that the model does not capture all the factors associated with *Salmonella* transmission and prevalence. Part of the reason for this may be data gaps associated with some of the parameters. Sometimes this is a lack of adequate quantitative data across all MSs (such as estimating the skin contamination at the start of the slaughter line and the effect stress). In other cases we have good data for some MSs and not others (e.g. the effect of cleaning of lairage, proportion of pigs kept overnight in lairage) so it was necessary to estimate the value based on data from another MS. However, it could well be due to issues at the farm level, it has been shown that the within batch prevalence before transport is more influential than any of the parameter distributions within the Transport & Lairage module.

Perhaps the most important data gap in the Transport & Lairage module is the effect of stress during transport, as well being a significant data gap, the sensitivity analysis suggests that it the most important factor in relation to lymph-node prevalence at the end of transport. There is little quantitative data on stress so expert opinion had to be used to estimate the proportion pigs that become stressed. On top of this, the effect that stress has in relation to *Salmonella* is not clear. We have assumed that it will results in a 1-3 log10 cfu/g increase in the amount of *Salmonella* shed in the faeces of lymph-node positive pigs, but this is not a published result. These parameters are included in the uncertainty analysis to ascertain their influence on the risk of illness.

It should be noted that while the effects described above are significant within the Transport & Lairage module, they actually have little effect on the risk of illness. This is highlighted by the logistic slaughter and cleaning of lairage interventions (see Chapter 13) which demonstrated a very low to negligible effect on the risk of illness. While it is clear that the conditions in lairage can have a significant effect on the prevalence of skin contamination at the start of the slaughter line, the intervention analysis suggests that this is of lesser importance to the effect that the various slaughter processes have (e.g. the increase in contamination at de-hairing and the decrease at singeing). This analysis suggests that intervention measures should focus on reducing on farm prevalence or carcass contamination in the slaughterhouse.

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Appendix 8.1 : Fitting a Beta Pert Distribution

It is not possible to get large accurate datasets to accurately estimate every parameter in the model. In some case we have to rely on expert opinion. In these cases the aim is to obtain a minimum (*a*), maximum (*b*) and most likely (*m*) value for the parameter. With this information we can fit a beta pert distribution on the interval [a, b]. To do this we first need to estimate the mean and variance and then the corresponding shape and scale parameters (α and β). The formulas required are shown in Table A8.9.

Table A8.9: Equations to estimate shape and scale parameters for beta distribution on the interval [a,b], given min, max and most likely data.

Value	Symbol	Equation
Minimum	a	Expert opinion
Most Likely	m	Expert opinion
Maximum	b	Expert opinion
Estimated mean	$\bar{x}$	$\bar{x} = \frac{a + 4m + b}{6}$
Estimated variance	v	$v = \frac{(b-a)^2}{36}$
Scaled mean	μ	$\mu = \frac{\bar{x} - a}{b - a}$
Scaled variance	σ^2	$\sigma^2 = \frac{v}{(b-a)^2}$
Shape parameter	α	$\alpha = \mu\left(\frac{\mu(1-\mu)}{v} - 1\right)$
Scale parameter	β	$\beta = (1-\mu)\left(\frac{\mu(1-\mu)}{v} - 1\right)$

Note that to implement this in Matlab, we do so via the pearson function. This requires an input of the skewness and kurtosis of the beta distribution as well as the mean and standard deviation. These can be calculated from the shape and scale parameters via standard methods.

9 Modelling the Slaughterhouse Environment and the Processing of Carcasses

9.1 Introduction

In this chapter we present the “Slaughter & Processing” module dealing with modelling of the slaughterhouse and cutting plant. We cover the route from slaughter pigs departing from lairage to half-carcasses which are the input to the next module: “Preparation & Consumption”.

Our model is built using the MPRM (Modular Process Risk Model) paradigm (Nauta 2008). The defining feature of an MPRM is splitting the model into several modules. These modules are sub-models in themselves and represent a well defined part of the real-world problem. In each module one or more of the basic microbiological processes of inactivation, growth, partitioning, mixing, removal and cross-contamination are modelled. The final model is obtained by chaining the modules, passing information from one module to the next.

Section 9.2 starts off with an overview of the type of models used to model the Slaughterhouse. We then continue by discussing each phase within the slaughterhouse in detail. For each phase, first a model is established, paying special attention to the justification of simplifications and modelling choices. This leads to a detailed mathematical definition of the processes. Next, technical implementation or mathematical solving steps will be discussed in a separate section, which may be skipped by the reader. Finally, the sources for the parameter values will be given. Results for the large slaughterhouse are presented in Section 9.4

The small slaughterhouse is discussed in Section 9.6, in the same vein as the description in Section 9.2, results are presented in Section 9.7.

The next step is development of a model for the cutting plant, where half-carcasses are cut into smaller parts and processed into meat products. This is presented in Section 9.8.

Section 9.3 deals with 'house flora', the persistent strains of *Salmonella* present throughout the slaughterhouse. We discuss the main sources and the modelling thereof.

We conclude with a discussion of the model and the results obtained. Also, we point to data gaps, which are the main obstacle in the accurate representation of the slaughter process.

9.2 Modelling the Large Slaughterhouse

9.2.1 Overview

This section describes the modelling of the large slaughterhouse, a facility that accepts live pigs, slaughters and processes them, delivering half-carcasses at the end of the procedure. In contrast to the small slaughterhouse, the process is automated to a large extent. Conceptually, the slaughterhouse consists of a line of carcasses which are subjected to several processing stages. We assume a single slaughterline per slaughterhouse. At each stage several relevant processes may increase or decrease the *Salmonella* concentration on the carcass.

At the start of the slaughterhouse procedure, live pigs enter the facility. The exterior of those pigs may be polluted with faeces or dirt containing *Salmonella*. If *Salmonella* is present on the exterior, we say that the pig is contaminated. The number of colony forming units (cfu) of *Salmonella* per contaminated pig is an input parameter for the Slaughterhouse model, coming from the Transport & Lairage model. Apart from being contaminated, the pig may be infected, meaning that *Salmonella* is present in the intestines of the animal. The number of *Salmonella* in the intestines is also an input parameter to our model.

Pigs are delivered to the slaughterhouse in batches originating from a single farm, where they may be split up into smaller sub-batches during lairage, depending on the size of the batch and the capacity of the pens in lairage (there is no mixing of pigs from different batches). The slaughterhouse processes a number of batches per day and we consider entire batches only. An important term in this context is prevalence. In this model we can speak of the prevalence of infected or contaminated pigs in a batch, but also of the prevalence of infected batches. Note that pigs with zero *Salmonella* are treated in the same way as positive pigs. It is not possible to single out only positive pigs for simulation, due to cross-contamination between pigs. The final output of the slaughterhouse model is the level of contamination per half-carcass, for a large number of half-carcasses, presented in the form of distributions. Also the percentage of contaminated half-carcasses (prevalence) is calculated from this and separately presented.

9.2.2 General slaughterhouse parameters

This section lists some general parameters that are not specific to any stage in the process.

We start with the percentage of slaughterhouses that are large and the capacities of small and large slaughterhouses. Using the same criterion as adopted in the cluster analysis, we define a slaughterhouse to be ‘large’ if it slaughters over 100,000 pigs per year. The data used is the same data as used for the cluster analysis (Chapter 6). We assumed 250 working days per year, for conversion from years to days, thus a large slaughterhouse slaughters over 400 pigs per day. As an upper limit we set 5000 pigs per day. For the small slaughterhouse we set a minimum of one pig per day. Table 9.1 summarises the percentages and capacities. Compare those numbers to some other data collected (Appendix 9.1) and note the numbers seem to be in the same order of magnitude.

Table 9.1: Slaughterhouse types and capacities for the case study MSs. See the main text for sources.

MS	Fraction Large	Number of pigs Slaughtered per day	Capacity Large (per slaughterhouse, per day)	Capacity Small (Per slaughterhouse, per day)
MS1	0.48	22500	U(400,5000)	U(1,400)
MS2	0.94	37400	$n_{2,l}$ (see below)	$n_{2,s}$ (see below)
MS3	0.14	16300	U(400,5000)	U(1,400)
MS4	0.24	16200	680, (EFSA 2008b)	3, (EFSA 2008b)

As an alternative, we also calculate the number of pigs slaughtered per day, from the annual slaughter volume (Table A9.34) and the carcass weight (Table 9.15). Unfortunately, these data are not compatible. For example, MS3 has 401 slaughterhouses, of which 56 are large. The minimum number of pigs per day would be 400 times 56 equals 22,400, which is already more than the total number of pigs slaughtered per day. Therefore we choose to use the numbers as given in the cluster analysis (Chapter 6).

For the capacity of the small slaughterhouse for MS2, denoted $n_{2,s}$, we have a detailed description, obtained from the questionnaire (EFSA 2008b). We describe the variation in capacity by means of a general distribution:

$$n_{2,s} = \Re(G([1,4,20,40,80,120,400], [0.26,0.33,0.11,0.09,0.06,0.06]/0.91) \quad (9.5)$$

This should be read as, e.g.: the probability that the capacity is between 1 and 4 is 0.26/0.91. For the capacities of the large slaughterhouses we have detailed information for Denmark,

$$n_{2,l} = \Re(G([0,5000,10.000,15.000][16/22,5/22,1/22])). \quad (9.6)$$

See also the histograms of the distributions in Figure 9.1.

Any probability distribution mentioned in this Chapter is listed in Table A5.1 in Appendix 5.1 (Chapter 5).

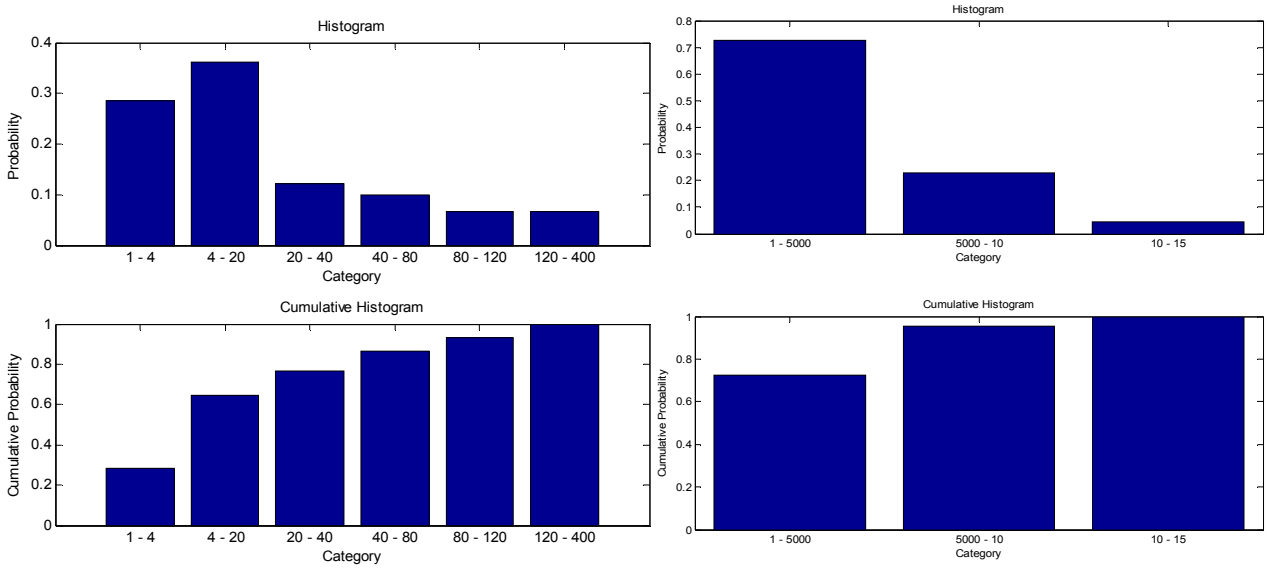

Figure 9.1: Histograms of the distribution of the capacities of small (left panel) and large (right panel) slaughterhouses in MS2.

9.2.3Cross-contamination and inactivation models

The two major relevant microbiological processes to be modelled in the slaughterhouse are (cross-)contamination and inactivation. Growth of bacteria is not relevant in the slaughterhouse environment, due to the short total time of processing as compared to the generation time of *Salmonella*. Also, at several stages lag-phases are probably induced due to stress (e.g. heat stress at singeing). We refer the reader to Titus, 2007 (Section 3.3), or Nauta *et al.* 2005 (Section 3) for more information on cross-contamination, inactivation and slaughterhouse models in general.

Cross-contamination is not a well-defined concept. We will define cross-contamination as the contamination of a carcass (or other unit under investigation) by means of a second agent (e.g. a cutting knife, or the scalding tank), which has previously been contaminated by another carcass.

We assume that contamination takes place in discrete time, thus the exchange of micro-organisms from pig P_k to an environment W is an instantaneous action. See Chapter 5 (including Appendix 5.1 and 5.2) for a list of all variables and quantities used throughout this report, including a description of the notation used. We have two entities that must be taken into account, these are given in Table 9.2.

Table 9.2: Typical quantities in a cross-contamination model.

Quantity	Domain	Unit	Description
$N_k(S)$	$\mathbb{N}$	cfu	The number of *Salmonella* on the k^{th} pig or carcass P_k, in phase S
$W_S(k)$	$\mathbb{N}$	cfu	The number of *Salmonella* in the environment W, in phase S, at time k.

The number k will run through all numbers from one to n, the total number of pigs going through the slaughterhouse per day[16]. Note the reversed roles of S and k for the environment and the pig. For the pig, time can be measured by the phase it is in, while in the environment, time is represented by the number of the pig it is interacting with.

We continue by defining rates of transfer, defining fractions of pathogen per time unit that move from P_k to W_S and vice versa. We define the rates of transfer in Table 9.3.

We are now in the position of formulating a simple model. Note that a carcass P_k at stage S has a contamination level equal to its contamination from the previous stage, multiplied by the fraction $1-\beta$ of *Salmonella* not moving to the environment, plus the contribution from the environment to the carcass. This conception may be expressed as

$$N_k(S) = \lfloor (1-\beta)N_k(S-1) + \alpha W_S(k-1) \rfloor. \quad (9.7)$$

Note the notation $\lfloor x \rfloor$, which means we round x down to the nearest integer. Similarly, the environment retains a fraction $(1-\alpha)$ of *Salmonella* that was present at time $k-1$ and increases with a fraction β of *Salmonella* from the carcass,

$$W_S(k) = \lfloor \beta N_k(S-1) + (1-\alpha)W_S(k-1) \rfloor. \quad (9.8)$$

We should supplement these equations with suitable initial conditions, i.e. we prescribe $N_k(1)$ for $k = 1, \ldots, n$ and $W_S(0)$ for all stages.

Note that, due to cross-contamination negative carcasses may become positive. Therefore, it is not possible to model only positive carcasses, adjusting for negative carcasses only. In our case, the splitting phase (Section 9.2.10) is the final point where cross contamination may occur, and from that point onward negative half-carcasses could be removed from the model. However, for practical implementation reasons, negative carcasses were not singled out.

Next, we discuss the inclusion of inactivation in the model. Inactivation is the reduction of the number of *Salmonella* on the carcass, or in the environment. Two inactivation models are relevant for our purposes, a linear model and an exponential inactivation model.

Let $N_k(t)$ denote the number of *Salmonella* at time $t \geq 0$ and arbitrarily set $N_k(0) = N_{k,0}$ for given values $N_{k,0}$. Then, the linear model, where the number of bacteria decreases with a constant value $\epsilon \geq 0$ each minute may be written as

$$N_k(t) = \lfloor N_{k,0} - \epsilon t \rfloor. \quad (9.9)$$

[16] We will also write $k = 1, \ldots, n$ and generally start numbering from one onward, instead of zero.

Table 9.3: Typical parameters in a cross-contamination model.

Quantity	Domain	Unit	Description
α	$[0,1]$	-	Fraction of *Salmonella* in the environment, moving from the environment to the carcass
β	$[0,1]$	-	Fraction of *Salmonella* on the carcass, moving from the carcass to the environment

Secondly, the exponential inactivation model is widely used. In the exponential model, the number of bacteria decreases with the same factor every time unit. Mathematically, the exponential model is given by

$$N_k(t) = \lfloor e^{-\epsilon t} N_{k,0} \rfloor. \qquad (9.10)$$

The exponential model is often more realistic than the linear model when modelling natural inactivation of micro-organisms. However, adding an exponential decay to the cross-contamination equations yields a mathematically intractable system. On the other hand this does not pose a problem numerically.

Due to the exponential nature of the natural growth and decay of micro-organisms, the number of cfu's is often measured in 'log cfu'[17]. An amount of n log cfu is equal to 10^n cfu. Converting $N_k(t)$ to log units we find that the exponential model becomes linear,

$$\log(N_k(t)) = \log(N_{k,0}) - \frac{\epsilon}{\ln(10)} t. \qquad (9.11)$$

Here we have not rounded down N_k to integers for simplicity. The models appearing in Section 9.2.4 are variations on the basic models established in this section and will be somewhat more complicated. However, the main reasoning is similar to the description in this section.

9.2.4Large slaughterhouse stages

The slaughterhouse environment varies throughout the EU and within slaughter stages the specific equipment and settings of the machinery are also not constant. Since it is not feasible to model variations in slaughterhouse stages, we have opted to identify the most relevant stages (in terms of risk of contamination) and restricted the model to those stages. The variability between machinery parameters however, is modelled using data from scientific literature.

Within a slaughterhouse several stages may be distinguished and are summarised in Figure 9.2.

Some of these stages have little or no impact on *Salmonella* contamination and will not be modelled. Others, however, are recognised as highly relevant. Those stages will be part of the model in the form of modules. Below we present a list of stages, accompanied by a brief description and a justification for inclusion or exclusion from the model. As such, this section acts as a qualitative risk assessment - a first step where we identify potential hazards and relevant processes

[17] All logarithms are in base 10, the natural logarithm (base 'e') is written 'ln'.

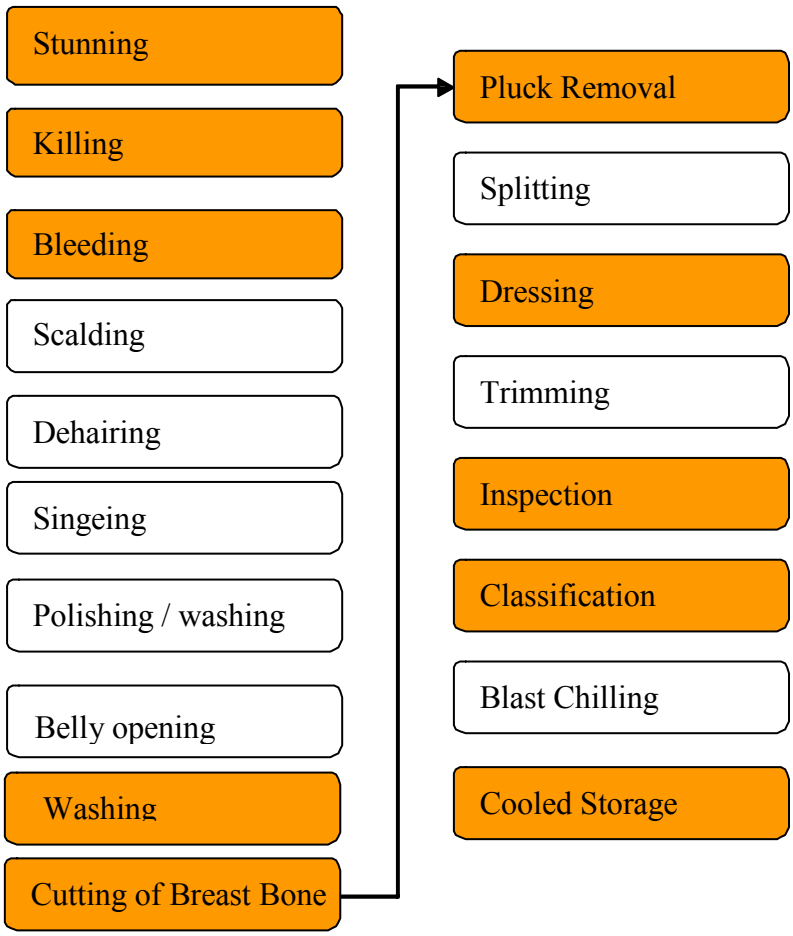

Figure 9.2: Slaughterhouse stages. Coloured stages are not modelled[18].

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[18] We thank the Q&A department of the Dutch slaughterhouse for allowing us to examine the slaughter process at their facility.

Stunning, sticking and bleeding

Upon entry to the Slaughterhouse facility, the pigs are stunned by means of an electric shock. No microbiological risks are present at this point. Apart from the electrical stunning method, a gas stunning method is in widespread use. In this second method a number of pigs (one at a time, or from 5 to 10) are stunned using gas (carbon dioxide). This treatment relaxes the muscles (as opposed to electrical stunning) and may lead to increased shedding. Furthermore, pigs have contact with each other and the slaughter floor, leading to cross-contamination. Unfortunately, no data are available to quantify this hazard. Data on increased shedding, *Salmonella* concentrations, cross-contamination and frequency of employment of the gas stunning method would be needed. Further research on this phase is needed, for it is potentially a highly relevant phase.

After stunning, the pigs are subsequently killed by severing the main artery in the neck ('sticking'). Also, methods are used where longer knives penetrate all the way through the heart. The main hazard at this point is contamination of the pig from the conveyor belt, which will be covered with blood and dirt. Again, due to lack of data, this cross-contamination will not be modelled. The knives themselves only touch a small part of the contaminated skin and are sterilized routinely. We therefore consider them as relatively safe.

Finally, the pig bleeds for some time before entering the scalding bath. Apart from contact with the conveyor belt there is no risk, and this phase is also not modelled.

Scalding (Section 9.2.5)

During the scalding phase, the pigs are submerged into the scalding bath, containing hot water. The primary objective is the loosening of hairs, but the hairs will still be attached and are removed in the following stage.

From a microbiological quality point of view, the high temperature potentially reduces the pathogen levels on the exterior of the pig. Also, *Salmonella* may be washed off. Relevant parameters are the temperature of the scalding water, the number of pigs sharing the scalding bath and the time spent in the scalding bath. A potential risk is contamination of the scalding water, which could contaminate the skin of subsequent pigs that enter the scalding bath.

An alternative to vat scalding is spray scalding, where the pig is not immersed in hot water, but rather a hot spray is applied. There is little data on the frequency of this scalding method and the effect of spray scalding on contamination levels. For these reasons spray scalding was not considered. Readers interested in scalding methods are referred to Troeger, 1993.

Dehairing (Section 9.2.6)

Dehairing takes place in the dehairing machine. This machine consists of a rotating drum with extensions (e.g. brushes or flaps) at the inside. This procedure removes the bulk of the hair. Due to the vigorous action of this machine, some faecal material will be extruded, from the interior of the pig, contaminating both the pig and the machine. It is evident that due to the many brushes or flaps, cleaning of the machine is difficult. For this reason, the dehairing machine has been identified as a significant source of contamination. See also Section 9.3 on the presence of unavoidable *Salmonella* cultures in the slaughterhouse: the house flora.

Note that scalding and dehairing may be combined in a single machine, as also considered in Section 9.6.2.

Singeing (Section 9.2.7)

The singeing stage aims to remove any remaining hairs left after the dehairing phase. The pigs are subjected to very high temperatures (approximately 800-1000 ºC) in a singeing machine, where remaining hairs are burnt. As a side effect, it also burns or dries dirt and faecal material. Typically, a singeing machine consists of two heated half shells closing on the pig, or a heated tunnel.

Singeing is considered to be the most effective stage for microbial inactivation. Its efficiency is dependent on the time spent in the machine and the temperature to which the pig is subjected.

Note that it is possible to have dehairing and singeing combined in one single machine.

Polishing (Section 9.2.8)

The polishing machine, also known as the 'wet scraper', is a tunnel with a car-wash like series of brushes with flaps. Almost all dried dirt and other contamination (e.g. debris of hairs after singeing) is removed or loosened during this phase. Similar to the dehairing phase, there is a risk of cross-contamination at this step and some faecal extrusion may take place. Note that the polishing stage is not implemented in all countries. Sometimes the polishing phase is replaced by a manual washing step (Bolton *et al.* 2002).

Belly opening (Section 9.2.9)

During this step, the belly is opened by machine, using a small hook. Here the infection of the gut becomes relevant, since there is a risk of puncturing the colon, or rupturing the stomach, thereby re-contaminating the carcass or the hook. In between the processing of consecutive pigs the hook is “sterilised” inside the machine, i.e. washed with water at 82 °C.

Evisceration

After belly opening, the gut (colon, small intestine, stomach, spleen) is loosened manually and put in a container. The main hazard is the spilling of faecal material and/or additional puncturing during manual loosening. We consider these risks as part of the belly opening phase, i.e. the risk of puncturing during belly opening includes the evisceration risks.

Cutting the breast bone

The chest cavity is opened from the front, exposing the interior of the pig. Little data on this step is available, but no further cross-contamination seems to occur.

Pluck removal

The ‘pluck’ is a term encompassing the tongue, pharynx (including tonsils), oesophagus, trachea, heart, lung and liver. During this phase, the pluck is removed manually, after which the pluck is put in a container. This phase probably has some risk as the pharynx, tonsil and

tongue are very often heavily infected, as pigs during lairage tend to investigate their surroundings orally (including excretions from other pigs)

Furthermore, during scalding the contaminated water can get into the lungs, and when the pluck is removed it splashes over carcass, thereby further contaminating it.

Workers are not supposed to touch the carcass, they mainly contaminate the plucks. This could pose some risk to consumers, the liver is a part of the pluck that may be consumed, possibly unheated (e.g. liver sausage). However, since liver sausage is not a part of this risk-assessment (see Chapter 10) we do not model this step.

Splitting (Section 9.2.10)

During this phase, the carcass is split in two, top down by machine-saw, stopping at the neck. Between carcasses the saw is cleansed inside the machine. However, the inside of the machine is unreachable and therefore hard to clean and the saw might therefore be contaminated. This step is also risky because of *Salmonella* present in the oral cavity.

Dressing

During this phase, the kidney plus surrounding fat is removed. This is mostly done manually. Like the pluck-removal phase, we assume a negligible risk of cross-contamination during this step.

Trimming (Section 9.2.11)

We define trimming as the inspection, by slaughterhouse personnel, of the carcass. If any visible contamination is found, it is removed. The trimming is done manually, with a knife which is sterilised in-between actions. Care is taken to remove a large portion around the contamination, not touching any of the contamination.

Inspection

Meat inspectors examine carcass, intestines and pluck, to see if the carcass contains any risk for human health, when consumed. This includes looking for indicators for disease or infection. See for example Table 1 in Mousing *et al.* 1997 for an extensive overview of what is inspected. Inspectors will handle the carcass manually, make incisions and perform palpations. The inspector uses his hands and also knives for making incisions in lymph nodes. It is estimated in Pointon *et al.* 2000 that approximately one out of 360 carcasses have lymph nodes cut. These lymph nodes have a *Salmonella* prevalence of 2%. Note that lymph nodes might be heavily contaminated with *Salmonella* and therefore the knife and hands should be cleaned between carcasses. If the inspector found visible contamination, abscesses, swellings, etc. then the carcass is put on a separate line. The offending deviation is removed by knife. A risk during meat inspection is cross-contamination, although the meat is in principle not cut. We assume that hygiene is such that in total the meat inspection step has little risk.

Classification

During classification, the fat and muscle contents are measured by inserting a probe. We assume there to be a negligible risk of cross-contamination at this step.

Post-evisceration washing

As described by Bolton *et al.* 2002, some slaughterhouses perform a final wash of the carcass before chilling. Counter-intuitively, post-evisceration washing increased bacterial counts by 1 log cfu ml^{-1}. This was also observed for the pre-evisceration wash (2.5 log cfu cm^{-2}) which is sometimes performed instead of polishing. Possibly, the washing step does not remove the bacteria, but rather redistributes them, spreading them over the carcass and thereby increasing recovery. The reported numbers refer to total bacterial counts, not specifically *Salmonella*. Specific data were not found in the literature and the redistribution effect is as yet speculative. For this reason this step was not modelled. However, the increase may of course be added to the simulation by means of a hypothetical intervention.

Blast chilling (Section 9.2.12)

Blast chilling is the fast cooling of carcasses by means of blowing cold air. This phase takes place in a room with low ambient temperature. Some *Salmonella* inactivation will take place due to the low temperature and drying of the pig skin. The amount of inactivation is dependent on the temperature and time duration.

Cooling (chilling)

This phase is actually a storing phase, keeping the half-carcasses cooled at 4°C for an extended period of time, until the carcasses are transported to the cutting plant. *Salmonella*, theoretically, does not grow, nor inactivate, at 4°C. Therefore, we do not model this phase. In Bolton *et al.* 2002 it was confirmed experimentally that *Salmonella* do not grow during cooled storage.

In the following, each phase is discussed in three subsections:

- 'Problem definition', describes the process and establishes the equations that model the process.
- 'Solution and implementation', contains the solution procedure for the equations and the efficient implementation in the model. These sections may be skipped by the casual reader who is not primarily interested in the technical details.
- 'Parameter estimation', describes the sources of the parameters used and any assumptions and simplifications made in the process of transforming the data to a form that fits our model.

Parts of the models described in the following sections were adapted from Titus, 2007 and Nauta *et al*. 2005.

9.2.5Scalding

Problem definition

In addition to the quantities defined in Section 9.2.3, we define the following variables, where the subscript '1' refers to scalding being the first stage and a subscript 'k' means that the value of the variable differs between pigs P_k due to variability,

Table 9.4: Quantities used in the scalding phase.

Quantity	Domain	Unit	Description
$N_{1,k}(t)$	$\mathbb{R}^+$	cfu	Number of *Salmonella* on P_k, at time $t \geq 0$
$W_1(t)$	$\mathbb{R}^+$	cfu	Number of *Salmonella* in the environment W_1, at time $t \geq 0$.
n_l	$\mathbb{N}$	-	Total number of pigs, slaughterhouse capacity
$\mathcal{T}_1$	$\mathbb{R}^+$	°C	Temperature of the scalding water
$\alpha_{1,k}$	$[0,1]$	-	Fraction of bacteria moving from the water to the pig
β_1	$[0,1]$	-	Fraction of bacteria moving from the pig to the water
τ_1	$\mathbb{R}^+$	1/min	Rate of pathogen inactivation on the pig
γ_1	$\mathbb{R}^+$	1/min	Rate of pathogen inactivation in the water
T_1	$\mathbb{R}^+$	min	Time spent in the scalding bath

Note that $N_{1,k}(t)$ and $W_1(t)$ take values in the real numbers, not the integers. This reflects our decision of rounding only in between stages. Also note that all fractions are $\mathcal{T}_1$-dependent, although this has been suppressed in the notation.

We set $t = 0$ to be the time at which pig P_1 is introduced into the scalding bath. This is assumed to be an instantaneous action. For convenience, we define

$$t_i = (i-1)T_1. \tag{9.12}$$

Suppose that P_i enters the scalding bath at time t_i. It will then have just left the bath at time t_{i+1}. The equation for the change of the number of pathogens on pig P_k needs to take into account the rate of inactivation, the fraction migrating to the water and the fraction migrating from the water. We have chosen not to model transfer rates from the pig to the scalding bath per minute, since direct data on this parameter was not available. Instead we just assume that a particular fraction of the *Salmonella* on the carcass will be transferred instantaneously to the bath when the pig is entering it. Therefore, the fraction moving from the pig to the tank is modelled as a first step, occurring at $t = t_k$. The number of *Salmonella* remaining is then

$$N_{1,k}(t_k) = (1-\beta_1)N_{0,k}. \tag{9.13}$$

Here $N_k(0) = N_{k,0}$, with $N_{k,0}$ an output from the Transport & Lairage model, being the initial number of *Salmonella* on the pig.

For the dynamics after the initial transfer of *Salmonella*, we need to take into account the time slot $[t_k, t_{k+1})$ during which P_k occupies the tank. In terms of an ordinary differential equation the inactivation during scalding may be modelled as

$$N_k'(t) = -\tau_1 N_k(t) + \alpha_{1,k} W(t), \quad \text{for } t \in (t_k, t_{k+1}), \tag{9.14}$$

$$N_k'(t) = 0, \quad \text{for } t \notin [t_k, t_{k+1}], \tag{9.15}$$

which is valid for $k = 1, \ldots, n$. Note that, in order not to overwhelm the reader with subscripts, we have dropped the '1' subscript in N_k, we will continue to do so when no danger of confusion exists.

We now turn to a description of the number of pathogens in the scalding tank. This is dependent on the number of pigs that have entered the tank up to time t, since each pig deposits a fraction β_1 of its pathogens in the water, while at a rate $\alpha_{1,k}$ the pathogens in the scalding water move to the pig. In between each of the pigs, exponential decay of *Salmonella* in the scalding water takes place. This yields the equations

$$W'(t) = -\gamma_1(1-\alpha_{1,k})W(t), \quad \text{for } t \in (t_k, t_{k+1}), \tag{9.16}$$

$$W(t_k) = \beta N_k(t_k) + \lim_{t \to t_k} W(t). \tag{9.17}$$

The expression $\lim_{t \to t_k} W(t)$ stands for the number of *Salmonella* in the water just before pig P_k enters. The initial condition for the scalding water is $W(0) = 0$, stating no initial contamination of the scalding water at the beginning of the day.

The following section is concerned with numerical solution and implementation of the derived equations and may be skipped by the casual reader at first reading.

Solution and implementation

Firstly, the differential equation for $W(t)$ is easily solved,

$$W(t) = W(t_k)e^{-\gamma_1(1-\alpha_{1,k})(t-t_k)}, \quad \text{for } t \in [t_k, t_{k+1}). \tag{9.18}$$

Next, the equation for $W(t_k)$ may be inserted and subsequently the equation for $N_k(t_k)$, yielding

$$W(t) = [\beta_1(1-\beta_1)N_{k,0} + W^-(t_k)]e^{-\gamma_1(1-\alpha_1,k)(t-t_k)}, \quad \text{for } t \in [t_k, t_{k+1}). \tag{9.19}$$

Here we introduced the notation $W^-(t_k) = \lim_{t \to t_k} W(k)$. The above equation also implies that

$$W^-(t_{k+1}) = [\beta_1(1-\beta_1)N_{k,0} + W^-(t_k)]e^{-\gamma(1-\alpha_{1,k})T_1}. \tag{9.20}$$

The solution to this recursion can be used to find the $W^-(t_k)$ in the formula for $W(t)$.

The differential equation for $N(t)$ now reads

$$N_k'(t) = -\tau_1 N_k(t) + \alpha_{1,k}[\beta_1(1-\beta_1)N_{k,0} + W^-(t_k)] e^{-\gamma_1(1-\alpha_{1,k})(t-t_k)}. \tag{9.21}$$

Using (9.20), this may be written shorter as,

$$N_k'(t) = -\tau_1 N_k(t) + \alpha_{1,k} W^-(t_{k+1}) e^{-\gamma(1-\alpha_{1,k})(t-t_{k+1})}. \tag{9.22}$$

In essence, this is an equation of the form $N'(t) = aN(t) + be^{ct}$, which may be solved by the method of integrating factors, giving,

$$N(t) = e^{at}[\frac{b}{c-a}e^{(c-a)t} + C]. \quad (9.23)$$

Substituting the proper a, b and c we obtain

$$N(t) = -W^-(t_{k+1})^{\frac{\alpha_{1,k}}{\eta}} e^{\gamma(1-\alpha_{1,k})(t_{k+1}-t)} + Ce^{-\tau_1 t}, \quad (9.24)$$

where we defined $\eta = \gamma_1(1-\alpha_{1,k}) - \tau_1$. The constant C is determined by using the initial condition (9.13) and some rewriting gives

$$C = W^-(t_{k+1})^{\frac{\alpha_{1,k}}{\eta}} e^{-\gamma(1-\alpha_{1,k})T_1 + \tau_1 t_k} + (1-\beta_1)N_{0,k}e^{\tau t_k} \quad (9.25)$$

Now we are primarily interested in the number of pathogens at time t_{k+1}, which is easily calculated,

$$N_k(t_{k+1}) = -W^-(t_{k+1})^{\frac{\alpha_{1,k}}{\eta}} + Ce^{-\tau_1 t_{k+1}}. \quad (9.26)$$

Inserting the expression for C we obtain

$$N_k(t_{k+1}) = W^-(t_{k+1})^{\frac{\alpha_{1,k}}{\eta}}(e^{\eta T} - 1) + (1-\beta_1)N_{0,k}e^{-\tau_1 T}. \quad (9.27)$$

Combining this with the recursion (9.20), we have a solution to the scalding equations.

Parameter estimation

In this Section we will discuss estimation of the parameters used for the scalding phase. Each parameter listed in Table 9.4 will be given attention in the following subsections

$N_{1,k}(t)$, the number of *Salmonella* on P_k, at time $t \geq 0$ and n_l, the slaughter capacity

Of course, this quantity is calculated for $t > 0$. However, $N_{0,k} = N_{1,k}(0)$, the initial contamination, comes from lairage, the output of the Transport & Lairage model.

The quantity n_l, the number of pigs processed comes from the slaughterhouse capacity (see Section 9.2.2).

$\mathcal{T}_1$, the temperature of the scalding water, α_1 and β_1, the fraction of bacteria moving from the water to the pig and vice versa.

The temperature of the scalding water according to Wilkin *et al.* 2007, ranges from 58 to 64°C with an average of 60°C. These values were measured in a slaughterhouse in MS2. The distribution of the temperatures is not explicitly stated. For lack of a better alternative we use the 'beta pert' distribution for the MS2, a distribution based on the beta distribution, but scaled to a prescribed minimum, maximum and mode (Vose, 2000). We use the mean 60 for the mode (or, most likely value). For the other MSs we use the former European Guidelines. Although these guidelines are no longer in effect, they are still widely adhered to. Below we present a table of estimates for each MS (Table 9.5). Other temperatures found from the literature review are given in Appendix 9.1.

The fraction of bacteria moving from the pig to the water is determined from the attachment strength of bacteria to the skin. From Namvar & Warriner 2005 we find that 2% of *E. Coli* on pork skin samples is loosely bound to the skin and removed by very modest washing. We therefore set $\beta_1 = 0.02$.

The value of α_1 is determined from data presented in Notermans & Kampelmacher, 1974 Figure 9.3 presents attachment rates dependent on temperature, for an initial concentration of 10^5 *E. coli* per ml. We assume that *Salmonella* and *E. coli* behave similarly, both being flagellated micro-organisms.

Table 9.5 Temperature of the scalding water for each member state.

Member State	Quantity	Source
MS1	$\mathcal{T}_1 = \Re(U(58, 60))$	European Guideline
MS2	$\mathcal{T}_1 = \Re(BP(58, 60, 64))$	Wilkin *et al.* 2007
MS3	$\mathcal{T}_1 = \Re(U(58, 60))$	European Guideline
MS4	$\mathcal{T}_1 = \Re(U(58, 60))$	European Guideline

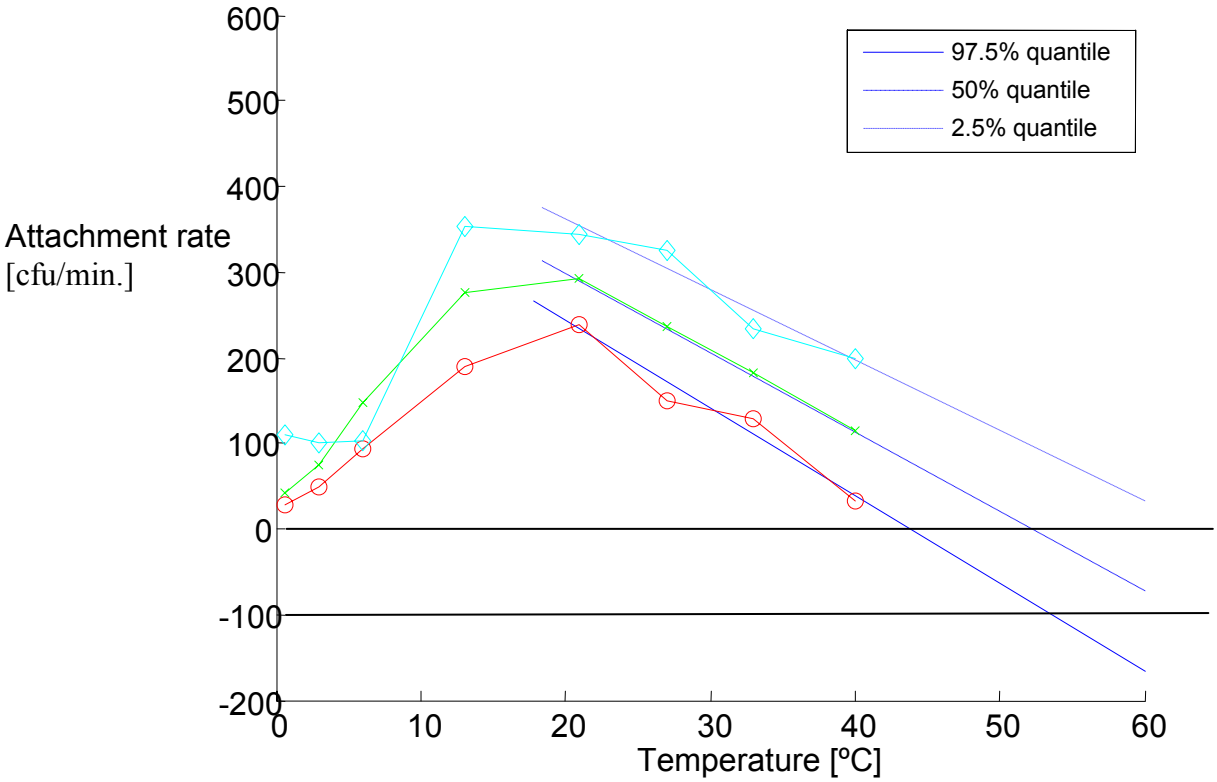

Figure 9.3 Attachment of *E. coli* to chicken skin.

The curves represent the 2.5, 50 and 97.5 percentiles. We interpret the spread in measured values as variability (in skin characteristics), rather than uncertainty. From 20°C onward, the curves seem to approximate straight lines. We used a least squares estimate to fit a straight line through the last four data points. The coefficients for these lines are tabulated in the Table 9.6. Also the variance is listed.

Now, there is also a dependence on the initial concentration N_0, determined by Notermans & Kampelmacher 1974 to be almost linear:

$$\frac{dN(t)}{dt}|_{\mathcal{T}=21} = mN_0^r. \qquad (9.28)$$

Here m is a constant which will later drop out of the equations and $r = 1.05$. On the other hand, the line describing the attachment (for high temperatures), at $N_0 = 10^5$ is described by

$$\frac{dN(t)}{dt}|_{N_0=10^5} = a_2\mathcal{T} + b_2. \qquad (9.29)$$

Table 9.6 Linear interpolation coefficients of attachment data.

Line	a	b
$P_{2.5}(\mathcal{T}) = a_1\mathcal{T} + b_1$	-10.2	446.7
$P_{50}(\mathcal{T}) = \mu(\mathcal{T}) = a_2\mathcal{T} + b_2$	-9.35	489.0
$P_{97.5}(\mathcal{T}) = a_3\mathcal{T} + b_3$	-8.19	523.5
$\sigma^2(\mathcal{T}) = [(a_3 - a_1)\mathcal{T} - (b_3 - b_1)]/(2 * 1.96) = a_4\mathcal{T} + b_4$	0.52	19.60

We may combine those dependences by assuming the attachment rate to be a product of a function depending on $\mathcal{T}$ and a function depending on N_0. Some elementary algebra then yields

$$\frac{dN(t)}{dt} = (\frac{N_0}{10^5})^r(a_2\mathcal{T} + b_2). \quad (9.30)$$

Instead of using the description for the mean, we incorporate the variability by drawing from a normal distribution. Furthermore, we approximate $r \approx 1$ for simplicity.

Then, the attachment parameter (a parameter α such that $N(t) = \alpha N_0$) can be drawn from a normal distribution, provided the value is not below zero,

$$\alpha_k(\mathcal{T}_1) = \max(0, 10^{-5}\Re(N(\mu(\mathcal{T}_1), \sigma^2(\mathcal{T}_1)), k)) \quad (9.31)$$

The numbers presented should be interpreted with care, since several factors influence the attachment rate. Some factors unaccounted for are: the change of skin composition during scalding, the change of organic material in the scalding water or any chemical added to the scalding water.

τ_1 and γ_1 pathogen inactivation on the pig and in the water

Pathogen inactivation in the water is calculated using the D-values and Z-Values for *Salmonella* in water. The values reported by Soerquist 1990, Yang *et al.* 2001 and Bolton *et al.* 2003 are given in Table 9.7.

Table 9.7 Pathogen inactivation rates in water as reported by several authors.

Author	D_{60} [min.]	Z [°C]
Soerquist 1990[19]	0.29	6.03
Yang et al. 2001[19]	0.4	4.95
Bolton *et al.* 2003	1.4	5.61

The D-value may be used as follows to obtain the reduction in concentrations

$$N(t) = N(0)10^{-t/D_{\mathcal{T}}} \quad (9.32)$$

The dependence of the D-value on temperature involves the Z-value and an arbitrarily chosen reference D-value, customarily D_{60}. The relation is as follows

[19] For *S. Typhimurium*

$$D_{\mathcal{T}} = D_{60} 10^{\frac{60-\mathcal{T}}{Z}}. \quad (9.33)$$

For the concentration now the following relation holds

$$\frac{dN(t)}{dt} = -\frac{\ln(10)}{D_{\mathcal{T}}} N(t). \quad (9.34)$$

From this relation we find that we can define the rate of pathogen inactivation as a function of time by

$$\gamma_1(\mathcal{T}) \equiv \frac{\ln(10)}{D_{\mathcal{T}}} = \frac{\ln(10)}{D_{60}} 10^{\frac{\mathcal{T}-60}{Z}}. \quad (9.35)$$

We interpret the variation in the results for the D-values and Z-values as a matter of variability between strains and draw values from a general distribution. We keep the D-values and Z-values together and to this end we draw pairs $(D_{\mathcal{T}}, Z)$ from the distribution,

$$(D_{60}, Z) = \Re(DG([(0.29, 6.03), (0.4, 4.95), (1.4, 5.61)], [1/3, 1/3, 1/3])). \quad (9.36)$$

Rates of pathogen inactivation on pig skin are not reported in the literature. However, (Yang *et al.* 2001) report D-values at several temperatures for chicken skin. We recognize the difference between pig skin and poultry skin, but for lack of better data we use the reported value of $D_{60} = 2.5$. Furthermore, from interpolation of the reported D-values at 50 and 55°C, we find $Z = 5.4$. From these values, the inactivation parameter for *Salmonella* on pig skin becomes

$$\tau_1(\mathcal{T}_1) = 0.92 * 10^{\frac{\mathcal{T}_1-60}{5.4}}. \quad (9.37)$$

T, the time spent in the scalding bath.

For the time spent in the scalding bath only data were available for MS2, which had a minimum time of 2.77 mins and maximum time of 7.5 mins. Information on time in the scalding bath from five other studies was averaged to provide an estimate for the other 3 MSs (see Appendix 9.1). The resulting parameter estimates for T_1 for each MS are provided in Table 9.8.

Table 9.8: Time in minutes spent in the scalding bath, for each MS.

MS	Quantity	Source
MS1	$T_1 = 5.9$	Table A9.
MS2	$T_1 = \Re(U(2.77, 7.5))$	Wilkin *et al.* 2007
MS3	$T_1 = 5.9$	Table A9.
MS4	$T_1 = 5.9$	Table A9.

9.2.6Dehairing

Problem definition

The dehairing phase involves interaction between the dehairing machine and one pig at a time. We suppose that P_k interacts with the dehairing machine in timeslot $t \in [t_k, t_{k+1})$. The dehairing machine exerts some pressure on the pig, which may lead to faecal extrusion, contaminating both the pig and the dehairing machine. The total resulting bacterial load B_k is the product of the amount A_k of faeces extruded per pig, the infection status I_k and the concentration C_k of *Salmonella* in the faeces. Also taking into account transfer from the pig to the machine (at rate β_2), transfer from machine to pig (a fraction $\alpha_{2,k}$) and the bacterial load on the machine $W_2(t)$ we arrive at the following list of parameters given in Table 9.9.

As was the case in the scalding stage, there is initial transfer from the pig to the machine. In this case a fraction β_2 at time t_k. We assume that initially only the pig is contaminated by the extruded faeces, by an amount $B_{2,k}$, then transfer to the machine occurs. Therefore, the initial concentration on the carcass is given by $M_k = N_{k,1} + B_{2,k}$, where $N_{k,1}$ stands for the number of *Salmonella* at the end of stage 1. Thus, the equation for the concentration on the carcass at time t_k is

$$N_{2,k}(t_k) = (1 - \beta_2)M_k \qquad (9.38)$$

Table 9.9: Quantities used in the dehairing phase.

Quantity	Domain	Unit	Description
$N_{2,k}$	$\mathbb{R}^+$	cfu	Number of *Salmonella* on the k^{th} pig during phase 2
$W_2(t)$	$\mathbb{R}^+$	cfu	Number of *Salmonella* on the machine at time t, at phase 2
T_2	$\mathbb{R}^+$	min.	Time spent in the dehairing machine.
$B_{2,k}$	$\mathbb{R}^+$	cfu	Number of *Salmonella* extruded by pig P_k
A_k	$\mathbb{R}^+$	g	Amount of faeces extruded
Ω_k	$\{0,1\}$	-	Status of the pig: infected (1), not infected (0).
C_k	$\mathbb{R}^+$	cfu/g	Concentration of *Salmonella* in faeces
β_2	$[0,1]$	-	Fraction of *Salmonella* transfer from pig to machine
$\alpha_{2,k}$	$[0,1]$	-	Fraction of *Salmonella* transfer from machine to pig

At times before, during and after interaction with the dehairing machine the equations are

$$N'_{2,k}(t) = \alpha_{2,k} W_2(t), \quad \text{for } t \in (t_k, t_{k+1}) \tag{9.39}$$
$$N'_{2,k}(t) = 0, \quad \text{for } t \notin [t_k, t_{k+1}]. \tag{9.40}$$

The dehairing machine loses a fraction $\alpha_{2,k}$ to the pig and obtains what the pig transferred at $t = t_k$ (see (9.38)).

$$W'_2(t) = -\alpha_{2,k} W_2(t), \quad \text{for } t \in (t_k, t_{k+1}), \tag{9.41}$$
$$W_2(t_k) = \beta_2 M_k + \lim_{t \to t_k} W_2(t). \tag{9.42}$$

Note how $W'_2(t) + N'_k(t) = 0$ and $N(t_k) + W_2(t_k) = N_{k,1} + B_{2,k} + \lim_{t \to t_k} W_2(t)$, as required.

Implementation

The differential equation for W_2 has as its solution

$$W_2(t) = \gamma e^{-\alpha_{2,k} t}, \quad \text{for } t \in (t_k, t_{k+1}). \tag{9.43}$$

The constant γ is determined by examining the initial condition (9.42), giving

$$W_2(t) = [\beta_2 M_k + W_2^-(t_k)] e^{-\alpha_{2,k}(t - t_k)}, \quad \text{for } t \in (t_k, t_{k+1}). \tag{9.44}$$

Inserting in equation (9.39) yields

$$N'_k(t) = \alpha_{2,k} [\beta_2 M_k + W_2^-(t_k)] e^{-\alpha_{2,k}(t - t_k)}, \tag{9.45}$$

with solution

$$N_k(t) = -[\beta_2 (M_k + W_2^-(t_k)] e^{-\alpha_{2,k}(t - t_k)} + c. \tag{9.46}$$

The undetermined constant c should be found by the initial condition (9.38) yielding

$$N_k(t) = [\beta_2 M_k + W_2^-(t_k)](e^{-\alpha_{2,k}(t-t_k)} - 1) + (1-\beta_2)M_k. \quad (9.47)$$

Now we are left with finding W_2^-, which may be done by taking the limit $t \to t_{k+1}$ in (9.44), giving the recursion

$$W_2^-(t_{k+1}) = [\beta_2 M_k + W_2^-(t_k)]e^{-\alpha_{2,k}T_2}. \quad (9.48)$$

The previous two equations may be combined into a simpler equation for $N_k(t)$, which may be evaluated at t_{k+1}, yielding the final result

$$N_k(t_{k+1}) = (e^{\alpha_{2,k}T_2} - 1)W_2^-(t_{k+1}) + (1-\beta_2)M_k. \quad (9.49)$$

Parameter estimation

Parameters Ω_k, infection status of the pig, A_k, Amount of faeces extruded and C_k, concentration of *Salmonella* in faeces.

Whether the pig is infected or not is an input from the Transport & Lairage model. The concentration in faeces, C_k is also an input from the Transport & Lairage model (denoted there as $\varepsilon_L(k, j)$).

Data on A_k was not available from the literature. Expert opinion suggested that it would be in the order of magnitude of 10 grams (data obtained from QA department of a Dutch slaughterhouse).

Parameter T_2, time spent in the dehairing machine.

For the time spent in the dehairing machine we use for all MS

$$T_2 = \Re(U(0.48, 2.13)), \quad (9.50)$$

which is taken from Wilkin *et al.* 2007, a UK study.

Parameters $\alpha_{2,k}$ and β_2, the transfer rates from machine to pig and pig to machine.

As in Section 9.2.6 we estimate the value of β_2 using data from Namvar and Warriner 2005. Where in the scalding bath we assumed that loosely bound *Salmonella* would be removed instantaneously, we now assume that the dehairing machine removes all of the firmly attached *Salmonella*. This value was reported as 18%, thus we arrive at a value of $\beta_2 = 0.18$.

Estimation of $\alpha_{2,k}$ is not easy, since it is thought of as stemming from a distribution. Appendix 9.2 presents a number of simple approaches for estimation of $\alpha_{2,k}$, that unfortunately do not result in useful estimates. We are therefore forced to perform a complex calculation, which we present below.

We start with the recursion (9.48) for W_2, which can be solved in closed form as

$$W_{2,k+1} = \beta_2 \sum_{i=1}^{k} M_i \, e^{-T_2 \sum_{j=i}^{k} \alpha_{2,j}}. \qquad (9.51)$$

Inserting this expression into (9.49) yields

$$N_k(t_{k+1}) = (1-\beta_2) M_k + \left(e^{\alpha_{2,k} T_2} - 1\right) \beta_2 \sum_{i=1}^{k} M_i \, e^{-T_2 \sum_{j=i}^{k} \alpha_{2,j}} \qquad (9.52)$$

Next, we limit ourselves to estimating the average $\bar{\alpha}_2$ and set each $\alpha_{2,k}$ equal to this average. Also we neglect the first term in the above equation, which is only of importance for very high values of M_k. Finally, we assume all M_k to be equal to their average value: $M_k = \bar{M}$. This quantity can then be pulled out of the summation. We then have

$$N_k(t_{k+1}) = \left(e^{\bar{\alpha}_2 T_2} - 1\right) \beta_2 M_k \sum_{i=1}^{k} e^{-(k-i+1) T_2 \bar{\alpha}_2}, \qquad (9.53)$$

and therefore

$$\begin{aligned}
\frac{N_k(t_{k+1})}{M_k} &= \left(e^{\bar{\alpha}_2 T_2} - 1\right) \beta_2 \sum_{i=1}^{k} e^{-i T_2 \bar{\alpha}_2} \\
&= \left(e^{\bar{\alpha}_2 T_2} - 1\right) \beta_2 \sum_{i=0}^{k-1} e^{-(i+1) T_2 \bar{\alpha}_2} \\
&= \left(1 - e^{-\bar{\alpha}_2 T_2}\right) \beta_2 \sum_{i=0}^{k-1} e^{-i T_2 \bar{\alpha}_2} \\
&= \left(1 - e^{-\bar{\alpha}_2 T_2}\right) \beta_2 \frac{1 - e^{-k T_2 \bar{\alpha}_2}}{1 - e^{-T_2 \bar{\alpha}_2}} \\
&= \beta_2 (1 - e^{-k T_2 \bar{\alpha}_2}) \qquad (9.54)
\end{aligned}$$

Several authors report a number of log-increases of microbial counts. We therefore need to express $\bar{\alpha}_2$ in terms of the average of $\log(N_k/N_{1,k})$. This forces us to neglect the contribution of B_k in the calculation of $\bar{\alpha}_2$. Taking the logarithm of the above equation and computing the average gives

$$\overline{\log(N_2/N_1)} = \log(\beta_2) + \frac{1}{n} \log\left(\prod_{k=1}^{n} 1 - e^{-k T_2 \bar{\alpha}_2}\right). \qquad (9.55)$$

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n} \sum_{k=1}^{n} \log\left(1 - \frac{1}{e^{ka}}\right). \qquad (9.56)$$

For convenience we defined $a \equiv T_2\bar{\alpha}_2$. For small values of the exponent, the exponential function can be reasonably approximated by a linear function, $e^x \approx 1 + x$. This applies here, resulting in

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\sum_{k=1}^{n} \log(1 - \frac{1}{(1+a)^k}), \quad (9.57)$$

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\sum_{k=1}^{n} \log(\frac{(1+a)^k - 1}{(1+a)^k}), \quad (9.58)$$

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\sum_{k=1}^{n} \log((1+a)^k - 1) - \log((1+a)^k), \quad (9.59)$$

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\sum_{k=1}^{n} \log((1+a)^k - 1) - \frac{\log(1+a)}{n}\sum_{k=1}^{n} k. \quad (9.60)$$

Now, assume that k is odd, making the average of $1 + 2 + \ldots + k$ simply $k/2$. Furthermore, approximate $(1+a)^k \approx 1 + ka$. Then,

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\sum_{k=1}^{n} \log(ka) - \frac{1}{2}\log(1+a), \quad (9.61)$$

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\log(\prod_{k=1}^{n} ka) - \log(\sqrt{1+a}), \quad (9.62)$$

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\log(a^n n!) - \log(\sqrt{1+a}), \quad (9.63)$$

$$\overline{\log(N_2/N_1)} - \log(\beta_2) = \frac{1}{n}\log(n!) + \log(\frac{a}{\sqrt{1+a}}). \quad (9.64)$$

In Appendix 9.3 we establish that the log of the factorial term in the above equation may be reasonably approximated by $\log(n) - \log(e)$ and our final result becomes

$$\log(\frac{a}{\sqrt{1+a}}) = \overline{\log(N_2/N_1)} - \log(\beta_2) - \log(n) + \log(e). \quad (9.65)$$

At this point all quantities on the right hand side are known. Denote the right hand side by $\log(y)$, then,

$$\frac{a}{\sqrt{1+a}} = y, \quad (9.66)$$

$$a^2 - y^2 a - y^2 = 0, \quad (9.67)$$

$$a = y^2(1 + \sqrt{1 + 4/y^2})/2\,, \quad (9.68)$$

$$\bar{\alpha}_2 = y^2(1 + \sqrt{1 + 4/y^2})/(2T_2), \quad (9.69)$$

Data on the log-increase of micro-organisms due to dehairing was reported by several authors. Unfortunately, these data are not specifically for *Salmonella*, but for several other types of bacteria, which we list in Table 9.10.

Since these are relative numbers, we assume that these increases may also be applied for *Salmonella*. Some of the numbers are clearly too high, and would yield an unrealistically high *Salmonella* load. Therefore, we set an upper bound of $\alpha = 1$. Then, the estimates for $\bar{\alpha}_2$ are well approximated by a uniform distribution, when taken in log-scale,

$$\alpha_{2,k} = 10^{\Re(U(-1.5,0),k)}. \qquad (9.70)$$

9.2.7Singeing

Problem definition

During singeing, the pigs are subjected to high temperatures for a short time period. This causes an exponential reduction in the number of *Salmonella*. Table 9.11 provides a description of the parameters needed.

The formula describing the exponential decay is now simply

$$N_{k,3}(T_3) = N_{k,2}e^{-\epsilon_3 T_3}. \qquad (9.71)$$

Table 9.10: Reported increases in microbial numbers on the pig, due to the dehairing machine.

Source	**Measured**	**Increase (log units)**	**Number of pigs (sample size)**	$\log(\bar{\alpha}_2)$
Spescha *et al*. 2006	Enterobacteriaceae	3.4	1000	3.0
Rivas *et al.* 2000	Enterobacteriaceae	0.7	1600	-1.4
Pearce *et al.* 2004	Coliform counts	1	1000	-0.9
Warriner *et al.* 2002	Enterobacteriaceae	1.6	140	1.2

Table 9.11: Quantities used in the singeing phase.

Quantity	**Domain**	**Unit**	**Description**
ϵ_3	$\mathbb{R}^+$	cfu/min	Inactivation parameter at phase 3
T_3	$\mathbb{R}^+$	min	Time spent in the singeing machine

Parameter estimation

The time spent in the singeing machine is unknown for any of the representatives of the clusters. We use Belgian data (Delhalle *et al.* 2008), being the only European data available (see Table A9.54),

$$T_3 = \Re(U(0.07, 0.27)) \quad (9.72)$$

The inactivation parameter can be inferred from data in Pearce *et al.* 2004, who obtained the enumeration data (see Table 9.12) after a 16 second singeing step.

We average all numbers and find an average of 4.03 log cfu before singeing and 1.45 log cfu after singeing. Using equation (9.71) this yields an inactivation parameter of

$$\epsilon_3 = 23.7 \quad (9.73)$$

Table 9.12: Log cfu micro-organisms reported by before and after singeing**.**

Log cfu before	Log cfu after	Type	Location
4.75	2.20	AMC*	Ham
4.46	2.25	AMC	Belly
4.65	1.80	AMC	Neck
3.64	1.03	CC**	Ham
3.32	1.33	CC	Belly
3.54	0.84	CC	Neck
4.16	1.28	CRC***	Ham
3.82	1.33	CRC	Belly
3.91	1.03	CRC	Neck

*AMC: Aerobic mesophilic count; **CC: Coliform count ***CRC: Coliform resuscitation count

9.2.8 Polishing

Problem definition

The polishing machine is conceptually like the dehairing machine, only the parameters will differ. In contrast to the dehairing machine, no water is used and the machine does not exert a large amount of pressure. Therefore, only a small amount of faecal matter is assumed to extrude from the pig during polishing. The parameters given in Table 9.13 are used.

Table 9.13: Quantities used in the polishing phase.

Quantity	Domain	Unit	Description
$W_4(t)$	$\mathbb{R}^+$	cfu	Number of *Salmonella* on the machine at time t, at phase 4
T_4	$\mathbb{R}^+$	min.	Time spent in the polishing machine
$B_{4,k}$	$\mathbb{R}^+$	cfu	Number of *Salmonella* extruded by pig P_k at phase 4
β_4	$[0,1]$	-	Fraction of *Salmonella* transferred from pig to machine
$\alpha_{4,k}$	$[0,1]$	-	Fraction of *Salmonella* transferred from machine to pig

The governing equations are similar to the equations describing the dehairing machine, but the initial conditions for the contamination on the pig differ. Previously, in Section 9.2.6, we had classified the *Salmonella* into three groups: loosely bound, firmly bound, irremovable and used these groups to estimate β. An amount $\beta_2(N_{2,k}+B_{2,k})$ would be transferred to the dehairing machine. At this point however, we have only irreversibly bound *Salmonella*e left. Now no fraction of $N_{3,k}$ will move to the machine, but a fraction β_4 of $B_{4,k}$ will do so. The resulting equations are a minor modification of the dehairing equations,

$$N_{4,k}(t_k) = (1-\beta_4)B_{4,k} + N_{3,k}, \tag{9.74}$$

$$N'_{4,k}(t) = \alpha_{4,k}W_4(t), \text{ for } t \in (t_k, t_{k+1}), \tag{9.75}$$

$$N'_{4,k}(t) = 0, \text{ for } t < t_k \text{ and } t \geq t_{k+1}, \tag{9.76}$$

$$W'_4(t) = -\alpha_{4,k}W_4(t), \text{ for } t \in (t_k, t_{k+1}), \tag{9.77}$$

$$W_4(t_k) = \beta B_{4,k} + \lim_{t \to t_k} W_4(t). \tag{9.78}$$

Implementation

We follow the exposition from Section 0 but will not repeat the steps here. The equivalents of equations (9.48) and (9.49) are

$$W_4^-(t_{k+1}) = [\beta_4 B_{4,k} + W_4^-(t_k)]e^{-\alpha_{4,k}T_4}, \quad (9.79)$$

$$N_k(t_{k+1}) = (e^{\alpha_{4,k}T_4} - 1)W_4^-(t_{k+1}) + (1-\beta_4)B_k + N_{3,k}. \quad (9.80)$$

Parameter estimation

Parameters Ω_k, infection status of the pig, A_k, Amount of faeces extruded and C_k, concentration of *Salmonella* in faeces

The parameters Ω_k and C_k are the same as the values derived in the dehairing section. However, A_k will differ, due to the difference between the dehairing and polishing machine. We assume the value $A_k = 1$ gram, based on data obtained from the QA department of a Dutch slaughterhouse.

T_4, time spent in the polishing machine

For this quantity, we only have data available from a Belgian study (Delhalle *et al.* 2008). The data was found to be well approximated by a uniform distribution between 28 and 95 seconds,

$$T_4 = \Re(U(0.47, 1.58)). \quad (9.81)$$

Parameters $\alpha_{4,k}$ and β_4, the transfer rates from machine to pig and pig to machine.

Estimation of $\alpha_{k,4}$ will follow the procedure from Section 9.2.6. Values for the log-increase in microbial numbers were reported by several authors, as summarised in the Table 9.14.

The second value is too high, and we follow the same procedure as before and truncate it to zero. We estimate the resulting distribution of $\bar{\alpha}_4$ as

$$\bar{\alpha}_4 = 10^{\Re(U(-0.5,0))}. \quad (9.82)$$

The value of β_4, is not reported in the literature. We consider that contamination in the faecal material behaves like the loosely attached *Salmonella* in the scalding bath, which yields

$$\beta_4 = \beta_1 = 0.02. \quad (9.83)$$

Table 9.14: Reported increases in enterobacteriaceae numbers on the pig during the polishing phase.

Source	**Increase (log units)**	**Number of pigs (sample size)**	$\log(\bar{\alpha}_4)$
Rivas *et al.* 2000	0.6	1600	-0.4
Spescha *et al.* 2006	2.9	1000	4

9.2.9 Belly opening

Problem definition

During this phase, the belly of the pig is opened by an automated cutting machine. Next, during evisceration, the gut is loosened and removed manually.

After processing of a pig, the cutting hook is retracted into the machine and auto-sterilized using hot water treatment. The temperature of the water should be 82 °C by EU regulation (Eustache *et al.* 2007). However, Delhalle *et al.* 2008 arrive at a distribution, BP(47,77,81). Taking the most conservative estimate of 47°C, already yields a 2 log decrease per second (see equation (9.35). In Maribo *et al.* 1998 a sterilizing time of 8 seconds is reported. Clearly, the resulting reductions are sufficient for elimination of all *Salmonella*e on the knife. This however, does not take into account the possible formation of biofilm, or recontamination of the knife after sterilizing, by dripping of condensed water. See also Peel & Simmons 1978, who experimentally establish that at least 10 seconds are needed at 82°C.

However, since Peel & Simmons 1978 do not mention any counts, we assume a sterile cutting hook (which we also call 'knife' in the following). See Section 9.3.2 for the treatment of house flora (biofilm) in this phase.

This brings us to the relevant quantities, being the transfer coefficients and inactivation coefficients. Additionally, we also need to take into account the area of the pig's exterior touched by the knife, since only from this area can *Salmonella* be transferred.

Finally, there exists the possibility of puncturing the gut, thereby spilling faecal matter on the knife and pig.

Table 9.15: Quantities used in the belly opening phase.

Quantity	Domain	Unit	Description
$N_{k,5}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the kth pig or carcass P_k, in phase 5.
$\beta_{5,k}$	$[0,1]$	-	Fraction of *Salmonella* on the kth pig moving to the knife
δ_5	$[0,1]$	-	Fraction of *Salmonella* in the faecal spillage moving to the knife
$l_{5,k}$	$\mathbb{R}^+$	cm	Length of the incision
b_5	$\mathbb{R}^+$	cm	Width of the incision
$O_{5,k}$	$\mathbb{R}^+$	cm2	Surface area of the pig
$A_{5,k}$	$\mathbb{R}^+$	g	Amount of faeces spilling from the gut
$G_{5,k}$	$\{0,1\}$	-	Status of the gut, punctured (1), or not punctured (0)
$B_{5,k}$	$\mathbb{R}^+$	cfu	Number of *Salmonella* spilling from the gut
$\Omega_{5,k}$	$\{0,1\}$	-	Status of the pig: infected (1), not infected (0).
$C_{5,k}$	$\mathbb{R}^+$	cfu/g	Concentration of *Salmonella* in faeces

Note that potentially a fraction $l_{5,k}b_5/O_{5,k}$, being the ratio of touched surface area to total surface area, of the *Salmonella* on the exterior can be transferred from and to the knife. The equation describing cross-contamination and faecal spillage is

$$N_{k,5} = (1 - d_{5,k})N_{k,4} + (1 - \delta_5)B_{5,k}, \tag{9.84}$$

with

$$B_{5,k} = G_{5,k}\Omega_{5,k}C_{5,k}A_{5,k}, \tag{9.85}$$

and

$$d_{5,k} = \beta_{5,k}l_{5,k}b_5/O_{5,k}. \tag{9.86}$$

Implementation

The recursion derived in the previous section can be directly implemented in Matlab.

Parameter estimation

$\beta_{5,k}$, transfer rate from the pig to the knife

Unfortunately, very little data has been published on the Belly Opening or Evisceration phases in pig slaughter. Therefore, estimation of transfer parameters on the basis of measured *Salmonella* counts can not be done. As an alternative, we use transfer parameters reported in Kusumaningrum *et al.* 2003 on transfer from stainless steel surfaces to roasted chicken and sponges to stainless steel.

The transmission from sponges to stainless steel was measured by contaminating a wetted sponge and wiping a steel surface. Certainly a sponge has a different structure than pig skin, but the effect of wiping is somewhat comparable to the cutting action of the knife. The authors found a transfer rate of 21±8 percent. Modelling the variability using a normal distribution may result in negative values, or values over 100%. For this reason we use a beta pert distribution with most likely value 0.21 and minimum and maximum equal to 0.13 and 0.29,

$$\beta_{5,k} = \Re(BP(0.13, 0.21, 0.29), k). \quad (9.87)$$

$l_{5,k}$, b_5 and $O_{5,k}$, incision length and width and the surface area of the pig

In Titus, 2007 a incision length varying from 129 to 146 cm was reported, with an average of 137 cm. It is unclear in what way this variation was modelled. Since no further information is available we choose each outcome to be equally likely and set

$$l_k = \Re(U(129, 146), k). \quad (9.88)$$

A typical width of $b = 0.1$ cm was found. The surface area of a pig was measured in Kelley *et al.* 1973, where the following relation between body mass $m_{5,k}$ and surface area $O_{5,k}$ was obtained

$$O_{5,k} = 734 m_k^{0.656}. \quad (9.89)$$

From the EFSA baseline study (EFSA 2008a; annexes Table VI.5), we have data for each MS on weights of pig carcasses, in the form of means and minimum and maximum values (see Table 9.16).

The weight of an individual pig is obtained by sampling from a beta pert distribution with the parameters taken from the above table.

$B_{5,k}$, $\Omega_{5,k}$, $C_{5,k}$ and δ_5, puncturing of the gut, resulting faecal extrusion, infection status of the pig, Salmonella concentration in faeces and proportion moving to the knife.

The frequency with which the gut is punctured is not reported in the literature. However, expert opinion from a Dutch slaughterhouse suggest that the probability of faecal leaking lies somewhere in the range $[0.012, 0.02]$. Therefore we set

$$p_k = \Re(U(0.012, 0.02), k). \quad (9.90)$$

Table 9.16: Carcass weight per MS in kg.

	Min	Mean	Max
MS1	60	94	121
MS2	54	79	128
MS3	57	89	126
MS4	75	80	84

and

$$G_{5,k} = \Re(B(1, p_k), k). \quad (9.91)$$

In order to estimate the proportion of *Salmonella* moving to the knife and to the pig we use data from Titus, 2007 who reports

- If the knife becomes contaminated, the mass of the contamination lies between 0.0125 and 0.5 g.
- On carcasses, faecal contamination was found ranging from 6.6 to 19.8g.

Thus, let us take the total contamination (carcass and knife) uniformly distributed, rounded to one digit:

$$A_{5,k} = \Re(U(6.6, 20.3), k) \quad (9.92)$$

Assuming that low (high) values on the knife are linked with low (high) values on the carcass, we can derive an approximate fraction δ_5. We derive this factor by a least squares fit through the points (0,0), (0.0125,6.6) and (0.5, 19.8), yielding[20]

$$\delta_5 = 0.02. \quad (9.93)$$

The infection status and concentration in the faeces were determined before, in Section 9.2.6.

9.2.10 Splitting

Problem definition

The splitting phase constitutes the halving of the carcass, top-down, by an automated saw. The saw stops at the head, which is removed later. The relevant parameters are largely the same ones as used in the Belly Opening phase, with the difference that faecal leakage no longer plays a role; the gut has been removed at this point. Thus, the following parameters are needed (Table 9.17),

Table 9.17: Parameters used in the splitting phase.

Quantity	Domain	Unit	Description
$N_{6,k}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the k^{th} pair of half carcasses obtained from P_k, in phase 6
$H_{6,k}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the k^{th} half carcass in phase 6
$\beta_{6,k}$	$[0, 1]$	-	Transfer fraction from pig k to the saw
$O_{6,k}$	$\mathbb{R}^+$	cm^2	Surface area of the pig
$l_{6,k}$	$\mathbb{R}^+$	cm	Length of the incision
b_6	$\mathbb{R}^+$	cm	Width of the incision

Note there are twice as many half carcasses as there are carcasses. We assume that the distribution of resulting contamination over the half carcasses is proportional. Note that the factor relating the incision length to the total area is not $l_{6,k}b_6/O_{6,k}$, but $2l_{6,k}b_6/O_{6,k}$, since the saw cuts the exterior on the front and back side of the pig. The governing equation is similar to the Belly Opening equation:

$$N_{k,6} = (1 - 2d_{6,k})N_{k,5}, \quad (9.94)$$

with

$$d_{6,k} = \beta_{6,k}l_{6,k}b_6/O_{6,k}. \quad (9.95)$$

[20] Actually, we fit (0.0125, 6.6) and (0.5, 19.8) to a line of the form y=ax, making sure the line goes through the origin: no contamination should imply no contamination on the knife.

Next, we distribute the *Salmonella* load over the half-carcasses, using a binomial distribution. Let X_k be realized from the binomial $B(N,p)$ distribution with parameters $N = N_{k,6}$ and $p = 1/2$. Then the *Salmonella* load on each half-carcass is

$$H_{k,6} = X_k, \quad \text{for } 1 \leq k \leq n_l, \tag{9.96}$$

$$H_{k,6} = N_{6,k-n_l} - X_{k-n_l}, \quad \text{for } n_l + 1 \leq k \leq 2n_l. \tag{9.97}$$

Implementation

Similar to the Belly Opening phase, these recursions can be directly implemented in the model.

Parameter estimation

$\beta_{6,k}$, transfer rate from the pig to the saw

In Section 9.2.6 this parameter was estimated from published literature on cross contamination from steel surfaces to sponges and roasted chicken (Kusumaningrum *et al.* 2003). The saw used in the splitting phase is comparable to the knife used in the Belly Opening phase and thus we set

$$\beta_{6,k} = \Re(BP(0.13, 0.21, 0.28), k). \tag{9.98}$$

$l_{6,k}$, b_6 and $O_{6,k}$, incision length and width and the surface area of the pig

The surface area of the pig was already determined in Section 9.2.6. The incision length and width is however different. The incision length is equal to the length of a carcass, which was determined in Titus, 2007 to range from 137.7 to 164.5cm, with an average of 152 cm. We fit these values to a beta pert distribution (using 152cm for the most likely value) and obtain

$$l_{6,k} = \Re(BP(137.7, 152, 164.5), k). \tag{9.99}$$

Also from Titus, 2007 a value of $b_6 = 0.1$ cm was found for the width of the saw.

9.2.11 Trimming

Problem definition

Trimming is an inspection, by slaughterhouse personnel, for abnormalities on the half carcasses. For our purposes the most important element of the trimming procedure is the detection of faecal contamination and its removal. Potentially, there is the risk of cross-contamination, either by the knife, or by the hands of the handler. Table 9.18 lists the quantities that will be of interest.

Any visible faecal material on the carcass must have originated from either the farm, lairage, dehairing, polishing, or belly opening. The material originating from the farm, transport, lairage or dehairing phases is very likely to have been removed or spread during subsequent stages and no longer visually detectible. We assume only material due to the polishing and belly opening phase is detectable.

After polishing, an amount $(1-\beta_4)A_{4,k}$ is deposited on the pigs' exterior. The contribution from belly opening is $(1-\delta_5)A_{5,k}$. Thus

$$A_{7,k} = (1-\beta_4)A_{4,k} + (1-\delta_5)A_{5,k}. \quad (9.100)$$

Table 9.18: Quantities used in the trimming phase.

Quantity	Domain	Unit	Description
$H_{7,k}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the k^{th} half carcass
$A_{7,k}$	$\mathbb{R}$	g	Amount of faeces on the k^{th} carcass
$D_{7,k}$	$\mathbb{R}$	g	Amount of faeces on the k^{th} half carcass
$R_{7,k}$	$\mathbb{R}$	g	Amount of faeces removed from the k^{th} half carcass
$M_{7,k}$	$\mathbb{N}$	-	Number of trimming actions
$V_{7,k}$	$\mathbb{R}$	g	Faecal contamination detection limit
$B_{7,k}$	$\mathbb{R}$	cfu	Number of *Salmonella* in faeces on the k^{th} half carcass
$C_{7,k}$	$\mathbb{R}^+$	cfu/g	Concentration of *Salmonella* in faeces
$I_{7,k}$	$\{0,1\}$	-	Status of the pig: infected (1), not infected (0).

The contamination will be distributed over both half-carcasses, with no preference for either,

$$D_{7,2k} = \Re(U(0,1),k)A_{7,k}, \quad (9.101)$$

$$D_{7,2k+1} = A_{7,k} - D_{7,2k}. \quad (9.102)$$

Experiments with the model indicate that low levels (originating from polishing) of approximately 1 gram occur rather frequently. High levels, of around 5 to 10 grams (originating from belly opening) occur infrequently.

The faecal contamination is partially removed, depending on a number of factors. We assume that faecal contamination is divided over the half carcass in a number of 'chunks'. Also, we assume that larger chunks are easier to spot by the trimmer. Furthermore, we know a typical number of trimming actions (a random variable M_k). Finally, we assume some threshold value at which the faecal material is just visible (a random variable V_k). Figure 9.4 schematically depicts the situation.

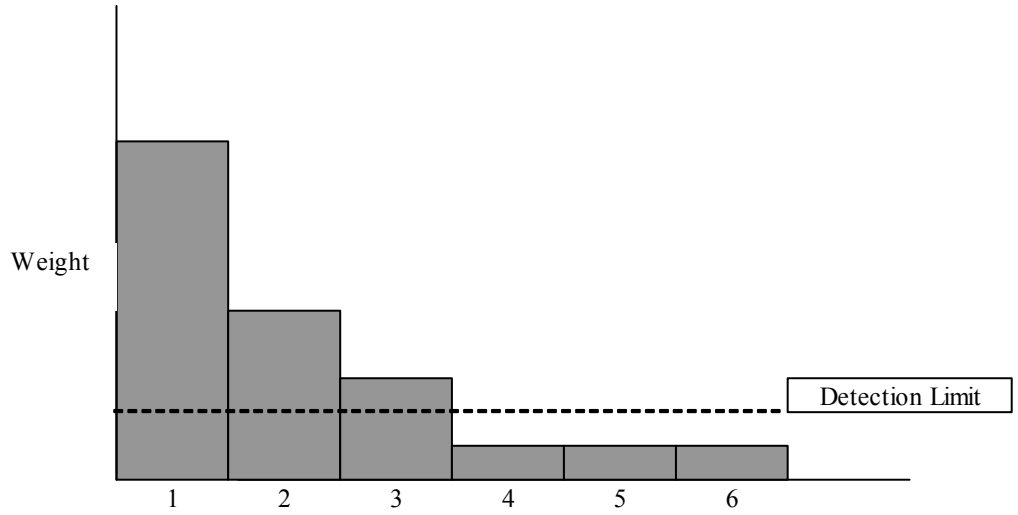

Figure 9.4: Schematic distribution of faecal material at the trimming stage. The x-axis labels the chunks of faecal material.

What we would like is to determine the weights of the first few pieces, those that are above the detection limit, which are those that are detected. We treat the number of detected pieces as a given (observed).

To this end we need to assume some distribution of feacal material over the half-carcass. We choose an exponential distribution of the weights. This has the drawback that the x-axis (labeling the chunks in Figure 9.4) is now continuous. Also, for distributions the total area is one, while we would like $D_{7,k}$ grams. But this is easily solved by interpreting $D_{7,k}$ as one 'weight unit', with $V_k/D_{7,k}$ the threshold. In this continuous setting we have a situation like Figure 9.5.

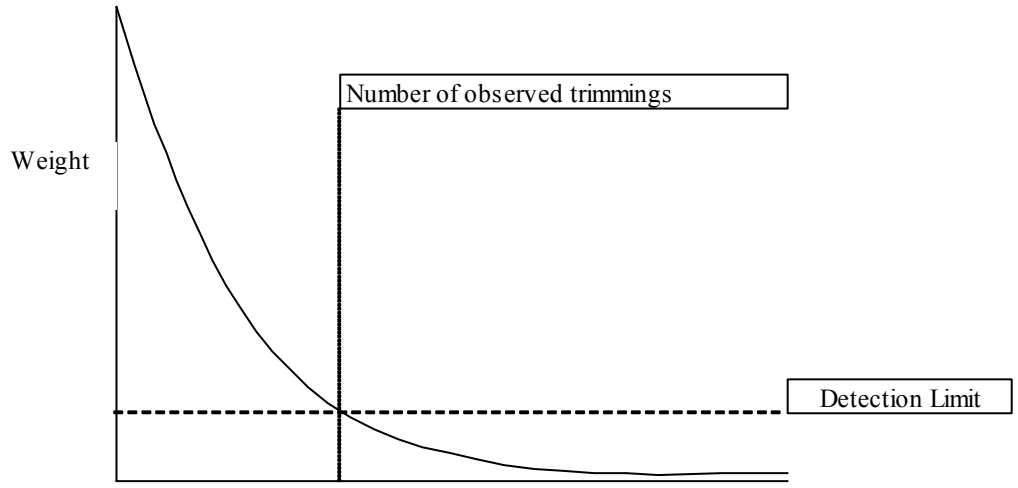

Figure 9.5: The trimming phase as a continuous process. Compare with Figure 9.4.

Now, given the detection limit and number of observed trimmings, we can calculate the parameter of the exponential distribution. Then, we integrate this distribution from zero to M_k, which is the amount of material removed $R_{7,k}$ (in accordance to both the detection limit and the number of observed trimmings). This amount is simply subtracted,

$$H_{7,k} = H_{6,k} - R_{7,k}. \quad (9.103)$$

Solution and implementation

In order to find the parameter $\lambda(k)$ of the exponential distribution, we assume that at a detection limit of $V_{7,k}$ grams, exactly $M_{7,k}$ pieces are removed at a total weight of $D_{7,k}$ grams of faecal material on the k-th half carcass,

$$V_{7,k} = \lambda(k) D_{7,k} e^{-\lambda(k) M_{7,k}}. \quad (9.104)$$

Unfortunately, finding λ from this equation cannot be done analytically. However, introduce $\gamma(k) = \lambda(k) M_{7,k}$, then

$$e^{\gamma(k)} = \gamma(k) \frac{D_{7,k}}{V_{7,k} M_{7,k}}. \quad (9.105)$$

Also, assume $\gamma(k) \ll 1$, then $e^{\gamma(k)} \approx 1 + \gamma(k) + \gamma(k)^2/2$, yielding

$$1 + (1 - \frac{D_{7,k}}{V_{7,k} M_{7,k}})\gamma(k) + \gamma(k)^2/2 \approx 0 \quad . \quad (9.106)$$

This quadratic equation is easily solved, giving

$$\gamma(k) \approx \frac{1}{2}(\frac{D_{7,k}}{V_{7,k} M_{7,k}} - 1) \pm \frac{1}{2}\sqrt{(\frac{D_{7,k}}{V_{7,k} M_{7,k}} - 1)^2 - 2} \quad . \quad (9.107)$$

Of these two solutions, only the positive one is acceptable. This gives for λ, also depending on k,

$$\lambda(k) = \frac{1}{2M_{7,k}}\left[(\frac{D_{7,k}}{V_{7,k} M_{7,k}} - 1) + \sqrt{(\frac{D_{7,k}}{V_{7,k} M_{7,k}} - 1)^2 - 2}\right] . \quad (9.108)$$

Finally, the amount to be removed is found by integrating the distribution from zero to $M_{7,k}$,

$$R_{7,k} = \lambda(k) D_{7,k} \int_0^{M_{7,k}} e^{-\lambda(k)x}\, dx = D_{7,k}(1 - e^{-\lambda_k M_{7,k}}). \quad (9.109)$$

Parameter estimation

We need to estimate the parameter $V_{7,k}$, the detection limit of faecal material on pig skin. Such a number was not found in the available literature. However, in Evers *et al.* 2008 a similar situation is described. This paper deals with children visiting petting-zoos. After a visit, the parent will inspect the hands of the children for faecal contamination. From laboratory experiments it was derived that 3mg of material would be visible upon inspection.

However, this number is rather uncertain. Also, it is not directly applicable to the trimming procedure. Therefore we consider that this number indicates terms of magnitude only and set the detection limit between 1 and 10 mg randomly for each pig,

$$V_{7,k} = \Re(U(0.001, 0.01), k). \quad (9.110)$$

The number of trimming actions was estimated to be two or three by the QA department of a large Dutch slaughterhouse,

$$M_{7,k} = \Re(DU([2, 3]), k). \quad (9.111)$$

The remaining parameters (concentration, infection status) were already discussed in previous sections.

9.2.12 Blast chilling

During blast chilling the temperature of the exterior of the pig is lowered rapidly to very low freezing temperatures. The temperature decrease is effected by blowing cold air at the half-carcass. At temperatures below the freezing point, ice crystals have the potential of killing *Salmonella* cells. The application of cold air also dries the exterior of the pig, lowering the a_W. Also, chemical reactions in the cell may destroy the lipid bilayer, causing permanent damage to the cell (Chang *et al.* 2003).

Not all slaughterhouses have implemented the blast chilling phase and the time-temperature combinations vary between slaughterhouses. A few examples of reported parameters are given in Table 9.19.

Since the variation in times and temperatures is large, the range of processes is large (drying, cooling, ice crystal formation), data is scarce and blast chilling is not implemented in every slaughterhouse, it was decided to implement blast chilling simply as a one log reduction. This number may be changed in the model when more accurate reductions factors are available. Also, the above table can act as a guide for those who wish to enter approximate reductions into the model.

Table 9.19: Several time-temperature combinations used for blast chilling.

Source	Time (min)	Air temperature	Approximate log reduction.	MS
Spescha *et al.* 2006	45	-8	1 (TVC*)	Switzerland
Chang, *et al.* 2003	60-180	-20 to -40	1 (*S.* Typhimurium)	None (laboratory study)
Cutter, 2003	150 120 110 90	-15 to -10 -15 -15 -18 to -21	1.7 (Coliforms) 2.2 (E. Coli)	None (laboratory study)
Maribo *et al.* 1998	75	-10 to -20	-	Denmark
Borch *et al.* 1996	60-90 60-180	-10 to -30 -20 to -40	-	Denmark, Sweden Norway

.* TVC: Total Viable Counts

9.3 House Flora

9.3.1 Introduction

For the purpose of this study we define house flora as *Salmonella* contamination of the equipment, machines or other objects in the slaughterhouse that is never completely removed. Thus, house flora acts as a permanent source of potential contamination of carcasses.

House flora is reduced by cleaning, usually performed at the end of a work-day. The efficiency of cleaning is hard to assess. Easily reachable surfaces, such as floors, can be effectively cleaned and disinfected. On the other hand, there are many sites that are hard to reach, e.g. the inside of machines, or any rails, beams, etc. that are located high up.

During the night, any micro flora which was not removed by cleaning may grow to larger numbers. The temperature is certainly favourable and the humidity is high. We will model the combined effect of cleaning and subsequent growth using one factor for bacterial numbers, for those machines that are known to become heavily contaminated (dehairing machine, polishing machine). The resulting contamination will then be a model input for the start of the next day.

9.3.2 Belly opening and splitting

Previous studies indicate that the evisceration knife and halving saw are important sites of persistent micro flora (Swanenburg, 2000). This is not compatible with estimated decimal reduction times, which imply that the knife will be sterile in a matter of microseconds (see Section 9.2.9). On the other hand, Warriner *et al.* 2002 find only small numbers of E. *coli* and Enterobacteriaceae on the evisceration knife and halving saw. Possibly, contamination in terms of prevalence is high (many carcasses are contaminated), while contamination in terms of concentration is low (they are contaminated only moderately). This would reconcile the differing conclusions drawn by authors, whether the belly opening and splitting phases are important cross-contamination events or not.

Possible circumstances not accounted for in our model, that would contaminate carcasses even if the knives are sterilised efficiently, could be:

- Dripping of condensed water, inside of the machine, recontaminates the knife.
- Formation of a protective biofilm on the saw and knife that inhibits sterilisation
- Formation of a biofilm inside the machine, so that once in a while “pieces” of the biofilm will get loose and contaminate the knife.

All of these hypotheses lead to a persistent moderate contamination of carcasses. We will model both effects as an addition to the bacterial load on the skin. To be completely explicit, the sequence of events is modelled as follows,

1. The carcass is cut
2. The knife is retracted and sterilized

3. The knife is recontaminated / unsuccessfully sterilized

4. The next carcass is cut

This effect may be implemented by addition of an extra term $+h_{5,k}$. The distribution of this extra *Salmonella* load is unknown, so we take very modest values,

$$h_{5,k} = \Re(U(1,100),k) \quad (9.112)$$

9.3.3 Dehairing and polishing

According to Warriner *et al.* 2002, the dehairing machine is heavily contaminated at the start of a working day. It is a key factor in cross-contamination of carcasses (as confirmed in this model). The authors report Enterobacteriaceae counts at the beginning of the day (5.6 log / 100cm^2) and at the end of the day (7.2 log / 100 cm^2). Assuming that days at the slaughterhouse are similar, we have a rough estimate of the cleaning and growth effect, a decrease of 1.6 log.

The unpublished report by Richards & Dodd 2009 presents Enterobacteriaceae counts, sampled during the day on several sites on the polishing machine. Averaged, the polishing machine contains 2.0 log cfu/cm^2 at the start of the day and 0.96 log cfu/cm^2 at the end of the day. This results in a one log effect of combined cleaning and growth during the night.

These numbers were put into the model. When house flora is enabled, the initial load on a machine (i.e. at the start of a new iteration) will not be zero but the end of the previous iteration plus 1.6 log (dehairing) or plus 1.0 log (polishing).

9.3.4 Other house-flora

Another source of endemic *Salmonella* in the slaughterhouse could be airborne bacteria, as suggested by Bolton *et al.* 2002. These authors mention numbers up to 3000 cfu/m^3. The problem in modelling this phenomenon is that the mechanism of attachment to carcasses or slaughter equipment is unknown.

9.4 Results for the Large Slaughterhouse

In this section we discuss the results of the large slaughterhouse model. The results for the 4 case study MSs are given in Figure 9.6. The input to each model is the output of the previous phase, i.e. from the Transport & Lairage module.

The figures show the average contamination of positive products (top panel), and the prevalences (lower panel), over the phases. The phase and unit under investigation is listed at the ticks of the x-axis. The vertical axis is in units of average 'log cfu'. Here the geometric average is taken over all products within one iteration (typically 10,000), and an arithmetic average over the iterations. The iterations induce variability in the results. This variability is represented by 'variability bars', having ticks at the 5th percentile, 50th percentile (median) and 95th percentile.

It should be kept in mind at all times that the prevalence graphs and bar chart should be considered as a whole. If a slight drop is observed in average log cfu contamination per positive product/carcass, it can very well be that there is also a tremendous drop in

prevalence, and the decrease of *Salmonella* numbers might be higher than it seems when superficially considering the bar charts only.

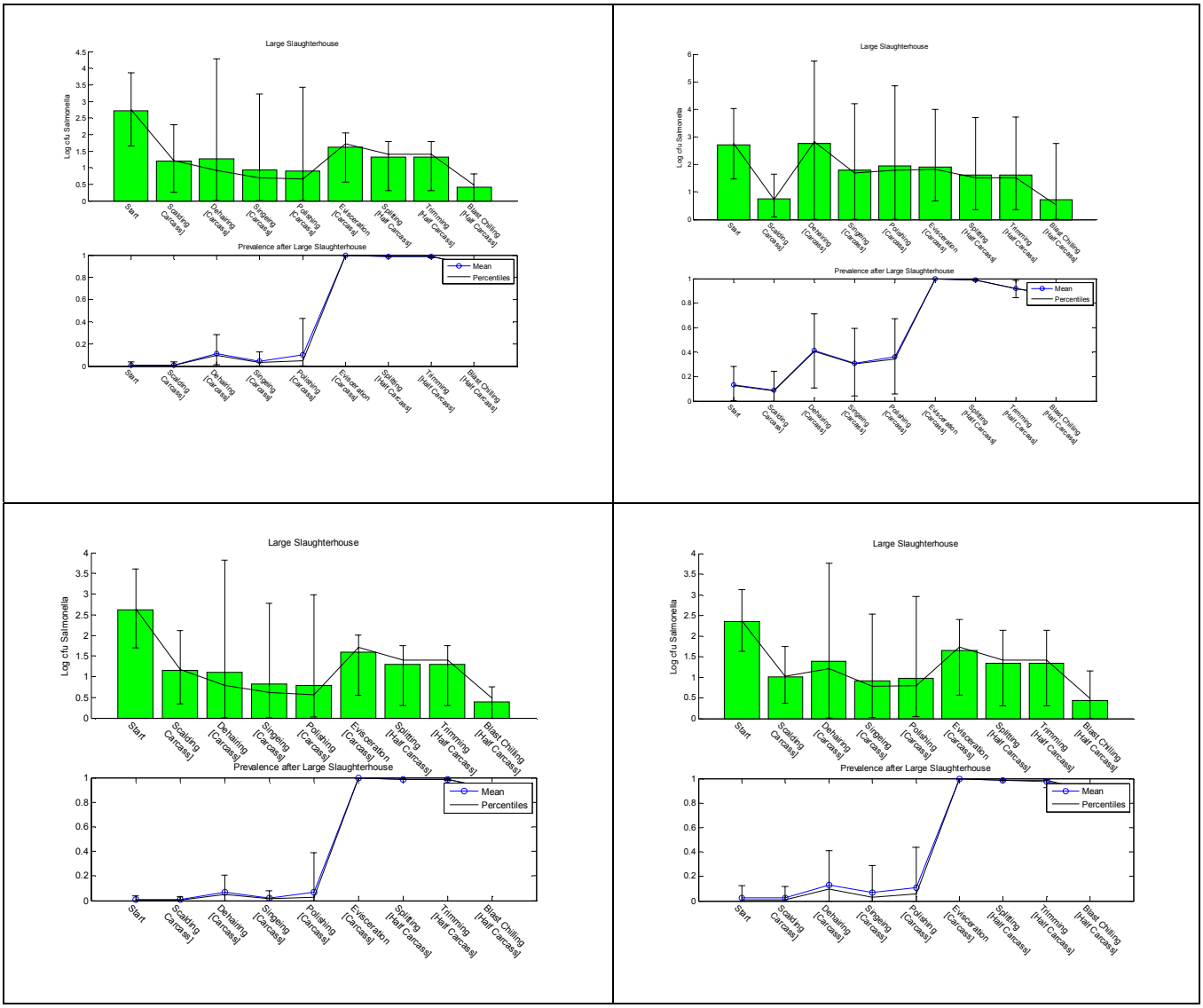

Figure 9.6: *Salmonella* numbers (top panel) and prevalence (bottom panel), during stages of the large slaughterhouse for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right)

At the start of the slaughter process, which is just after lairage, we have a low prevalence for MS1, MS3 and MS4, though the contamination on the skin can be substantial. As seen from Table 8.7, the prevalence of infection at this stage is higher for MS2 and therefore the probability of skin contamination will also be higher. The first process is the scalding step. Scalding has little effect on the prevalence (except for MS2), but numbers decrease considerably. We must conclude that the scalding water is sufficiently hot to properly kill off any *Salmonella* transferred from pigs to the scalding water, thereby prohibiting cross-contamination.

At dehairing prevalence increases and numbers increase, especially for MS2. Here we see the effect of cross-contamination, plus added faecal contamination. It turns out that the dehairing stage introduces a large amount of variability, probably due to a rare event (faecal extrusion) having a large impact (heavily contaminated).

Next is the singeing stage, which has the effect of about a 1.7 log reduction (on average), as seen from the formula and parameter values. However, the result from the graph seems to be much lower. This effect may be explained by means of an example. Suppose we have the following distribution of *Salmonella* over 100 carcasses

Nr. of carasses	40	10	10	10	10	10	10
Bacterial load	0	10^0	10^1	10^2	10^3	10^4	10^5

The prevalence is 60%, and the average log cfu per contaminated carcass is 2.5. Now, after singeing, suppose a two log reduction, any number below one is rounded to zero and we obtain

Nr. of carcasses	60	10	10	10	10
Bacterial load	0	10^0	10^1	10^2	10^3

Now, the prevalence is 40% while the average log cfu per carcass is 1.5. Observe how a two log reduction manifests itself as a one log reduction of contaminated carcasses!

Singeing is followed by polishing, a phase which is comparable to the dehairing phase. The main difference is the lower amount of extruded faecal contamination. We see from the bar chart that this lower load yields a small decrease in numbers for MS1 and MS3 and a small increase in numbers of MS2 and MS4. For all MSs the prevalence does increase slightly.

The following phase is evisceration. A striking feature is the increase of the prevalence to 100%. This is because of the implementation of house flora, contaminating every carcass with a small amount of *Salmonella*. Also, numbers of *Salmonella* increase and particularly for MS1, MS3 and MS4. This is the result of the faecal contamination resulting from puncturing of the gut. Although this is a rare event, the resulting contamination is very high.

During splitting, the contamination is divided randomly over two half-carcasses. This will cause average contamination to go down, but also lowers the prevalence slightly, since some half carcasses originating from a slightly contaminated carcass will end up with zero *Salmonella*. The process of trimming doesn't seem to have much effect. Since trimming basically consists of removal of detected faecal contamination (from polishing or evisceration), it must be the case that either the contamination is not detected, or there is not much contamination on average.

Finally, blast chilling, which is an inactivation step much like singeing, brings down both prevalence and numbers by a large amount. The results from the large slaughterhouse can be compared to results from other QMRAs.

Consider Figure 9.7, taken from Bolton *et al.* 2002. The figure shows total aerobic counts, but noting that we only compare relative amounts we can state that the results in the figure are consistent with ours, with regards to: a decrease due to combined scalding and dehairing, a decrease due to singeing and little effect from evisceration. We do not capture the remarkable increase during power hosing, it is not a part of our model, and not comparable to the polishing stage.

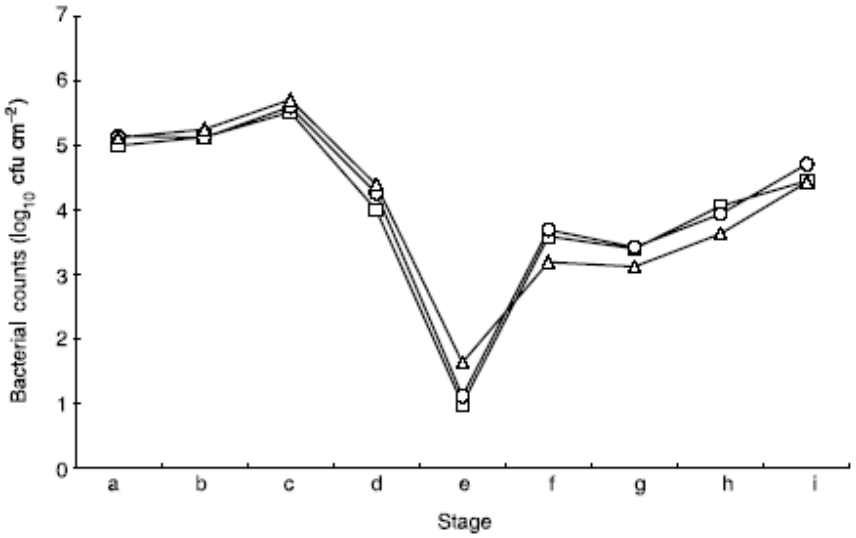

Figure 9.7 Total aerobic counts (log_{10} cfu cm^{-2}) on pork carcasses at the ham (□), belly (○) and neck (△) (a) on the farm and after (b) washing; (c) bleeding; (d) scalding-dehairing; (e) singeing; (f) power-hosing; (g) evisceration; (h) washing and (i) chilling (Bolton, Pearce et al. 2002). The workers swabbed an area of 0.05 m^2 on each of the ham, belly and neck, combining all three swabs in a 100 ml volume of BPW. Total aerobic counts and *Salmonella* prevalence (RV broth, BG and MLCB agar) were used for sample analysis.

Furthermore, we present in Figure 9.8 results of (Pearce, Bolton et al. 2004).

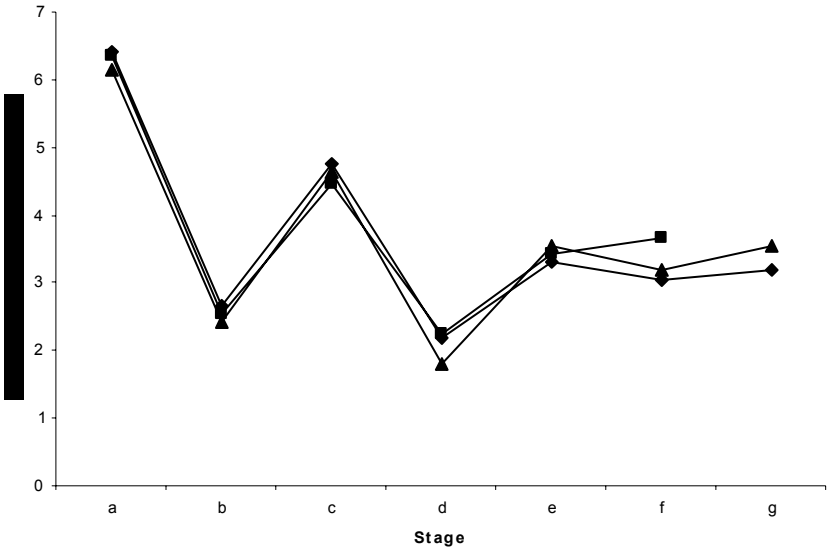

Figure 9.8 Total counts (log_{10} cfu cm^{-2}) on pork carcasses after (a) bleeding; (b) scalding; (c) dehairing; (d) singeing; (e) polishing; (f) evisceration; (g) chilling. After scalding, a 0.05 cm^2 area of the ham, belly and neck was swabbed. Afterwards each used swab was stomached individually in 100 ml MRD. The TACs, as enumerated on plate count agar. Taken from Richards and Dodd (2009), who adapted from Pearce, Bolton *et al.* (2004)

The correspondence with our model is even more striking here. We observe a marked decrease during scalding, increase from dehairing, decrease from singeing, increase from polishing and little effect from the remaining stages. A numerical comparison should not be attempted due to uncertainties and sampling methods.

As a final remark on the slaughterhouse model we mention that the modelling of house flora was based on little data, and mostly on expert opinion. As a result we are not confident that we have captured all house flora dynamics in sufficient detail. Yet, experts believe that it is an important factor within the slaughterhouse environment. Therefore we strongly advise further research into slaughterhouse house flora and biofilm formation on slaughter equipment.

9.5 Sensitivity analysis

The sensitivity analysis methodology is described in Chapter 5. For the slaughterhouse module we use the number of *Salmonella* on the half carcass at the point of chilling as the response variable. The results for the four MSs are shown in Figure 9.9 to Figure 9.12. There are many variables to consider in the Slaughterhouse module, so we label the variables using the parameter notation from the previous sections and also identify the stage at which the variable is used. It can be seen that the variation in the length of incision at belly opening is the most significant factor for MS1 and MS4, while for MS3 it is the body mass of the pig (used to determine the surface area of the carcass during belly opening) and for MS2 it is the time spent in the dehairing machine. Parameters associated with the scalding water are also relatively significant for many MSs, as are a number of α and β parameters (it may be useful to remind the reader here that the α parameters are the fraction of *Salmonella* moving from the environment to the carcass at a particular stage and

the *β* parameters are the fraction of *Salmonella* moving from the carcass to the environment). For MS2 it is the time the pigs spend in different stages that seems to be more important than parameters associated with the mechanics of the stages (e.g. temperature of scalding water).

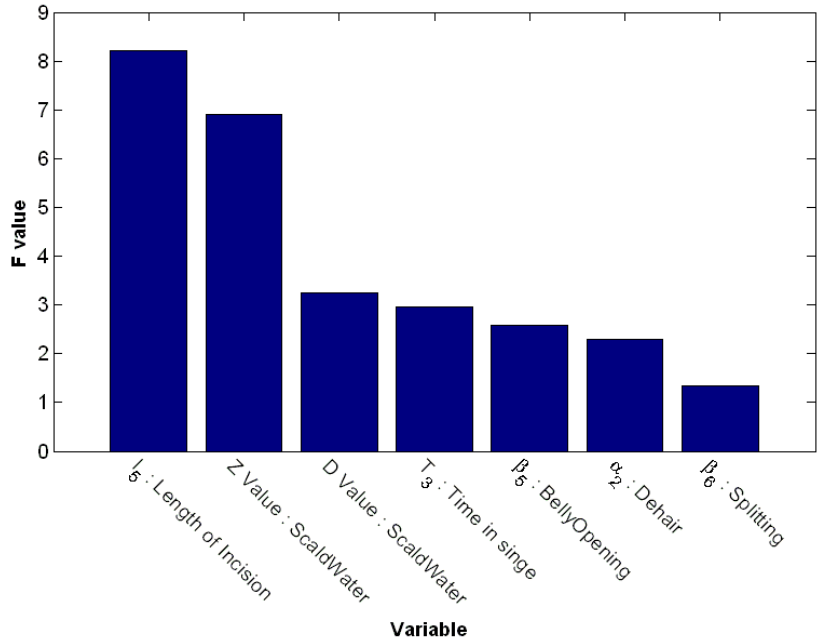

Figure 9.9: Slaughterhouse sensitivity analysis for MS1

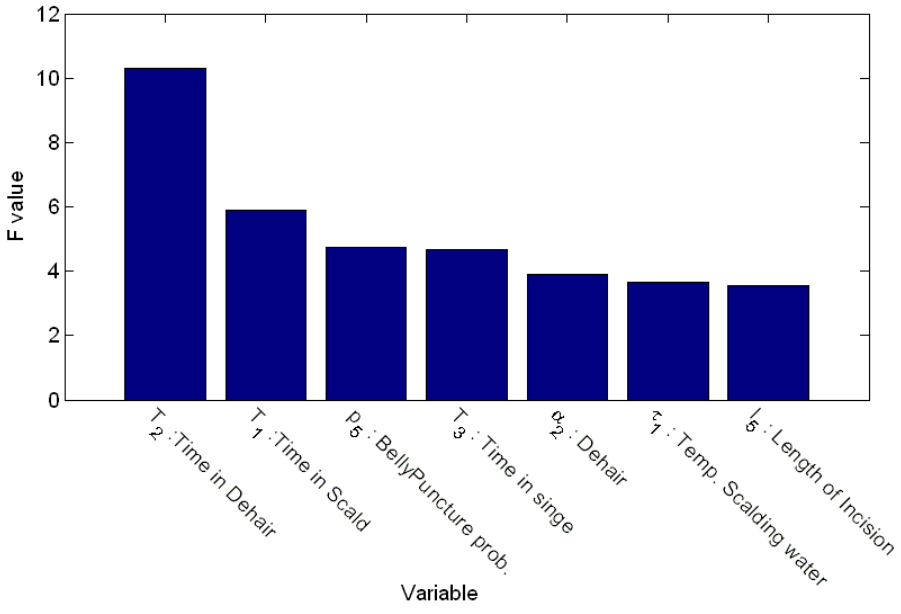

Figure 9.10: Slaughterhouse sensitivity analysis for MS2

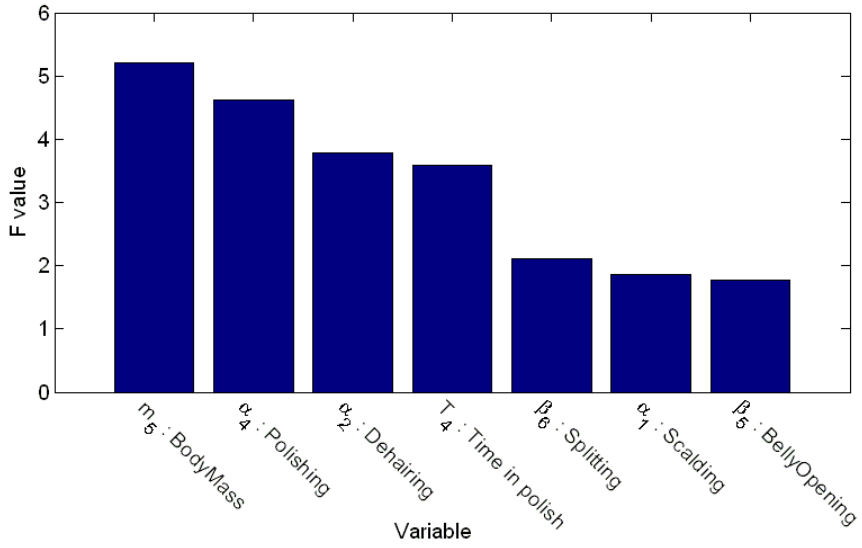

Figure 9.11: Slaughterhouse sensitivity analysis for MS3

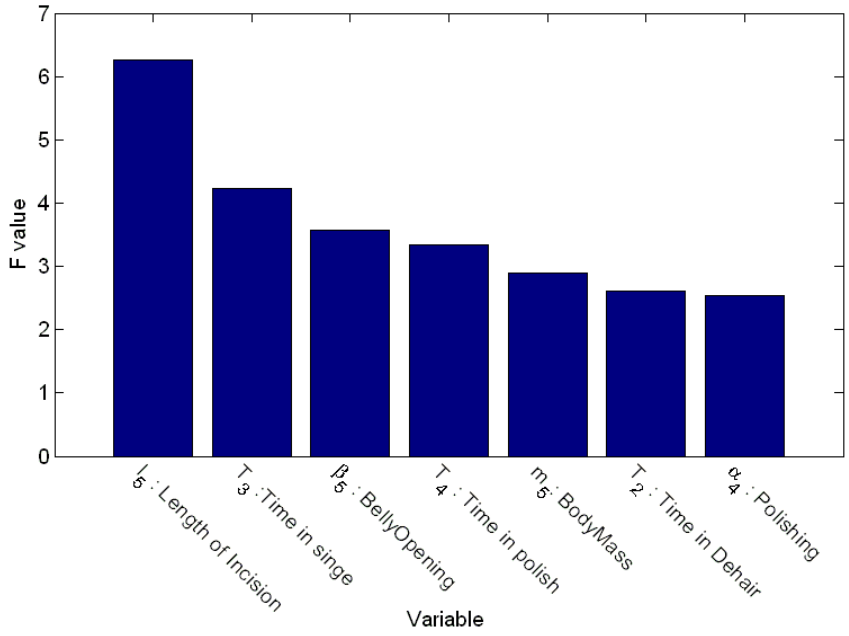

Figure 9.12: Slaughterhouse sensitivity analysis for MS4

9.6 Modelling the Small Slaughterhouse

The slaughterhouse model established in the previous sections described large continuous facilities. Most MSs also have smaller slaughterhouses in operation. These small slaughterhouses handle a much smaller number of pigs on a daily basis, use less dedicated

machinery and do not have a continuous slaughter line. This category in itself is ill-defined, encompassing the range from floor slaughter of only a few pigs per day to semi-automated slaughterhouses. Accounting for this range is not feasible within the setting of this project and we have chosen to model one specific setup. To this end we have observed the slaughter process in a small Dutch slaughterhouse. We will describe the process and the modelling thereof using the same template as used for the large slaughterhouse: description, solution and parameter estimation.

9.6.1 Small slaughterhouse phases

Before describing each phase in detail, we will first present a short overview. The process is based on the slaughter procedures as implemented at 'Slagerij Kenkhuis'[21]. The floor plan of the facility is as sketched in Figure 9.13.

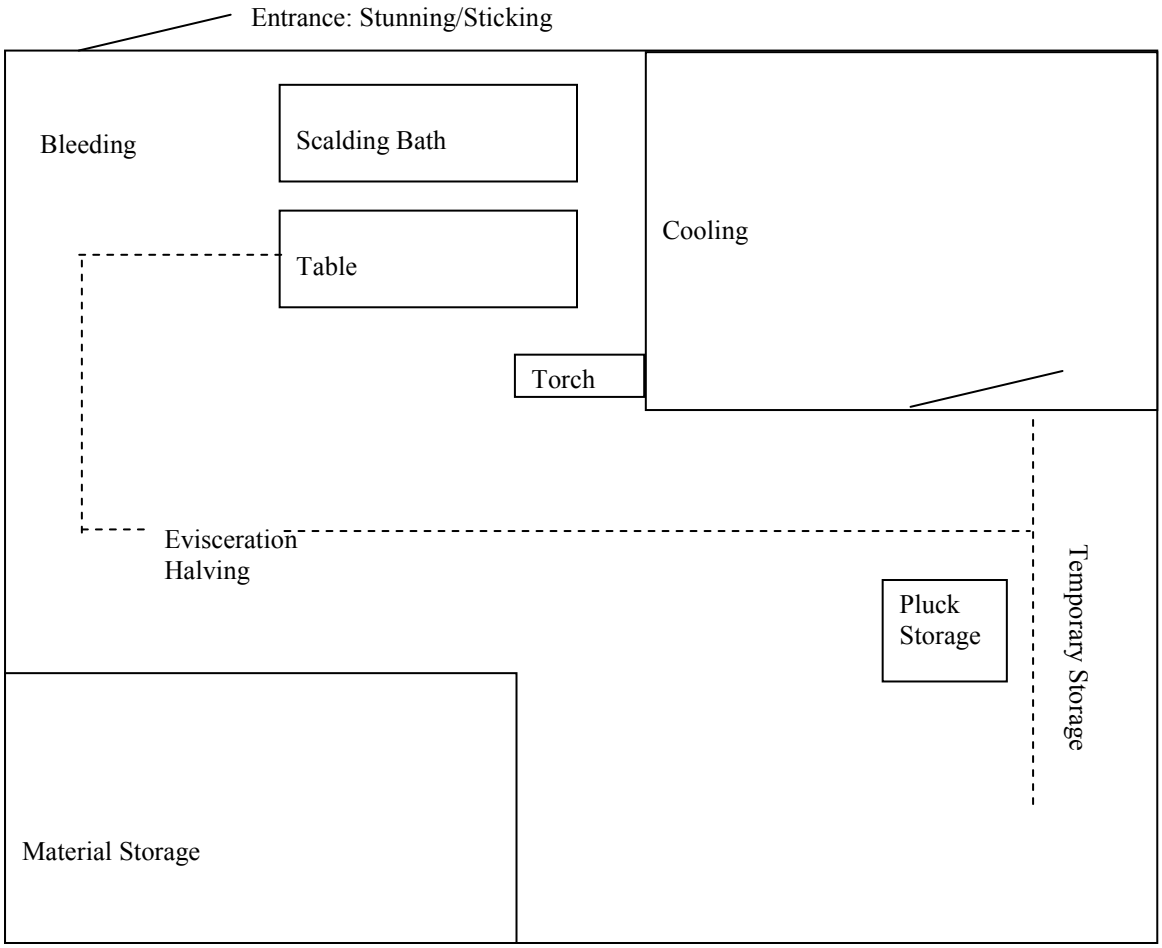

Figure 9.13: Example floor plan of a small slaughterhouse.

23..1.1 [21] *We are grateful to the people at 'Slagerij Kenkhuis', Vriezenveen, the Netherlands for generously allowing us to visit the abattoir and for answering our questions regarding the details of the slaughter process.*

The process starts outside of the building, at the entrance, where pigs are stunned. Pigs are kept in a little stable before slaughter. Next, the pigs are dragged inside, where they are bled, hauled up and inserted into the scalding bath. There is only a small amount of time for bleeding: the time that the previous pig spends in the scalding bath.

The scalding bath contains only one pig at a time. Inside the scalding bath, there are rotating rubber flaps. In a sense, it is a scalding bath and a dehairing machine in one. After four minutes, the scalding tank is opened, the pig is automatically lifted to the level of the table next to the scalding bath and two workers drag the pig onto the table.

When the pig is on the table, the claws and the ear pits are removed. Also, visible remaining hair and dirt is scraped off using knives.

Next, one side of the pig is singed using a hand held torch. The pig is then turned to its other side and the other side is singed. This is followed by loosening the rectum, to facilitate the removal of the gut later.

The hind legs of the pig are now incised. Using hooks through the incisions the pig is hauled up to a rail suspended from the ceiling, until it hangs head down. The pig is pushed towards the third worker, who proceeds with belly opening, evisceration and splitting.

Firstly the belly is opened. Then the gut is removed and kept apart (for later inspection). Then the pluck is removed and kept at a special storage area, some parts are also used for consumption (e.g. liver). The next step is the halving of the carcass. This is done manually using a large knife-shaped axe. Also the head is halved. Finally, some final scraping and cutting is performed and the carcasses are moved to temporary storage. This storage is at ambient temperature and takes from a couple of minutes up to a few hours, until meat inspection. Thereafter the meat is stored in a cooled room (4 °C).

9.6.2 Scalding

Problem definition

The scalding procedure is similar to the scalding process at the large slaughterhouse. The main differences are in the higher temperature of the scalding water, the number of pigs in the tank and a longer time spent in the scalding bath.

The integrated rubber flaps, acting as a dehairing mechanism, do not have the same potential of getting contaminated as the dehairing machine in the large slaughter line. This is because of the high temperature of the water in which the flaps are submerged. The dehairing flaps do have an effect on the contamination on the pig skin, which will be explored in Section 9.6.3.

Table 9.20 provides quantities in the small slaughterhouse. Note that we have mostly used the same symbols as used in the large slaughterhouse. Some parameters are described by the same formula and are not repeated here.

The equations describing the dynamics are the same as those of the large slaughterhouse,

$$N_{k,1}(t_k) = (1-\beta_1)N_{k,0}, \tag{9.113}$$

$$N_k'(t) = -\tau_1 N_k(t) + \alpha_{1,k} W(t), \text{ for } t \in (t_k, t_{k+1}), \tag{9.114}$$

$$N_k'(t) = 0, \text{ for } t \notin [t_k, t_{k+1}], \quad (9.115)$$

$$W'(t) = -\gamma_1(1-\alpha_{1,k})W(t), \text{ for } t \in (t_k, t_{k+1}), \quad (9.116)$$

$$W(t_k) = \beta N_k(t_k) + \lim_{t \to t_k} W(t), \quad (9.117)$$

$$W(0) = 0. \quad (9.118)$$

Table 9.20: Quantities used in the scalding phase (small slaughterhouse).

Quantity	Domain	Unit	Description
$N_{1,k}(t)$	$\mathbb{R}^+$	cfu	Number of *Salmonella* on P_k, at time $t \geq 0$
$W_1(t)$	$\mathbb{R}^+$	cfu	Number of *Salmonella* in the environment, at time $t \geq 0$.
n	$\mathbb{N}$	-	Total number of pigs in the current batch
$\mathcal{T}_1$	$\mathbb{R}^+$	ºC	Temperature of the scalding water
T_1	$\mathbb{R}^+$	min	Time spent in the scalding bath

Solution and implementation

We may follow the exact same procedure as outlined in Section 9.2.6. The resulting equations are,

$$W^-(t_{k+1}) = [\beta_1(1-\beta_1)N_{k,0} + W^-(t_k)]e^{-\gamma(1-\alpha_{1,k})T_1}. \quad (9.119)$$

$$N_k(t_{k+1}) = e^{-\tau_1 T_1}[(1-\beta_1)N_{0,k} - \frac{\alpha_{1,k}}{\eta}W^-(t_{k+1})(1-e^{\eta T_1})], \quad (9.120)$$

with $\eta = \gamma_1(1-\alpha_{1,k}) - \tau_1$.

Parameter estimation

The scalding tank was filled with water of 90-95 ºC (expert opinion of slaughterhouse personnel). The tank also has a heating system for keeping the water at the right temperature. However, the tank only contains 150 litres of water and a pig entering the tank can easily lower the temperature temporarily. Therefore we choose the minimum temperature somewhat lower. We consider 90°C the most likely, 95°C the maximum and 85°C the minimum and fit a beta pert distribution,

$$\mathcal{T}_1 = \Re(BP(85, 90, 95)). \quad (9.121)$$

The attachment and detachment parameters are similar to those of the large slaughterhouse, but now using the temperatures defined above,

$$\beta_1 = 0.02, \quad (9.122)$$

$$\alpha_k(\mathcal{T}_1) = \max(0, 10^{-5}\Re(N(\mu(\mathcal{T}_1), \sigma^2(\mathcal{T}_1)), k)). \quad (9.123)$$

Pathogen inactivation rates on the pig and in the water are adopted from equations (9.35), (9.36) and (9.37),

$$\tau_1(\mathcal{T}_1) = 0.92 * 10^{\frac{60-\mathcal{T}_1}{5.4}}, \quad (9.124)$$

$$\gamma_1(\mathcal{T}_1) = \frac{\ln(10)}{D_{60}} 10^{\frac{60-\mathcal{T}}{Z}}, \quad (9.125)$$

with the D-values and Z-values as in Equation (9.36).

The time spent in the scalding bath T_1, was measured on location to be around 4 minutes,

$$T_1 = 4. \quad (9.126)$$

9.6.3 Flaming/trimming

Problem definition

As described before the acts of flaming and trimming are performed alternately. First the pig is flamed, after which visible contamination is removed (trimming). Next, the pig is turned and the other side is singed and trimmed. Part of the model consists of the singeing and trimming phases developed before (Sections 9.2.7 and 9.2.11). Additionally, we model the contamination of the table and cross-contamination to the pig after turning. Thus, we model the following,

1. Assume even distribution of *Salmonella* between two sides of the pig
2. Singeing of side A
3. Dressing of side A
4. Cross-contamination between table and side B
5. Singeing of side B
6. Dressing of side B
7. Cross-contamination between table and side A

If needed, superscripts are added to the quantities to indicate to which step and side they refer.

The quantities for the flaming/dressing phase of the small slaughterhouse are described in Table 9.21

Table 9.21: Quantities used in the flaming/dressing phase of the small slaughterhouse.

Quantity	Domain	Unit	Description
$N_{2,k}^{A,s}(t_k)$	$\mathbb{R}^+$	cfu	Number of *Salmonella* on side A of pig k at phase 2, during step s, at time t_k.
$W_{2,k}$	$\mathbb{R}^+$	cfu	Number of *Salmonella* on the table.
ϵ_2	$\mathbb{R}^+$	cfu/min	Inactivation parameter at phase 2, for step 2 and 5.
$\mathsf{T}_{2,k}$	$\mathbb{R}^+$	min	Time spent singeing at phase 2, pig k.
J_2	$\mathbb{R}^+$	g	Amount of faecal material detectable by visual inspection.
$F_{2,k}$	$\mathbb{N}$	-	Number of dressing actions performed.
$A_{2,k}$	$\mathbb{R}^+$	g	Amount of faeces extruded
$B_{2,k}$	$\mathbb{R}^+$	cfu	Number of *Salmonella* extruded by pig P_k
$\Omega_{2,k}$	$\{0,1\}$	-	Status of the pig, infected (1), or not (0).
$C_{2,k}$	$\mathbb{R}^+$	cfu/g	Concentration of *Salmonella* in faeces[22]
t_{TP}	$[0,1]$	-	Transfer parameter from table to pig
t_{PT}	$[0,1]$	-	Transfer parameter from pig to table

As usual, we define t_k such that it is the time at which pig k starts the phase. The time t_{k+1} is the time at which pig k leaves the phase. We will also need to refer to some times halfway during this phase, e.g. $t_k + \mathsf{T}_{2,k}^2$ is the time just after pig k had side A singed.

We begin with step one, which is modelled as,

$$N_{2,k}^{A,1}(t_k) = N_{1,k}(t_{k+1})/2, \tag{9.127}$$

$$N_{2,k}^{B,1}(t_k) = N_{1,k}(t_{k+1})/2. \tag{9.128}$$

This is followed by dressing, removal of visible faecal contamination. Previously, this was modelled by collecting the faecal material extruded during polishing and spilled during belly opening and assuming the contamination was present in one spot. Then, a threshold for detection was used to determine whether the contamination was removed. Since in the small slaughterhouse these phases are not present we have to model this in a different way.

Since the scalding bath also acts as a dehairing machine, we assume an amount of faecal material to have leaked from the pig during this phase. During dehairing in the large slaughterhouse, this material would be directly deposited onto the pig. This is not realistic in the present case, the faecal material will be deposited on the pig via the water and the dehairing flaps. Estimating the amount of material remaining in the water, attaching via the water, or attaching via the flaps is unfeasible. However, we have observed the number of cleaning actions performed by the personnel. Also, we have an estimate of the amount of faecal material detected by visual inspection.

We propose an algorithm to model the routes 'water to pig', 'water to flaps', 'flaps to pig', 'pig to water', etc. The algorithm aims to construct a number of patches of faecal contamination in such a way that the predicted number of visible spots and a number of undetectable spots result. This is obtained by starting with a large number (100) of small patches of 10mg (summing to 1g), randomly combining them until the desired number of detectable patches

[22] These are the same quantities as those listed previously and are considered as an input from the Farm and Transport & Lairage model.

is obtained. The random recombination is meant to reflect the mixing and transfer occurring in the scalding bath.

The result of simulating the above algorithm for a large number of times is graphically depicted in Figure 9.14. The algorithm was run until 2,3, or 4 patches were present with more than 0.1 gram of faecal material. Then, the percentage of remaining faecal contamination, which is not removed, is plotted in a histogram. For example, when 2 patches of contamination were found and removed, it is most likely that approximately 75% of 1g remains on the carcass.

Surprisingly, the histograms are fitted very well by an extreme value distribution. We do not think this is a coincidence and suspect some deeper mathematics behind this observation. For now, we will work with the fitted parameters. Firstly, let F_k be the number of 'faecal contamination patches' found on pig k. From our observations we found that usually 2, 3, or 4 dressing actions took place:

$$F_k = \Re(DU([2,3,4]), k). \qquad (9.129)$$

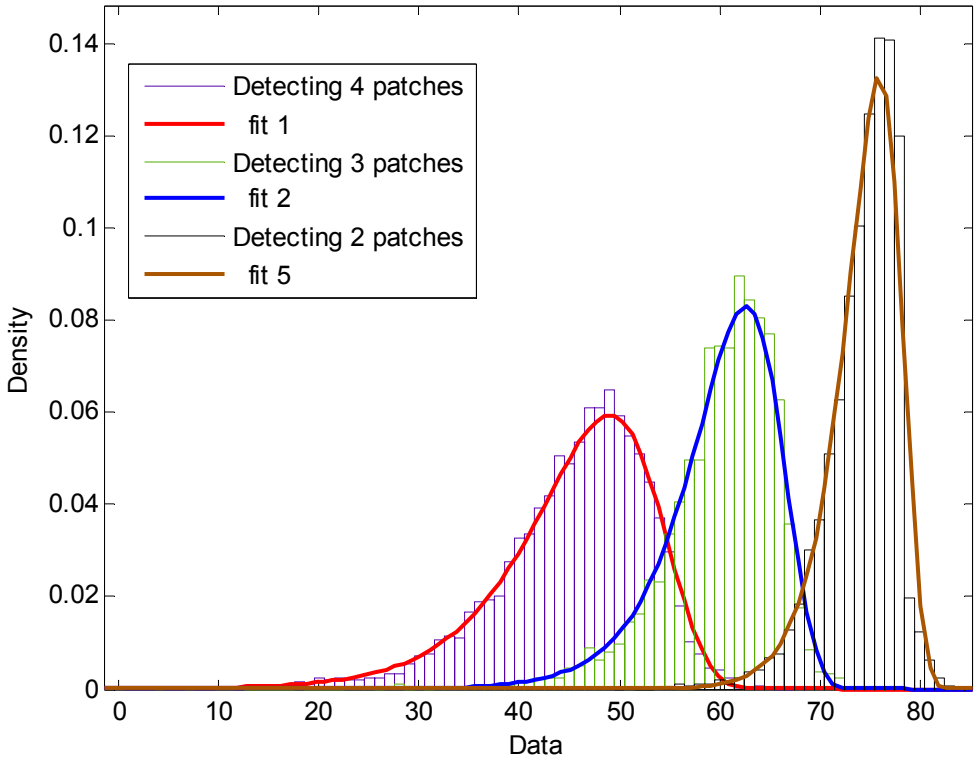

Figure 9.14: Percentage of remaining faecal contamination (out of 1g) after trimming, given a number of spots (of at least 0.1g) detected by personnel.

According to the result, we sample a percentage E_k from the extreme value distribution,

$$E_k = \Re(EV(\mu, \sigma), k), \tag{9.130}$$

using the fitted parameters given in Table 9.22 below.

The resulting non-detected material,

$$B_{2,k} = C_{2,k}\Omega_{2,k}A_{2,k}E_k/100, \tag{9.131}$$

is added to the skin contamination of side A,

$$N_{2,k}^{A,2} = N_{2,k}^{A,1} + B_{2,k}, \tag{9.132}$$

$$N_{2,k}^{B,2} = N_{2,k}^{B,1}. \tag{9.133}$$

Dressing is followed by flaming, modelled as in Section 9.2.7,

$$N_{2,k}^{A,3} = e^{-\epsilon_2 \mathsf{T}_{2,k}} N_{2,k}^{A,2}. \tag{9.134}$$

$$N_{2,k}^{B,3} = N_{2,k}^{B,2} \tag{9.135}$$

While singeing and dressing take place on side A of the pig, side B of the pig potentially cross-contaminates with the steel table.

- Pig k side B put on table, side A is flamed and singed, side B potentially cross-contaminates with the table, using the amount $W_2(t_k)$. Resulting in a new contamination $W_2(t_{k+1/2})$.

- Pig k is turned. Side A cross-contaminates with the table, using the amount $W_2(t_{k+1/2})$, resulting in a new contamination $W_2(t_{k+1})$. Side B is singed and trimmed.

Table 9.22: Parameters of the extreme value distribution, depending on the number of dressing actions.

F_k	**Mean (μ)**	**Variance (σ^2)**
2	74.3	12.6
3	60.0	32.3
4	45.4	63.1

During cross-contamination, a fraction t_{PT} is moved from the pig to the table and simultaneously a fraction t_{TP} is transferred from the table to the pig. This is conveniently expressed in matrix-vector notation as

$$\begin{bmatrix} N_{2,k}^{A,4} \\ N_{2,k}^{B,4} \\ W_{2,k+1/2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1-t_{PT} & t_{TP} \\ 0 & t_{PT} & 1-t_{TP} \end{bmatrix} \begin{bmatrix} N_{2,k}^{A,3} \\ N_{2,k}^{B,3} \\ W_{2,k} \end{bmatrix}. \tag{9.136}$$

After turning of the pig, cross contamination of side A with the table is given by

$$\begin{bmatrix} N_{2,k}^{A,5} \\ N_{2,k}^{B,5} \\ W_{2,k+1} \end{bmatrix} = \begin{bmatrix} 1-t_{PT} & 0 & t_{TP} \\ 0 & 1 & 0 \\ t_{PT} & 0 & 1-t_{TP} \end{bmatrix} \begin{bmatrix} N_{2,k}^{A,4} \\ N_{2,k}^{B,4} \\ W_{2,k+1/2} \end{bmatrix}. \quad (9.137)$$

Then, side B is trimmed and flamed,

$$N_{2,k}^{A,6} = N_{2,k}^{A,5}. \quad (9.138)$$

$$N_{2,k}^{B,6} = N_{2,k}^{B,5} + B_{2,2k}, \quad (9.139)$$

$$N_{2,k}^{A,7} = N_{2,k}^{A,6}, \quad (9.140)$$

$$N_{2,k}^{B,7} = e^{-\epsilon_2 \mathsf{T}_{2,k}} N_{2,k}^{B,6}. \quad (9.141)$$

Here we used

$$B_{2,2k} = C_{2,k} I_{2,k} E_{2k} / 100. \quad (9.142)$$

Note that E_{2k} is used, we don't want the same E_k as used in (9.132).

Solution and implementation

The equations (9.127 - 9.135) are trivially implemented. The cross-contamination equations can be written in full form as

$$N_{2,k}^{B,4} = (1 - t_{PT}) N_{2,k}^{B,3} + t_{TP} W_{2,k}, \quad (9.143)$$

$$N_{2,k}^{A,4} = N_{2,k}^{A,3}, \quad (9.144)$$

$$N_{2,k}^{A,5} = (1 - t_{PT}) N_{2,k}^{A,4} + t_{TP} W_{2,k+1/2}, \quad (9.145)$$

$$N_{2,k}^{B,5} = N_{2,k}^{B,4}, \quad (9.146)$$

$$W_{2,k+1/2} = t_{PT} N_{2,k}^{B,3} + (1 - t_{TP}) W_{2,k}, \quad (9.147)$$

$$W_{2,k+1} = t_{TP} N_{2,k}^{A,4} + (1 - t_{TP}) W_{2,k+1/2}. \quad (9.148)$$

From this system we can eliminate $W_{2,k+1/2}$, giving,

$$W_{2,k+1} = t_{TP} N_{2,k}^{A,3} + (1 - t_{TP}) t_{PT} N_{2,k}^{B,3} + (1 - t_{TP})^2 W_{2,k}. \quad (9.149)$$

Since the contamination from step 3 is known, we can use this formula to find all $W_{2,k}$, as a first step. Then we use the first equation and combine the second and third to obtain

$$N_2^{B,5} = (1 - t_{PT}) N_2^{B,4} + t_{TP} W_2, \quad (9.150)$$

$$N_2^{A,5} = (1 - t_{PT})N_2^{A,4} + t_{TP}t_{PT}N_2^{B,3} + t_{TP}(1 - t_{TP})W_2. \quad (9.151)$$

We've removed the indices k, to stress the fact that both equations can be solved in vector-form. Finally, equations (9.138 - 9.141) are again implemented in the model.

Parameter estimation

The time, in minutes, spent under the handheld flamer was observed to be,

$$\mathsf{T}_{2,k} = \Re(U(1,3)). \quad (9.152)$$

The inactivation parameter ϵ_2, is hard to estimate. In the large slaughterhouse the entire pig carcass is singed for some time. In the small slaughterhouse a small handheld torch is used, that may be less hot. Furthermore we have to take into account that only visible remaining hairs are flamed manually, and not all parts of the carcass are heated. We estimate the inactivation parameter to be 10 times lower as compared to the singeing inactivation parameter in the large slaughterhouse.

$$\epsilon_2 = 1.18. \quad (9.153)$$

This estimate is however highly uncertain. Next, we turn to the amount of visible faecal material J_2. As in Section 9.2.11 we use Evers *et al.* 2008. In this paper it was reported that an amount greater than 0.003g=3mg would be detected. Since it is unlikely that patches of contamination are that small (they were clearly visible) we used a value of $J_2 = 0.1$ g (approximately 10 times larger) for the weight of a removed patch.

The number of dressing actions was observed at the small slaughterhouse, usually between 2 and 4, we take it to be discretely uniformly distributed (see equation (9.129). For lack of better data we use for the amount of *Salmonella* extruded $B_{2,k}$ the value was used for the polishing phase in the large slaughterhouse, $B_{2,k} = 1$g.

Finally, we turn to the transfer parameters t_{TP} and t_{PT}. Again, these are unknown for our specific situation. As a substitute, we use the transfer rate 0.032 for pork cut to a cutting board from the consumer phase model (Chapter 10). The rate for cutting board to pork cut is unknown, and we set

$$t_{TP} = t_{PT} = 0.032. \quad (9.154)$$

9.6.4 Belly opening

Problem Definition

The manual belly opening process is, from a modelling point of view, very similar to the belly opening process by machine, the difference being the absence of a sterilising step. Therefore, cross-contamination plays an important role. The quantities used in the belly opening process in the small slaughterhouse are provided in Table 9.23.

As in Section 9.2.9 we define $d_{3,k} = \beta_{3,k} l_{3,k} b_3 / O_{3,k}$ and $B_{3,k} = G_{5,k}\Omega_{5,k}C_{5,k}A_{5,k}$. The equations describing cross-contamination with the knife and spilling of faecal material if the gut is punctured are then

$$N_{3,k} = (1 - d_{3,k})N_{2,k} + \alpha_{3,k}W_{3,k-1} + (1 - \delta_3)B_{3,k}, \quad (9.155)$$

$$W_{3,k} = d_{3,k}N_{2,k} + (1 - \alpha_{3,k})W_{3,k-1} + \delta_3 B_{3,k}. \quad (9.156)$$

The status of the gut is Bernoulli distributed according to the probability of puncturing the gut,

$$G_{3,k} = \Re(B(1, p_k), k). \quad (9.157)$$

Table 9.23: Quantities used in the belly opening process in the small slaughterhouse.

Quantity	Domain	Unit	Description
$N_{3,k}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the k^{th} pig or carcass P_k, in phase 3.
$W_{3,k}$	$\mathbb{N}$	cfu	Numberof *Salmonella* on the knife in phase 3.
$\alpha_{3,k}$	$[0, 1]$	-	Fractionof *Salmonella* on the knife moving to the k^{th} pig
$\beta_{3,k}$	$[0, 1]$	-	Fraction of *Salmonella* on the k^{th} pig moving to the knife
δ_3	$[0, 1]$	-	Fraction of *Salmonella* moving from spilled faecal material to the knife.
l_k	$\mathbb{R}^+$	cm	Length of the incision
b	$\mathbb{R}^+$	cm	Width of the incision
$O_{3,k}$	$\mathbb{R}^+$	cm^2	Surface area of the pig
$A_{3,k}$	$\mathbb{R}^+$	g	Amount of faecal material spilling from the gut
p_k	$[0, 1]$	-	Probabiltyof puncturing the gut
$G_{3,k}$	$\{0, 1\}$	-	Status of the gut, punctured (1), or not punctured (0)
$B_{3,k}$	$\mathbb{R}^+$	cfu	Number of *Salmonella* spilling from the gut
$C_{3,k}$	$\mathbb{R}^+$	cfu/g	Concentration of *Salmonella* infection

Solution and implementation

Since all parameters, $N_{2,k}$ and $B_{3,k}$ are known, the equation (9.156) can be iteratively solved, starting with $W_{3,0} = 0$. Then, quantities in (9.155) are known for all k, and N_3 (the vector containing all $N_{3,k}$) can be calculated in one step:

$$N_3 = (1 - d_3)N_2 + \alpha_3 W_3 + (1 - \delta_3)B_3. \quad (9.158)$$

Parameter estimation

Puncturing of the gut

The concentration of *Salmonella* is obtained from the farm phase. The amount of faecal material spilled from the gut is taken to be the same amount as obtained for the large slaughterhouse (equation (9.92),

$$A_{3,k} = \Re(U(6.6, 20.3), k). \quad (9.159)$$

The status of the gut (i.e. punctured or not) is Bernoulli distributed with parameter p_k. We have an expert opinion from the slaughterhouse personnel, who indicated a failure rate of about 1 in 2000. This is an expert opinion from just one small slaughterhouse, in reality this parameter will likely vary between slaughterhouses, due to e.g. the skill level of the personnel. We have no information on how this parameter might vary. However, this probability was assumed to be uniformly distributed between 0.012 and 0.02 with a mean of 0.015 for the large slaughterhouse (equation (9.90). We will copy the information that there is a factor of 1/3 between the upper/lower bounds and the mean. Combined with a mean of 1/2000 this yields

$$p_k = \Re(U(1/3000, 2/3000), k). \quad (9.160)$$

Transfer parameters

The transfer parameters to be determined are, $\alpha_{3,k}$ (machine to pig), $\beta_{3,k}$ (pig to machine), and δ_3 (spilled faecal material to machine). The latter two parameters were already determined in Section 9.2.9,

$$\beta_{3,k} = \Re(BP(0, 13, 0.21, 0.29), k), \quad (9.161)$$

$$\delta_3 = 0.02. \quad (9.162)$$

The parameter $\alpha_{3,k}$ will, like $\beta_{3,k}$, be based on Kusumaningrum *et al.* 2003). We use the reported transmission rates from stainless steel to roasted chicken. The experiments were performed with, and without, exerted pressure. Since the cutting action of the knife is rather vigorous, we use the number for the 'exerted pressure' case. The standard deviation was subtracted from the mean to act as a lower bound. The upper bound would be greater than one and was truncated at one,

$$\alpha_{3,k} = \Re(BP(0.52, 0.94, 1), k). \quad (9.163)$$

Incision dimensions

The incision dimensions, $l_{3,k}$, b_3 and the surface area of the pig $O_{3,k}$, needed for scaling the transfer parameter from pig to machine, were previously determined in Section 9.2.9. Assuming incision lengths to be equal for large and small slaughterhouses we have,

$$b_3 = 0.1, \quad (9.164)$$

$$l_{3,k} = \Re(U(129, 146), k), \quad (9.165)$$

$$O_{3,k} = 734 m_k^{0.656}. \quad (9.166)$$

Carcass weight m_k per cluster is tabulated in Table 9.16.

9.6.5 Splitting

Problem definition

The splitting phase at the small slaughterhouse resembles the splitting stage at the large slaughterhouse (Section 9.2.10). The difference is that there is no sterilization, and thus the

transfer from knife to pig plays a role. The rest of the model is analogous to the large slaughterhouse model.

Table 9.24: Quantities used in the splitting phase in the small slaughterhouse.

Quantity	Domain	Unit	Description
$N_{4,k}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the k^{th} half carcass P_k, in phase 4
$H_{4,k}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the k^{th} half carcass in phase 4
$V_{4,k}$	$\mathbb{N}$	cfu	Number of *Salmonella* on the halving saw in phase 4
$\beta_{4,k}$	$[0,1]$	-	Transfer fraction from pig k to the saw
$\alpha_{4,k}$	$[0,1]$	-	Transfer fraction from the saw to pig k
$O_{4,k}$	$\mathbb{R}^+$	cm^2	Surface area of the pig
$l_{4,k}$	$\mathbb{R}^+$	cm	Length of the incision
b_4	$\mathbb{R}^+$	cm	Width of the incision

The equations are

$$N_{4,k} = (1 - L_{4,k})N_{3,k} + \alpha_{4,k}V_{4,k-1}, \quad (9.167)$$

$$V_{4,k} = L_{4,k}kN_{3,k} + (1 - \alpha_{4,k})V_{4,k-1}, \quad (9.168)$$

where $L_{4,k} = 2l_{4,k}b_4/O_{4,k}$. Next, we distribute the *Salmonella* load over the half-carcasses, using a binomial distribution. Let X_k be realized from the binomial $B(N,p)$ distribution with parameters $N = N_{4,k}$ and $p = 1/2$. Then the *Salmonella* load on each half-carcass is

$$H_{4,k} = X_k \text{ for } 1 \leq k \leq n_s, \quad (9.169)$$
$$H_{4,k} = N_{4,k-n_s} - X_{k-n_s} \text{ for } n_s + 1 \leq k \leq 2n_s. \quad (9.170)$$

Solution and implementation

As in the previous phase, the equations are implemented by first calculating $V_{4,k}$ for all k, and subsequently calculating N_4 in one step. Using the vector X_1 of binomial realisations, determination of the *Salmonella* load on the half carcasses is simply $H = [X_1; N_4 - X_1]$, where the square brackets signify concatenation.

Parameter estimation

For the determination of the parameters we refer to Section 9.2.10, since there is virtually no difference with the large slaughterhouse. The results are

$$\beta_{4,k} = \Re(BP(0.13, 0.21, 0.28), k) \quad (9.171)$$

$$l_{4,k} = \Re(BP(137.7, 152, 164.5), k), \quad (9.172)$$

$$b_4 = 0.1, \quad (9.173)$$

$$O_{3,k} = 734 m_k^{0.656}. \quad (9.174)$$

The value for $\alpha_{4,k}$ is copied from the section on belly opening (9.2.9),

$$\alpha_{4,k} = \Re(BP(0.52, 0.94, 1)). \quad (9.175)$$

9.7 Results for the Small Slaughterhouse

Figure 9.15 shows the results for the small slaughterhouse for MS1, MS2, MS3 and MS4, in the same way as in Section 9.4

At entry level, the prevalence and log numbers are rather low as compared to the numbers for the large slaughterhouse. This is because of the lower prevalence and contamination for the small farm (and subsequent transport and lairage), see Chapter 7 & 8. The first step is combined scalding and dehairing. The effect of dehairing manifests itself in the following stage (it yields the faecal contamination which is removed in trimming). Thus, we see the effect of scalding, which is similar to the large slaughterhouse: very little cross-contamination combined with a significant reduction.

Next is the combined flaming/trimming phase. This was a rather complicated model (see Section 9.6.3), and it is hard to anticipate the result of the combined flaming and trimming in the presence of cross-contamination. It turns out that cross-contamination does increase the prevalence. However, singeing and trimming do achieve quite some log-reductions in numbers. Within the belly opening phase, both cross-contamination and faecal contamination is accounted for by the model. However, we see little effect. Apparently, the probability of puncturing the gut is low enough to have little overall effect. Also there doesn't seem to be much effect of cross-contamination, which is probably due to the very low prevalence and *Salmonella* concentration at this point.

Finally, splitting reduces the prevalence and numbers slightly, which is to be expected from a partitioning step.

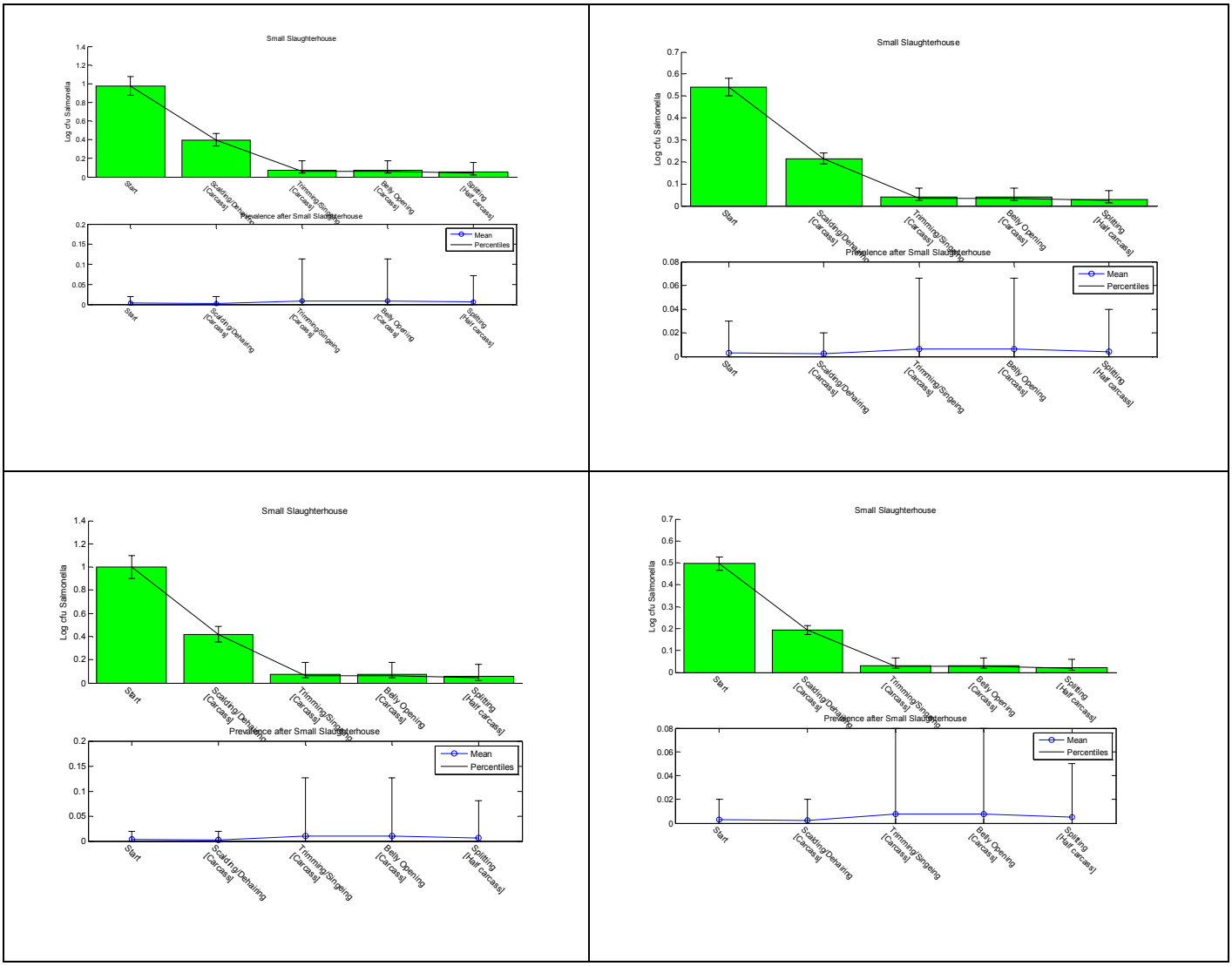

Figure 9.15: *Salmonella* numbers (top panel) and prevalence (bottom panel), during stages of the small slaughterhouse for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right).

9.8 The Cutting Plant

In our model, the cutting plant processes the half carcass and delivers the food product. Firstly, we combine output from small and large slaughterhouses into a single input to the cutting plant in 9.8.1. Then, a sample of half carcasses is cut into retail cuts (Section 9.8.2). Finally, each retail cut is split into meat used for pork cuts, minced meat or fermented sausage. This is arranged in such a way that we get a certain preset number of retail portions for each pork product. Those portions are the input for the next module, Preparation & Consumption, described in Chapter 10. Note that the cutting plant model will be a model describing variation in *Salmonella* densities over the portions. No cross-contamination between pork cuts is considered.

Transport from the slaughterhouse to the cutting plant, and from the cutting plant to retail, are not modelled. By regulation transport takes place at 4ºC, a temperature at which *Salmonella* numbers remain stable.

9.8.1 Combining production from small and large slaughterhouses

In each iteration of the model we run a large and a small slaughterhouse, according to their typical capacities. In reality, a percentage of production originates from small slaughterhouses and a percentage from large slaughterhouses. We will sample carcasses from the large and small slaughterhouses, in such a way as to obtain the proper fraction of pork from large and small slaughterhouses. We are interested in generating a number N of portions. The default in the current implementation of the model is *N=10,000*. Each portion is obtained from a half-carcass. The fraction f_1 of portions to be taken from half-carcasses from the large slaughterhouse and $f_2 = 1 - f_1$ of portions to be taken from the small slaughterhouse) can be found in Table 9.1. With probability f_1 we sample from the large slaughterhouse (or the small slaughterhouse otherwise). We assume the fraction f_1 to be the same for each pork product. This half-carcass is then used for further processing as described in the following section.

9.8.2 Retail cuts

Each meat product requires a number of 'cuts', these are important in the model, since cross-contamination might occur from the outside surface to the interior of the meat. We assume that initially only the exterior is potentially contaminated while the interior is sterile. Table 9.25 indicates how the carcass is divided and gives the main product obtained from it. For our purposes it is important to know the number of cuts required to obtain the final product. Cuts are not completely standardised but are generally done in the order described in Table 9.26.

Table 9.25: Naming of pig parts and associated main products

Part	Name	Main Products
1,2	Head	Soup, Stew
3	Loin/Rib	Pork Cuts
4,6	Loin	Pork cuts (rib roast, back ribs, cutlets)
7	Sirloin	Pork cut (Sirloin cut)
8	Tenderloin	Pork cut (Tenderloin cut)
5,9,10	Belly (or Side)	Spareribs, bacon, stir-fry meat
11	Shoulder/Blade	Ham
12	Leg	Ham, Schnitzel
13,14	Leg/Trotter	Soup/Stew

The top panel of Figure 9.16 shows an abstraction of the half carcass. The carcass is represented by a square and the cuts are perpendicular through the square, in the order as indicated in the rightmost column of Table 9.26. Cut number five, indicated schematically by a series of vertical lines, represents the cutting of the final cuts. This process takes place for each secondary cut.

Admittedly, this is an abstraction. In particular, note the positions of the legs (part 11) in both panels of Figure 9.. However, the abstractions capture the cutting of the carcass, dividing it

in several parts and allowing us to estimate the areas where cross-contamination may take place.

Table 9.26: Positioning and order of pork cuts.

Cut	**Between**	**and**	**Cut number in Figure 9.16**
At Slaughterhouse	1,2 13	3,4,5 14	1
Primal Cuts	4,5 6 7,8	11 9 10	2 2 2
Secondary Cuts (retail cuts)	3a 3b 3c 3a 4 3b 6	4 6 7,8 3b 6 3c 7,8	3 3 3 4 4 4 4
Tertiary cuts	Cutting each secondary cut		5

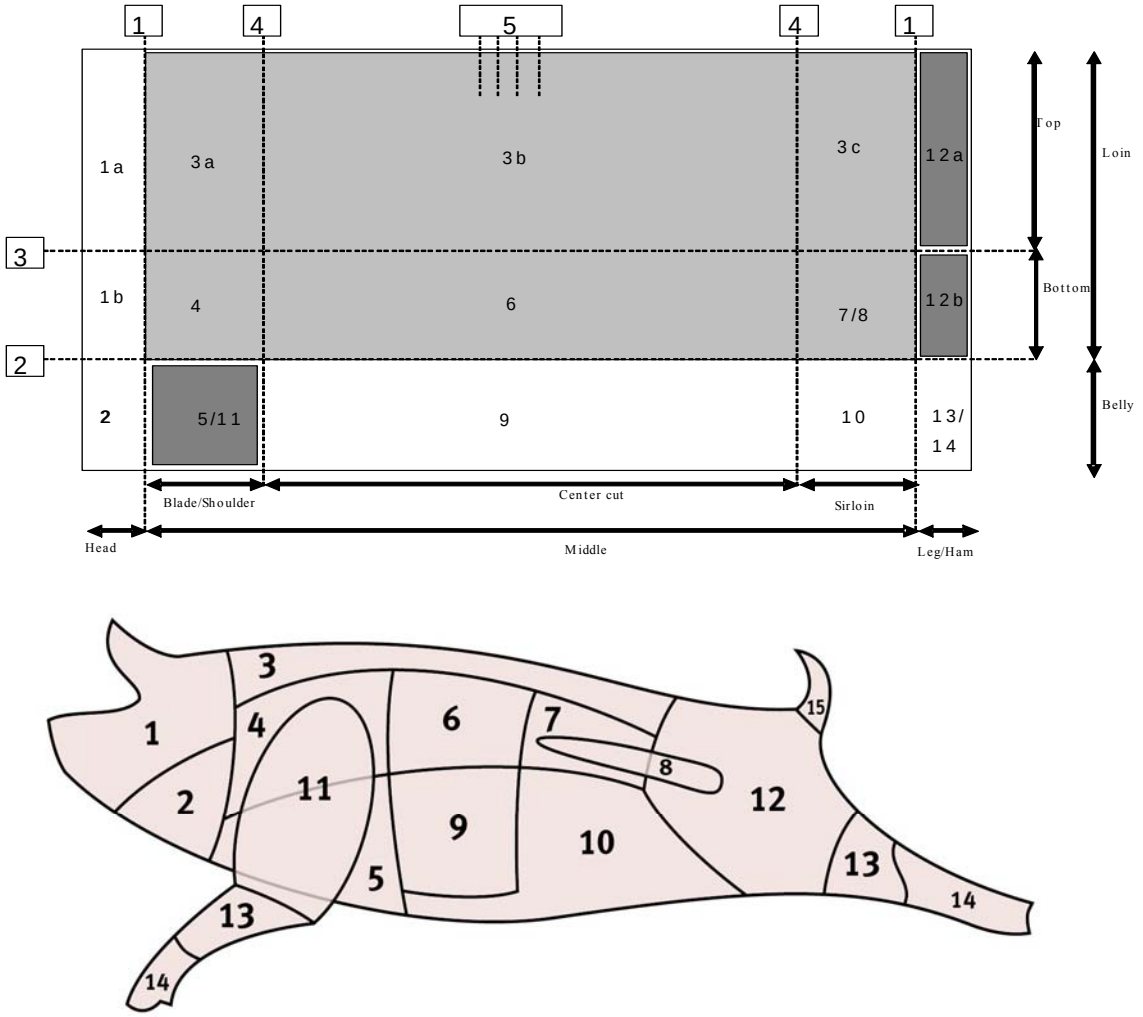

Figure 9.16: Origin of pork products. Light gray indicates meat used for pork. Dark gray indicates meat used for ham. The bottom panel shows the numbering of the pork cuts.

Minced meat is produced from virtually every part of the pig and leftovers from the cutting process. When sold, minced meat typically has a fat to meat ratio of 20 to 80. The head and legs may also produce minced meat, although this is only used as the basis for sausages.

In order to obtain a manageable cross-contamination model, we abstract the model a bit more, as in Figure 9.17. We re-number the cuts and label the resulting pieces of pork. The contamination is referred to by the label of the piece, subscripted by a direction. For example A_N is the number of *Salmonella* on the top of piece A, corresponding to a part of the loin. Width, height and depth (not shown) of the pieces are also subscripted. Later, we will also perform the final cuts, yielding the pork cuts as sold to the consumer. In this section we will work with relative *Salmonella* contamination, i.e. assuming a total of one *Salmonella*. Later this number can be scaled to the true value, but for the calculations it is only the relative distribution that counts.

For now we will work with the symbols, using their numerical values later. Also, we will use the reversed implication sign '$\Leftarrow$', which will mean 'becomes'. Thus, $A \Leftarrow A/2$, will mean that

A is halved. Define the total width $w = w_D + w_C + w_B + w_A$, the height (except part I) $h = h_D + h_H$ and the total circumference $t = 2(h + w + h_I)$.

Assume that the *Salmonella* is evenly distributed over the surface of the carcass, then the initial values of the *Salmonella* contamination is the initial concentration c_0 times the fractions in Table 9.27.

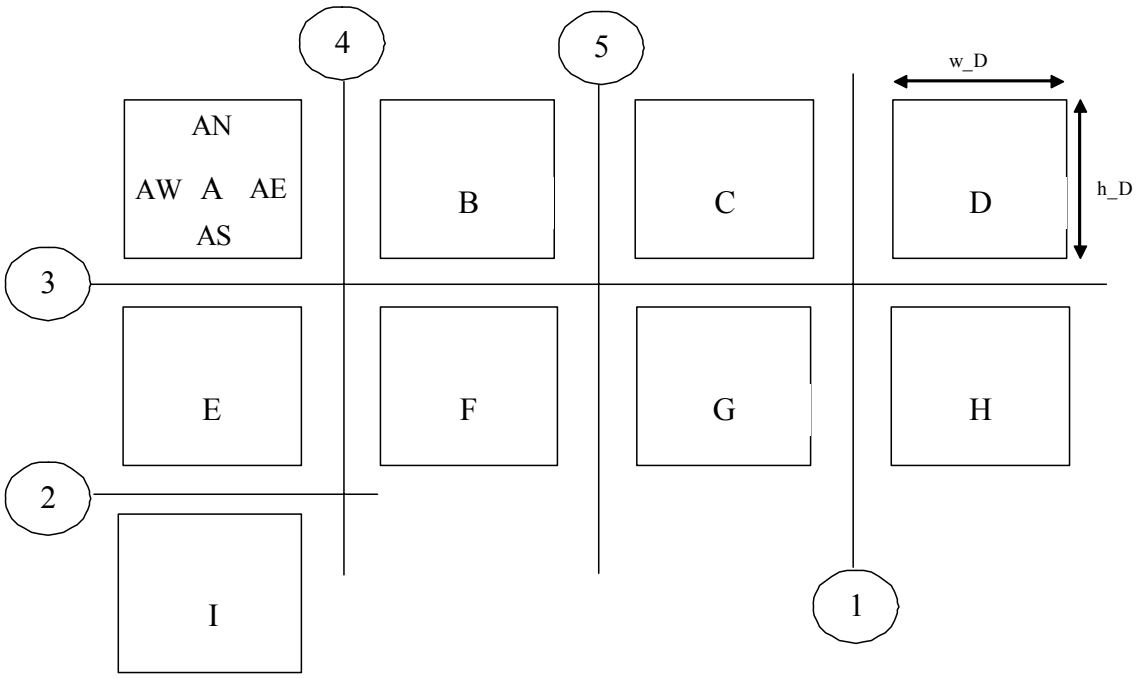

Figure 9.17: Schematic cutting process. The example indications AN, AS, AW, AE stand for A north, south, west, east. Abstracted from Figure 9.16.

Table 9.27: Initial contamination fractions on the exterior of pork cuts.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
N	w_A/t	w_B/t	w_C/t	w_D/t	0	0	0	0	0
W	h_A/t	0	0	0	h_E/t	0	0	0	h_I/t
E	0	0	0	h_A/t	0	0	0	h_E/t	h_I/t
S	0	0	0	0	0	w_B/t	w_C/t	w_D/t	w_A/t

Let us consider the first cut. Denote the width of the knife by k. The number of *Salmonella* picked up from C_N is $c_0(C_N/w_C)(k/2)$, and similar expressions for D_N, G_S and H_S. The removal of these amounts yields,

$$C_N \Leftarrow C_N - c_0(C_N/w_C)(k/2), \quad (9.176)$$

$$D_N \Leftarrow D_N - c_0(D_N/w_D)(k/2), \quad (9.177)$$

$$G_S \Leftarrow G_S - c_0(G_S/w_C)(k/2), \quad (9.178)$$

$$H_S \Leftarrow H_S - c_0(H_S/w_D)(k/2). \quad (9.179)$$

The total amount of *Salmonella* touched during cut one is

$$p_1 = \frac{c_0 k}{2}\left(\frac{C_N + G_S}{w_C} + \frac{D_N + H_S}{w_D}\right). \quad (9.180)$$

This amount is divided over the cutting line, according to the relative contribution of the sides of the pieces tot the total length of the cutting line. This leads to

$$C_E \Leftarrow C_E + p_1 \frac{h_A}{2(h_A + h_E)}, \quad (9.181)$$

$$D_W \Leftarrow D_W + p_1 \frac{h_A}{2(h_A + h_E)}, \quad (9.182)$$

$$G_E \Leftarrow G_E + p_1 \frac{h_E}{2(h_A + h_E)}, \quad (9.183)$$

$$H_W \Leftarrow H_W + p_1 \frac{h_E}{2(h_A + h_E)}. \quad (9.184)$$

Note that adding together the corrections yields zero, no *Salmonella* was lost or created. The remaining cuts are handled in a similar manner: remove *Salmonella* from the exterior and assign it to the cutting planes. These calculations are not shown, but are similar to the above equations.

Parameter estimation

From Hetzer *et al.* 1950 we find the approximate height (h), length (l) and width (w) of a half carcass as $(l, h, w) = (110, 40, 17)$. We obtained the relative heights and widths of the boxes (from Figure 9.17) from a poster showing pork cuts, ordered from KNS[23]. This yielded the values given in Table 9.28.

Results

The equations for *Salmonella* cross-contamination during the various cuts were solved using Mathematica[24]. The resulting relative contamination $T_A, \ldots, T_I$ per piece is given in Table 9.29.

9.8.3 Consumer Cuts

Whenever a consumer buys a portion of pork, it originates from one of the parts shown in Figure 9.16. However, since these parts are of unequal size, some parts have a larger probability of being the source of the consumer portion than others. This probability $P_A, \ldots, P_I$ is also the fraction of the total weight of the part and is determined from Figure 9.16 and Figure 9.28.

[23] Koninklijke Nederlandse Slagersorganisatie, Royal Dutch Butcher Association, http://www.knsnet.nl/

[24] Mathematica 7.0, Wolfram Research, Inc., Champaign, Illinois, 2008

Suppose we have picked a piece $X \in \{A, \ldots, I\}$ according to the probabilities above. From this piece consumer portions are produced. The following Sections will discuss the three pork types that are produced (in our model) from this piece: pork cuts, minced meat and fermented sausage.

Table 9.28: Relative widths and heights of pork cut parts of Figure.

w_A	w_B	w_C	w_D	h_A	h_E	h_I
7/34	8/34	8/34	11/34	2/7	2/7	3/7

Table 9.29: Relative contamination before consumer cuts.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
T	0.12	0.06	0.06	0.15	0.07	0.06	0.06	0.15	0.26

Table 9.30: Probabilities of choosing a pork cut.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
P	14/157	16/157	16/157	22/157	14/157	16/157	16/157	22/157	21/157

9.8.4 Pork cuts

The number of retail cuts taken from a piece is very uncertain. However, we realize that the main risk is having a consumer portion which is not taken from the interior. If we take the weight of the chosen piece, divide by the consumer portion size s_{PC} and round to the nearest integer, then we have an approximation of the number n_X of consumer portions taken from the current piece. The weight of a piece is the proportional fraction of the total weight $W/2$ of the half-carcass. The portion sizes s_{PC} are discussed in Section 9.8.7. The equation for n_X becomes,

$$n_X = \frac{W/2\frac{h_X w_X}{hw}}{s_{PC}}. \qquad (9.185)$$

Two of those portions have the highest number of *Salmonella*, they contain the sides. The relative *Salmonella* density in 1/m^2 of piece X is

$$c_X = T_X/[2(h_X + w_X)]. \qquad (9.186)$$

A simple calculation now yields,

- With probability $2/n_X$ we obtain an outer consumer cut having a relative *Salmonella* density of $c_{2,X} = c_X(h_X + 2w_X/n_X)$.
- With probability $1 - 2/n_X$ we obtain an inner consumer cut having a relative *Salmonella* density of $c_{1,X} = c_X\frac{2w_X}{n_X}$.

The relative *Salmonella* densities for one cut are shown below in Figure 9.48. For more than one cut, the densities scale approximately as $1/n_X$.

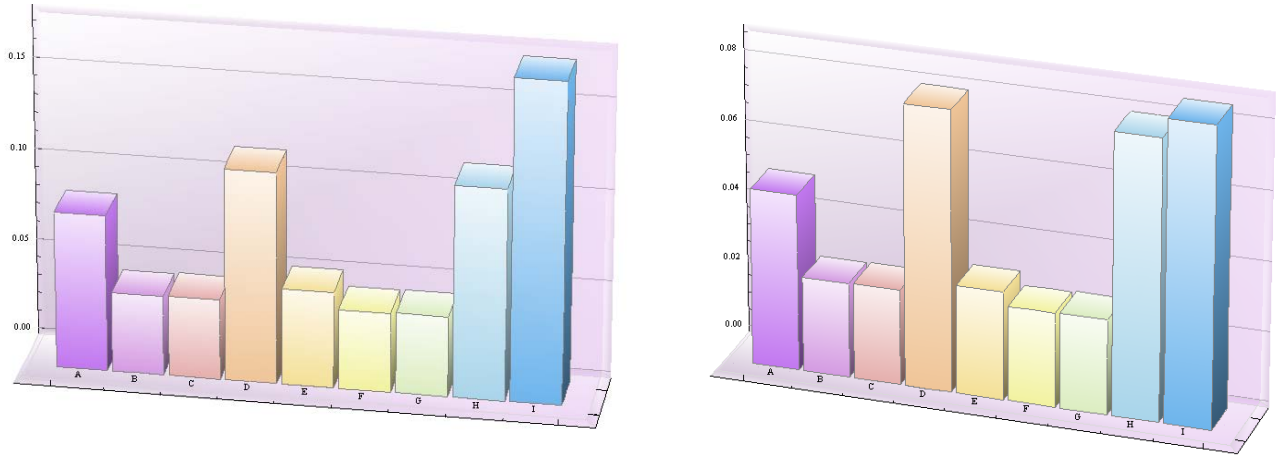

Figure 9.18: Relative *Salmonella* densities on consumer cuts, depending on primal cuts. To the left: consumer cut taken from outside of primal cut (C_2). To the right: taken from interior (C_1). Note the difference in scaling for the y-axes.

In order to determine the number of *Salmonella*, we follow this procedure, and do this for each cut needed in the remainder of the model:

1. Choose a cut X according to Table 9.30
2. Determine a carcass weight according to a beta pert distribution with parameters taken from Table 9.16.
3. Determine the number of cuts n_X
4. Determine either $c_{1,X}$ with probability $1 - 2/n_X$ or $c_{2,X}$ with probability $2/n_X$, see equation (9.186) and futher.
5. Multiply by $H_{7,k}$ (output of the slaughterhouse) to get the final result.

9.8.5 Minced meat

When producing a minced meat portion for use in the consumer model, we pick one half carcass at random. Since minced meat is produced from virtually every part of a pig, we assume the entire half carcass is used for minced meat. If s_{MM} is a portion size in grams and $W/2$ is the weight of the half carcass, then the number of *Salmonella* in the portion equals

$$H_{7,k}\frac{2s_{MM}}{W}. \qquad (9.187)$$

We have not considered the fact that one minced meat consumer portion will have originated from several half carcassess. Nor have we considered any cross-contamination via the mincing machine.

9.8.6 Fermented sausage

The procedure for fermented sausage is the same as for minced meat as minced meat is the basis for the sausage. In the consumer phase the fermentation process will be implemented, we view the fermentation as the "preparation" of the sausage. The number of cfu is,

$$H_{7,k}\frac{2s_{FS}}{W}. \qquad (9.188)$$

The effects of fermentation, drying and storage are considered in Chapter 10.

9.8.7 Pork consumption

This section describes the frequency of consumption and portion size per pork product.
We compiled data from several sources, using the following guidelines,

- We consider the population in general, that is consumer and non consumers.
- When data was split over men and women, we averaged the numbers
- We've taken the age group of approximately 18-40 years old, the group for which most data is available
- We indicated when data from other MS from within the same EU cluster were used
- When no data was found for any MS in the cluster, values from another cluster were used.

Table 9.31 and Table 9.32 provide details of the consumption data used on the QMRA. Further information is available in Appendix 12.1. Abbreviations of the EU MSs are provided in Appendix 9.4.

Table 9.31: Average amount of consumption of food products per day per cluster. (a) Lagiou & Trichopoulou 2001,(b) Anonymous, 2009a,(c) Koenig, 1999,(d) Anonymous, 2006,(e) Anonymous, 2009b,(f) Anonymous, 2008,(g) Anonymous, 2004

MS	Pork Chop [g/day]	Minced Meat [g/day]	Sausage [g/day]
MS1	33[a]	26[b]	53.15[d]
MS2	3.53[a]	14.72[e] (NL)	20.27[d]
MS3	Weekly[f] (LU)	4.34[a] (SI)	35.89[d]
MS4	Weekly[f] (LU)	6.0[g]	73,15[d] (EE)

Table 9.32: Portion sizes. (*) pork in general. See

Table 9. for references.

MS	Pork Chop [g/serving]	Minced Meat [g/serving]	Sausage [g/serving]
MS1	146[f]* (IR)	125[b] (SE)	150[b] (SE)
MS2	146[f]* (IR)	125[b] (SE)	150[b] (SE)
MS3	200[f] (LU)	76.7[g]	110[g]
MS4	200[f] (LU)	76.7[g]	110[g]

9.9 Sensitivity Analysis

For the cutting plant the response variable is the number of *Salmonella* on the pork cuts at the end of the cutting plant. As there are no parameters specifically related to minced meat or fermented sausage portions that have variability associated with them at the cutting plant, it is not necessary to conduct different analyses for the different product types. There are only 3 parameters with variability in the cutting plant module; 'pieces' (i.e. the pig part that the portion came from, see Table 9.25), 'dangerous cut' (i.e. the cut taken from the outside of the primal cut as opposed to the interior, see Figure 9.16) and carcass weight. The results are shown in Figure 9.19 to Figure 9.22 and are similar across all member states with the probability of a dangerous cut being the most significant factor and the carcass weight being the least significant.

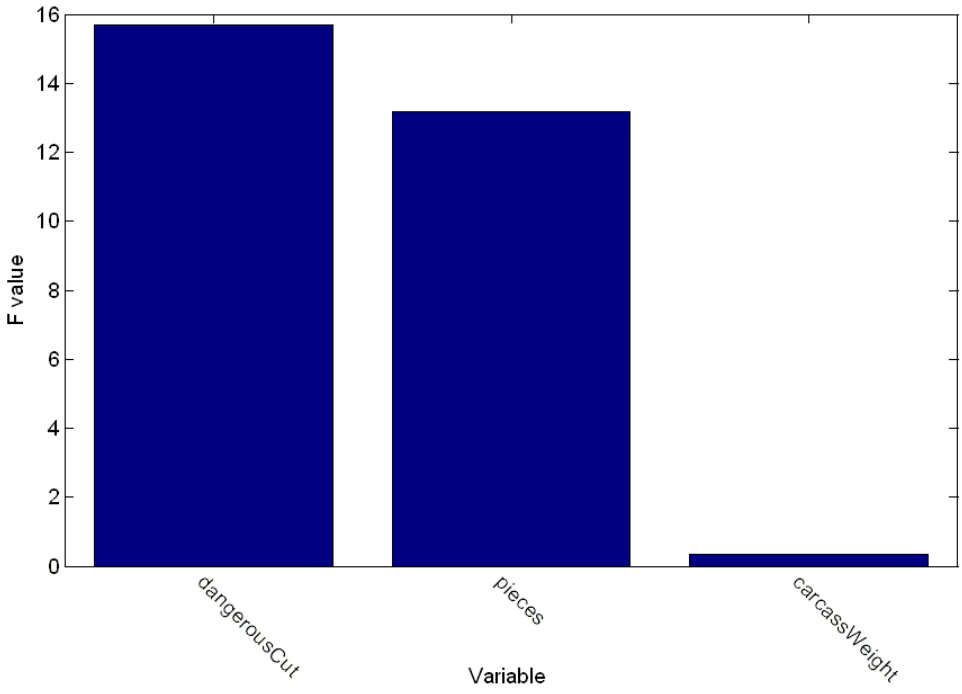

Figure 9.19: Cutting plant sensitivity analysis for MS1

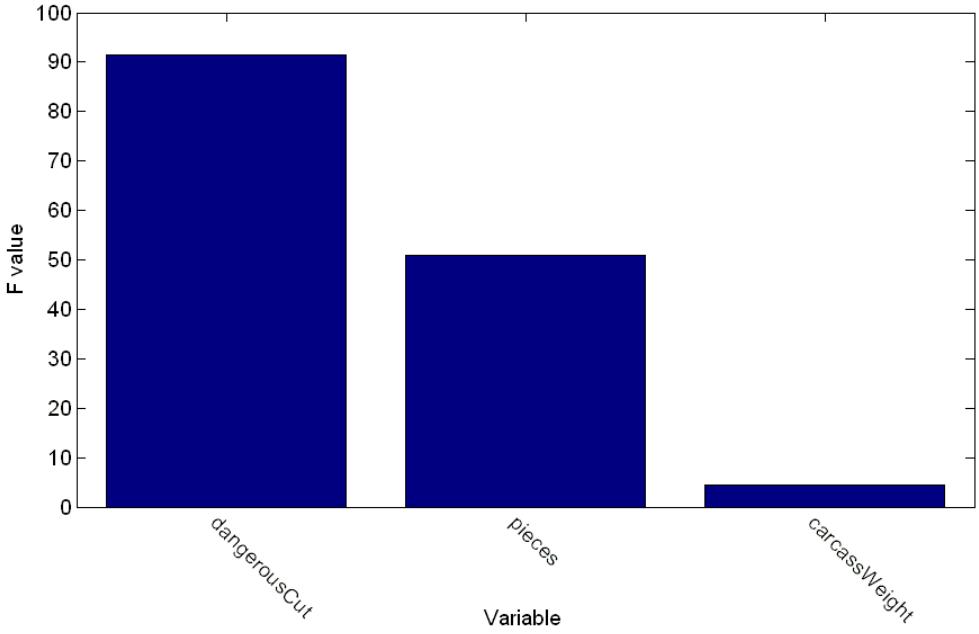

Figure 9.20: Cutting plant sensitivity analysis for MS2

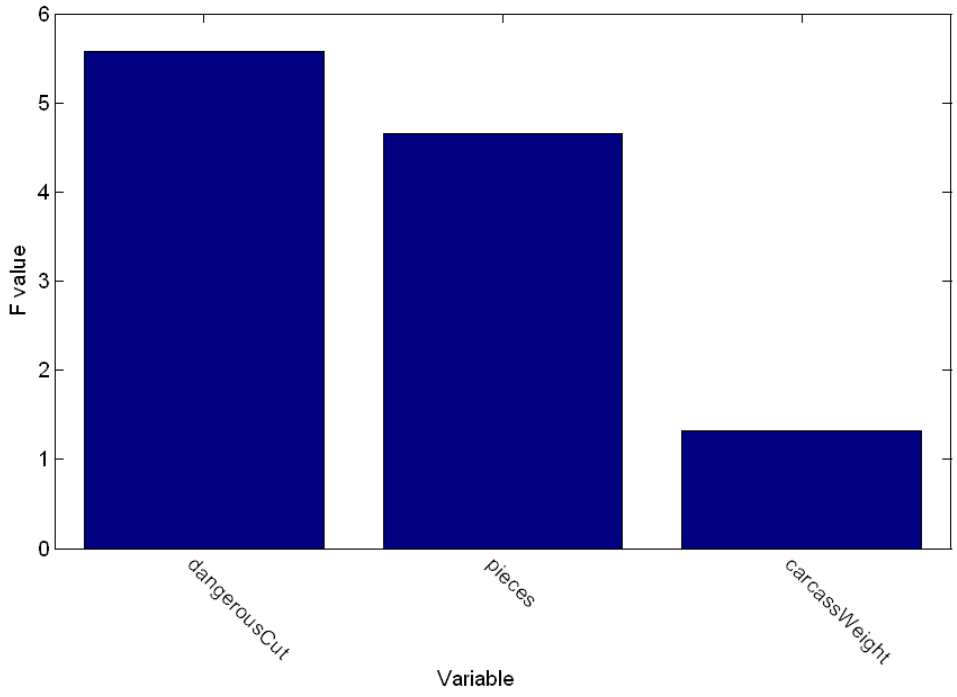

Figure 9.21: Cutting plant sensitivity analysis for MS3

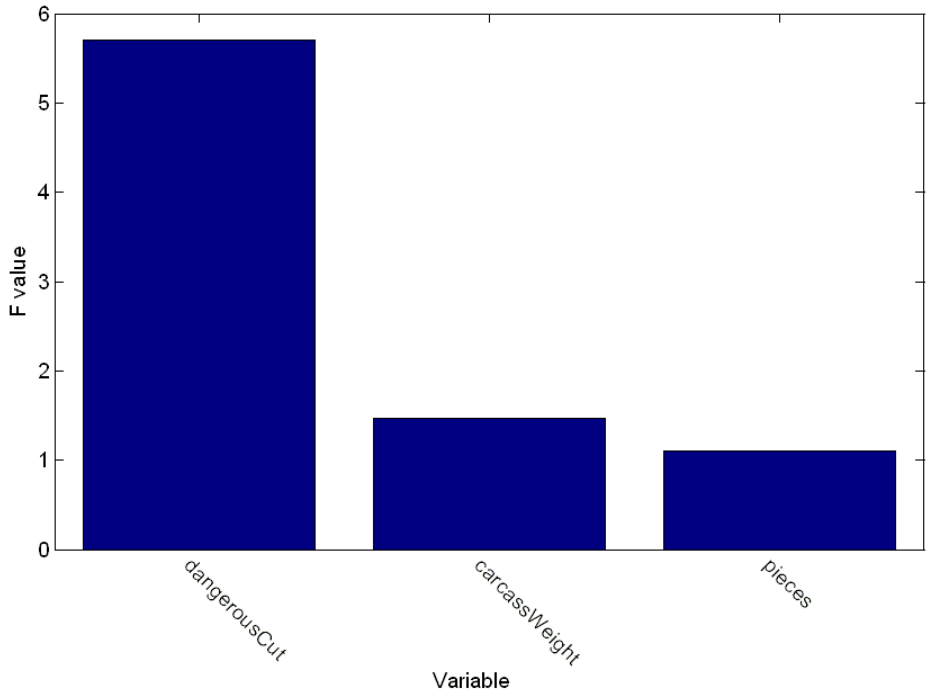

Figure 9.22: Cutting plant sensitivity analysis for MS4

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Appendix 9.1 Literature Survey Results

Table A9.33: Large slaughterhouse capacity for each MS. See Appendix A9.4 for the abbreviations of the EU MSs

Quantity	Source	Member State
$M \sim U(4000, 6400)$	Questionnaire	NL
$M \sim U(2000, 3000)$	Questionnaire	IE
$M \sim U(2400, 3200)$	Borch *et al.* 1996	DK
$M \sim U(560, 3120)$	Borch *et al.* 1996	SW

Table A9.34 Annual slaughterings, in thousands of tons per year, 2007, (Eurostat). See Appendix 9.4 for the abbreviations of the EU MSs

AT	530,9	IE	205,3
BE	1063	IT	1603
BG	41,24	LT	99,29
CY	54,98	LU	9,92
CZ	360,3	LV	40,43
DE	4985	MT	8,02
DK	1802	NL	1290
EE	37,8	NO	2091
ES	3439	PL	364,1
FI	213,3	PT	491,3
FR	2281	SE	264,9
UK	739	SI	33,19
EL	121,6	SK	113,8
HU	499,4		

Table A9.35: Temperature [ºC] of the scalding water. See Appendix 9.4 for the abbreviations of the EU MSs

Quantity	Source	Member State
$\mathcal{T}_1 \sim BP(58, 60, 64)$	Wilkin *et al.* 2007	UK
$\mathcal{T}_1 \sim U(58, 60)$		European guideline
$\mathcal{T}_1 \sim U(62, 70)$	Bolton *et al.* 2002	IE
$\mathcal{T}_1 \sim U(59, 62)$	Soerquist 1990	SE
$\mathcal{T}_1 \sim U(60, 61.5)$	Borch *et al.* 1996	Norway
$\mathcal{T}_1 \sim BP(59, 61, 64)$	Delhalle *et al.* 2008	BE

Table A9.36: The time [min.] spent in the scalding bath per MS. See Appendix 9.4 for the abbreviations of the EU MSs

Quantity	Sourcei	Member State
$T_1 = 7$	Maribo *et al.* 1998	DK
$T_1 \sim U(2, 3)$	Bolton *et al.* 2002	IE
$T_1 \sim U(2.77, 7.5)$	Wilkin *et al.* 2007	UK
$T_1 = 7$	Expert opinion QA dept.	NL
$T_1 \sim U(6, 8)$	Borch *et al.* 1996	Norway
$T_1 = 6.9$	Delhalle *et al.* 2008	BE
$T_1 = 5.9$	Average of the above	

Table A9.37: Time [min.] spent in the dehairing machine, for each MS

Quantity	Source	MS
$T_2 \sim U(0.48, 2.13)$	Wilkin *et al.* 2007	UK
$T_2 = 0.17$	Borch *et al.* 1996	Norway

Table A9.38: Time [min.] spent in the singeing machine, for each MS.

Quantity	Source	Member State
$T_3 \sim U(0.16, 0.23)$	Borch *et al.* 1996	Norway
$T_3 \sim U(0.07, 0.27)$	Delhalle *et al.* 2008	BE

Appendix A9.2 Estimation of Alpha

The steady state solution to the recursion for W_2,

$$(e^{\alpha_{2,k}T_2} - 1)W_2^-(t_{k+1}) = \beta_2 M_k, \qquad \text{(A9.2.1)}$$

is not useful, since substitution into (9.49) yields

$$N_k(t_{k+1}) = M_k\,, \qquad \text{(A9.2.2)}$$

which is trivial and does not contain $\bar{\alpha}_2$.

A second option is considering the extreme case of one pig, giving after a simple calculation an expression which does not contain $\bar{\alpha}_2$. This was to be expected, since the machine is clean at the start of the day and transfer from the machine to the pig does not play a role.

A thorough examination of average quantities for the limit of an infinite number of pigs also does not give any useful information. This is demonstrated below.

We start with the recursion for W_2, which can be solved in closed form as

$$W_{2,k+1} = \beta_2 \sum_{i=1}^{k} M_i e^{-T_2 \sum_{j=i}^{k} \alpha_{2,j}}, \qquad \text{(A9.2.3)}$$

where we set $M_i = N_{2,i} + B_{2,i}$. Inserting this expression into (9.49) yields

$$N_k(t_{k+1}) = (1 - \beta_2)M_k + \left(e^{\alpha_{2,k}T_2} - 1\right)\beta_2 \sum_{i=1}^{k} M_i e^{-T_2 \sum_{j=i}^{k} \alpha_{2,j}}. \qquad \text{(A9.2.4)}$$

Our goal is to estimate $\bar{\alpha}_2$ using reported average increases in micro-organisms. Therefore we sum the previous equation over k and divide by n,

$$\bar{N}_2 = (1 - \beta_2)\bar{M} + \beta_2 \frac{1}{n} \sum_{k=1}^{n} \sum_{i=1}^{k} M_i \left(e^{\alpha_{2,k}T_2} - 1\right) e^{-T_2 \sum_{j=i}^{k} \alpha_{2,j}} \qquad \text{(A9.2.5)}$$

Expanding the factor in the double sum, we find that we need to subtract two double sums of the type.

$$\frac{1}{n} \sum_{k=1}^{n} \sum_{i=1}^{k-1} M_i e^{-T_2 \sum_{j=i}^{k-1} \alpha_{2,j}}. \qquad \text{(A9.2.6)}$$

The second sum (not shown) has j running to k. The result is

$$\bar{M} - \frac{1}{n}\sum_{k=1}^{n} M_k e^{-T_2 \sum_{j=k}^{n} \alpha_{2,j}}. \qquad \text{(A9.2.7)}$$

This does however not help us, since for $n \to \infty$ this expression is equal to $\bar{M}$ and (A9.2.5) becomes $\bar{N}_2 = \bar{N}_1 + \bar{B}_1$. This is the same result as obtained when using the steady state solution and could be anticipated since (A9.2.3) implies that the steady state will be reached.

$$\bar{M} - \frac{1}{n}\sum_{k=1}^{n} M_k\left(1 - T_2 \sum_{j=k}^{n} \alpha_{2,j}\right) = \frac{T_2}{n} \sum_{k=1}^{n} M_k \sum_{j=k}^{n} \alpha_{2,j} \qquad \text{(A9.2.8)}$$

Appendix A9.3 Approximation of log of factorial

We wish to approximate $\frac{1}{n}\log(n!)$ for large n. A good starting point is Stirling's formula, $n! \approx \sqrt{2\pi n}(\frac{n}{e})^n$, from which we find $\frac{1}{n}\log(\sqrt{2\pi n}(\frac{n}{e})^n)$. Written as a sum of logarithms $\frac{1+2n}{2n}\log(n)+\frac{1}{n}\log(\sqrt{2\pi})-\log(e)$. For large n this is approximately $\log(n)-\log(e)$.

Appendix A9.4: Official abbreviations for the EU member states.

Table A9.39 Abbreviations of member states of the European Union (ISO 3166 alpha-2), except for Greece and the United Kingdom, for which the abbreviations EL and UK are recommended by the EU.

Short name in English (geographical name)	Country code
Belgium	BE
Bulgaria	BG
Czech Republic	CZ
Denmark	DK
Germany	DE
Estonia	EE
Ireland	IE
Greece	EL
Spain	ES
France	FR
Italy	IT
Cyprus	CY
Latvia	LV
Lithuania	LT
Luxembourg	LU
Hungary	HU
Malta	MT
Netherlands	NL
Austria	AT
Poland	PL
Portugal	PT
Romania	RO
Slovenia	SI
Slovakia	SK
Finland	FI
Sweden	SE
United Kingdom	UK

10 Preparation & Consumption Model

10.1 Introduction

This report describes the retail and consumer phase of the EFSA *Salmonella* in Pigs QMRA. The consumer phase is important in the sense that (in contrast to the farm, slaughter, or retail phases) it cannot be controlled by government (Nauta *et al.* 2002). Food preparation habits are highly variable and accurate data on daily life food handling practices are hard to obtain.

In Section 10.2 the food chain is described from retail to ingestion by the consumer. The input to the food chain, the number of *Salmonella*e per portion, for each case study MS and each product is obtained from the Slaughter and Processing module (Chapter 9). The output of the retail and consumer model is the number of *Salmonella* ingested per person per day, for each pig meat product and each case study MS. This output will, in turn, feed into the final model, where the risk of illness is modelled using a dose-response relation.

The model is directly coupled to the Slaughter & Processing model, the output of each iteration being matched to an iteration of the Preparation & Consumption model. The interpretation of one slaughter module iteration is 'pork produced in a realisation of typical production environment', the interpretation of variation over iterations being variation between production environments. The output of the slaughter model is a number of portions (10,000 at default) per iteration, randomly sampled from the total pork production. In the Preparation & Consumption module each of those portions is prepared and consumed. Any variation described in this report is within-iteration variation, and thus expresses variability over consumers, portions, etc.

Section 10.2.5 discusses the two major basic processes present in the consumer phase: growth and inactivation.

We continue with considering three types of pork: minced meat (Section 10.3), pork cuts (Section 10.5), and dry cured sausages (Section 10.6). This particular choice of products was made because each product represents a clear distinct hazard. Pork cuts are usually cooked well, but there is a chance of cross contamination during cutting and handling of the meat. Minced meat is thoroughly mixed, and *Salmonella*e may be present in the interior of hamburger patties (or meatballs, etc.). Since the core of a meat patty is less efficiently heated than the outside, undercooking may occur, and *Salmonellae* may survive. Dry cured sausages, including all variations therein like chorizo, salami, etc., are eaten uncooked. Any *Salmonella*e present after the fermentation process can potentially survive or grow, although salt concentration (lowering the a_W) and a low pH are limiting factors. Several outbreaks attributed to the consumption of fermented sausage have been reported (e.g. Pontello *et al.* 1998, Bremer *et al.* 2004, Gilsdorf *et al.* 2005, Emberland *et al.* 2006, Nygård *et al.* 2007, Luzzi *et al.* 2007).

10.2 The food pathway

In accordance with the MPRM methodology (Nauta, 2008), we split the food chain into several modules. Each module represents a distinct step, and is assigned a specific microbiological process: growth, inactivation, cross-contamination, partitioning, mixing or removal (Nauta 2001; Nauta 2008). Figure 10.1 shows the pathways for each of the products. The relevant process in indicated by (G)rowth, (I)nactivation and (C)ross-contamination.

Note that we only model products sold chilled, not frozen; therefore we do not model defrosting. There are several reasons for this. Firstly, it is a minor assumption, since *Salmonella* does not grow, nor is it inactivated, in a chilled or frozen environment. Secondly, there is little data on the percentages of products sold frozen. The few sources available indicate that the percentage sold frozen is negligible. Thirdly, there is no data on the type of defrosting practiced by consumers. There are three defrosting procedures that are usually considered: defrosting in the microwave, in the fridge, or at room temperature. Of those, only defrosting at room temperature could pose modest risk.

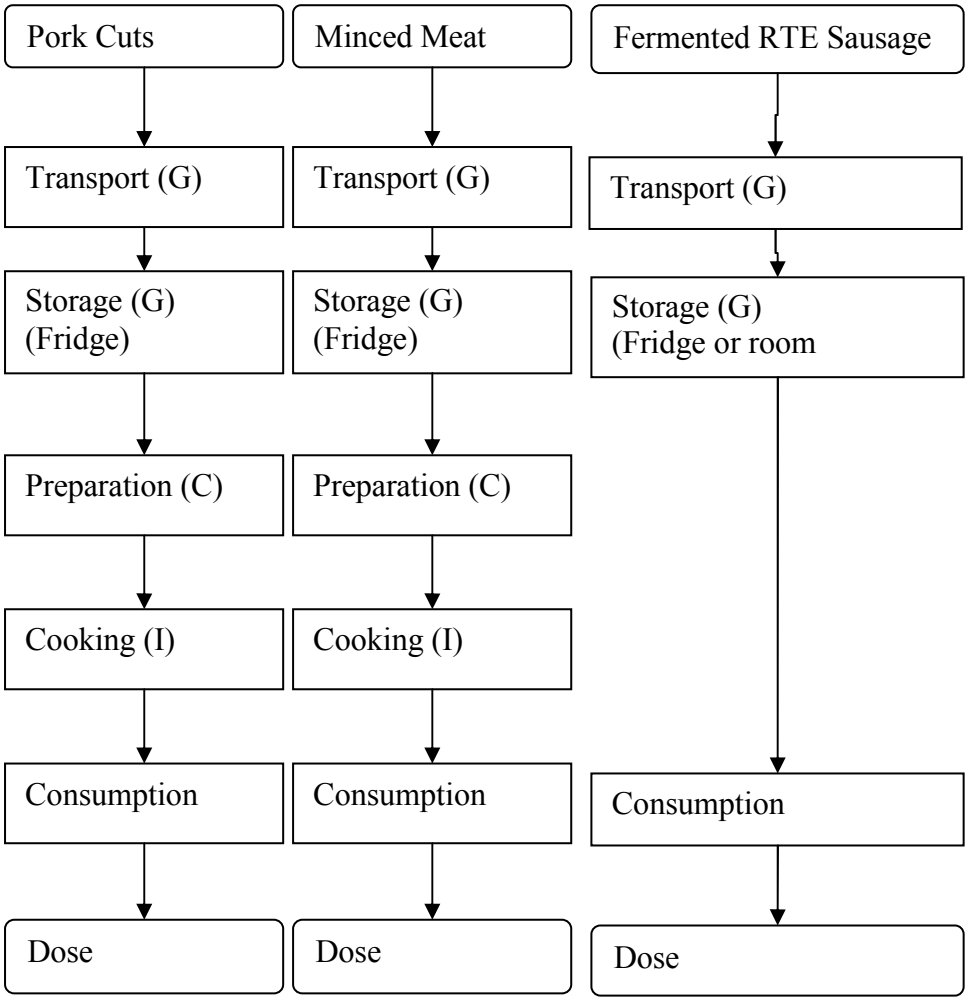

Figure 10.1: The food pathway for pork cuts, minced meat and fermented sausage.

10.2.1 Distribution and retail

During distribution, from the slaughterhouse to the store, the temperature is at a maximum of 6°C. This maximum is adhered to by virtually every MS. In the UK, 94% of the supermarkets, butchers, market stalls and other outlets adhere to the maximum temperature requirement (Peck *et al.* 2006).

The time spent in transport and retail was obtained from Derens *et al.* 2006. Due to poor data availability it is necessary to use the same data for all of the MSs.

The temperatures are not known separately for transport, wholesale or display, we only have some data on temperatures in lorries. Therefore, we choose to work with the total time, and assign it to the Transport category,

$$T_{*,1} = 110 \text{ hrs.} \qquad (10.189)$$

We have introduced a new notation here. The star (*) indicates all MSs, while the second index indicates the first phase in the model.

Temperatures in the lorries values were recorded in a French study (Peck *et al.* 2006). We describe those using a general distribution (see Chapter 5, Appendix 5.1).

$$\mathcal{T}_{*,1} = \Re(G([-2,-1,0,1,2,3,4,5,6,7,8], [0.9,2.5,7.7,12.6,21.4,25.3,18.5,7.4,2.8,0.9])/100). \qquad (10.190)$$

This notation should be read as follows: the temperatures for all MS have a certain probability of being in a range of values, e.g. the probability that the temperature is between -2 °C and -1 °C is 0.009. Within this interval the temperature is sampled uniformly.

10.2.2 Consumer transport and storage

In the following tables we summarise data on times and temperatures for the transport of meat products from the store to the domestic home. Also refrigerator temperatures and storage times are tabulated. See Appendix A10.1 for the sources of the data.

The travel time from store to domestic home is known for MS2. Due to lack of data it is assumed that MS1 & MS3 have similar travel times between the store and home. However, in the absence of any other data it is assumed that MS4 has the same travel time as Finland, which is also in Cluster 4 (see Chapter 6, Table 6.5).

$$T_{1,2} = T_{2,2} = T_{3,2} = \Re(G([0,30,50,120],[0.96,0.02,0.02])). \qquad (10.191)$$

$$T_{4,2} = \Re(BP(10,45,300)). \qquad (10.192)$$

Table 10.1: Duration of transport to, and storage in, retail

MS	Transport	Wholesale	Display Cabinet
All	5h	9h	96h

Storage times in the refrigerator were available only for MS2 which we use for the other case study MS,

$$T_{*,3} = \Re(DG([16, 72, 104, 29, 13, 3, 0, 11, 0, 0, 0, 0, 3, 0], [1/4.1/2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14])) \quad (10.193)$$

Data from freezers was not collected, since no growth or inactivation occurs. However, refrigerators often have varying temperatures (due to e.g. opening of the refrigerator door), and growth is an important process.

For temperature during transport and storage in the fridge, data are available in Appendix 10.1. For consumer transport temperature we used French data Derens *et al.* 2006 for MS1, MS2 and MS3 again, and Finnish data for MS4

$$\mathcal{T}_{1,2} = \mathcal{T}_{2,2} = \mathcal{T}_{3,2} = \Re(G([-2, 0, 2, 4, 6, 8, 10], [0.003, 0.023, 0.135, 0.242, 0.253, 0.344])), \quad (10.194)$$

$$\mathcal{T}_{4,2} = \Re(BP(6, 10, 20)). \quad (10.195)$$

For fridge temperatures, the best data available was for the UK. This data is used for all case study MSs, due to no data available for any other MS,

$$\mathcal{T}_{*,3} = \Re(G([0, 1, \ldots, 12], [0.01, 0.02, 0.05, 0.09, 0.11, 0.17, 0.21, 0.15, 0.12, 0.04, 0.01, 0.01])). \quad (10.196)$$

During transport from the supermarket to the domestic home, Evans *et al.* 1991 found that temperatures of meat products can easily assume an ambient temperature. Therefore, the temperature of pork during transport is highly dependent on the average temperature of the MS. In ambient temperature conditions, Evans 1998, estimates 0.6 cfu log increase (time and temperature not reported).

10.2.3 Preparation: cross-contamination

By cross-contamination we mean the contamination of a product, via an object that was contaminated by the *Salmonella* on the meat product. More specifically, during the cutting of the pork cuts the knife will be contaminated with *Salmonella*e from the meat. Later, these *Salmonella*e in turn may be transferred to another product (e.g. a salad). Other examples are cross-contamination via hands due to improper washing, or via a cutting board. Also there is the possibility of a direct dose of *Salmonella*, by touching of the lips, or licking of the finger. This dose is not expressed as 'per gram' but directly in cfu ingested.

The data relevant to cross-contamination, and the description of the model will be discussed later in the Chapter.

10.2.4 Consumption

The consumption phase does not include any basic process. Nonetheless, we list it since this is the phase in which we calculate the dose ingested due to the consumption of the food product and cross-contaminated RTE-product (if applicable).

10.2.5 Growth and Inactivation

During transport and storage there is an opportunity for bacterial growth. Depending on the duration of transport, temperature, pH, and water activity (amongst other factors), the number of *Salmonella*e in the product may increase. We use a growth model that takes into account these factors.

To give the reader an idea of the growth ranges for *Salmonella* spp. environmental factors, we list them in Table 10.2, taken from ICMSF 1996.

However, conditions in food products are different from growth media used in the laboratory. In order to construct a full model, we would need the minimum, optimal and maximum temperature, pH and a_W enabling growth for *Salmonella* in each of the food products under consideration. Furthermore we would need the temperature, pH and a_W of each of the food products under consideration. After a thorough literature search we concluded that these data are not all available.

A literature survey of published growth models was conducted. The results are presented in Appendix 10.2.

Oscar 2002 deduced that the hyperbola model yielded the best fit for the lag time parameter. For the specific growth parameter, the Ratkowsky model gave the best fit, while the CTM enjoyed the tightest confidence interval. According to the author, other studies have revealed mostly identical conclusions.

In addition to the data listed in the Appendix, the online database ComBase has a large amount of raw data on *Salmonella* inactivation on fermented sausage and pork cuts. This database is also actively maintained, and contains most of the data referenced in the literature. Appendix 10.3 contains an overview of the most common growth models used in literature. Both primary models (growth using a growth and lag-time parameter) and secondary models (T, pH and a_W dependent growth parameters) are reviewed.

Considering the available models and data, we chose the Baranyi model for the following reasons:

- Using the DMFit Excel add-on Baranyi and Roberts 1994, Baranyi 2006, we can calculate growth curves from raw data. This add-on uses the Baranyi model, and calculates all necessary parameters for the model. This is better practice than using parameters from the literature, that are fitted to specific models, for specific products.
- DMFit works very well with the ComBase database, opening up a large amount of data.

Table 10.2: *Salmonella* growth factors in laboratory conditions (ICMSF 1996).

***Salmonella* growth (Laboratory)**	**Minimum**	**Optimum**	**Maximum**
T	5.2	35-43	46.2
pH	3.8	7.0-7.5	9.5
aW	0.94	0.99	>0.99

- The Baranyi model is also suitable for curves without a lag phase. This is automatically detected by the DMFit software.
- The Baranyi model is also suitable for curves without an asymptote. This is automatically detected by the DMFit software.
- The model is also suitable for inactivation.
- The Baranyi model has a well-founded mathematical basis, in contrast to other models which are mostly ad-hoc fits to specific functions.

Table 10.3 lists the parameter values calculated with DMFit, using raw data from ComBase, that we use in our growth model. Note that if no lag phase or asymptote is listed, they were not present in experimental data. Also note that we have no data on fermented sausages. However, those data are not needed. Before fermentation, the constituent is minced meat, during fermentation we have a specialized model which does not depend on the Baranyi growth model.

The temperatures in the above table match very well with the temperatures needed for our models (transport temperatures and refrigerator temperatures). For improved accuracy, we linearly interpolate the growth rate for temperatures not listed. The lag phases are nearest-neighbour interpolated[25] (with all temperatures >23^{O}C interpolated to 23 ^{O}C and all temperatures below 4.4 ^{O}C interpolated to 4.4 ^{O}C). Due to lack of pH and a_W data at several temperatures, we do not use any secondary model.

Inactivation is the opposite of growth: a decrease in microbial numbers due to unfavourable environmental factors. This process is relevant during the cooking of pork cuts and minced meat, and is for those products the most important risk limiting factor. See Appendix 10.4 for an overview of D-values and z-values, the most common tools for inactivation calculation.

For pork cuts we assume complete inactivation due to cooking, which is justified since *Salmonella* is only present on the outside of the product (Section 10.5.2). Minced meat, however, is contaminated throughout the product and we use an exponential inactivation (Section 10.3.4). For fermented sausages we use a model depending also on pH and a_W, which are important factors during fermentation and drying of the sausage (Section 10.6.1).

[25] With nearest neighbour (also known as 'piecewise constant') interpolation, a value x is interpolated to the points $x_1, \ldots, x_n$ by choosing the x_i nearest to x.

Table 10.3: Growth rates for several pork products and environmental factors, taken from ComBase.

Product	T[°C]	Product	rate	lag	Asymptote
Pork Chop	23	Pork Chop	0,113347	2,691364	-
Pork Chop	10	Pork Chop	0,005066	-	-
Pork Chop	7,2	Pork Chop	0,001363	-	-
Pork Chop	4,4	Pork Chop	0,001729	-	-
Minced Meat	23	Minced Meat	0,250764	4,28946	-
Minced Meat	10	Minced Meat	0,029442	12,76222	5,301502
Minced Meat	7,2	Minced Meat	0,008143	-	-
Minced Meat	4,4	Minced Meat	0,00541	-	-

10.3 Minced Meat Model

10.3.1 Transport of minced meat

During transport, *Salmonella* in the minced meat has the opportunity to grow for a certain time period, under certain temperature conditions. Travel times were reported in Section 10.2.2. From Table 10.3 we have growth rates and lag phases for minced meat at several temperatures. These parameters are suitable for the description of growth in minced meat, including the lag phase.

10.3.2 Storage of minced meat

Duration of storage in the refrigerator and temperatures can be found in Section 10.2.2. Growth parameters for minced meat, at 4.4, 7.2 and 10 °C are given in Table 10.3. As discussed before, we will interpolate the growth rate between temperatures when needed.

10.3.3 Preparation of minced meat

The cross-contamination model for minced meat contains two phases. Firstly the meat is handled, preparing hamburger patties, or meat balls, from the mince. Secondly, a ready to eat (RTE) food product is prepared, possibly on the same board, possibly without washing hands. We model the cross-contamination process between two objects using transfer coefficients. Such a coefficient represents the fraction of *Salmonella* migrating from one object to another. We consider lettuce as the accompanying RTE food, since transfer data is available for this product.

The transfer coefficients given in Table 10.1 are relevant during these processes.

Next, we also need probabilities of improper handling, and frequency of preparation of a side dish which are adapted from

Table A10.. Values for the UK were used for each MS. When a range of values is listed in this table, the average is taken. When multiple sources were available, the geometric mean was taken rather than the arithmetic mean, because of large differences in orders of magnitude.

Let us define M, H, L, B as the random variables describing the number of *Salmonella* in the meat, on the hands, on the RTE product and on the cutting board. We add subscripts to distinguish the phases.

Table 10.4: Cross-contamination during handling, for minced meat (Wachtel, McEvoy *et al.* 2003)

To / From	Minced meat	Hand	Lettuce	Board
Minced Meat		0.04		0.02
Hand			0.06	
Lettuce				
Board			0.26	

Table 10.5: Probabilities of hazardous actions in the domestic kitchen

	Ph don't wash hands	Pk unsafe knife handling	Pb unsafe board handling	Ps prepare a salad
MS1, MS2, MS3, MS4	0.14	0.38	0.27	0.3

Also, define transfer matrices T_{ab} containing transfer rates from $a \in \{M, H, L, B\}$ to $b \in \{M, H, L.B\}$[26] as the matrix having t_{ab} as the entry corresponding to the positions of a and b in the vector having $1 - t_{ab}$ on the main diagonal in the same column as t_{ab}

having zeros at the remaining entries.

Then the first step, contaminating the hands and the board, and can be written

$$\begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_1 = T_{MH}T_{MB}\begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_0 = \begin{pmatrix} 1-t_{MH} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ t_{MH} & \mathbf{1} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} \\ 0 & \mathbf{0} & \mathbf{0} & 1 \end{pmatrix}\begin{pmatrix} 1-t_{MB} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ 0 & \mathbf{1} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} \\ t_{MB} & \mathbf{0} & \mathbf{0} & 1 \end{pmatrix}\begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_0. \quad (10.197)$$

This represents first a transfer from meat to board, followed by a transfer of what is left from meat to hand. On the other hand, one could also consider the reversed route,

$$\begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_1 = T_{MB}T_{MH}\begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_0 = \begin{pmatrix} 1-t_{MB} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ 0 & \mathbf{1} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} \\ t_{MB} & \mathbf{0} & \mathbf{0} & 1 \end{pmatrix}\begin{pmatrix} 1-t_{MH} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ t_{MH} & \mathbf{1} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} \\ 0 & \mathbf{0} & \mathbf{0} & 1 \end{pmatrix}\begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_0, \quad (10.198)$$

[26] e.g. t_{MH} represents transfer from meat to hands

which is not equal to the first one! Finally, one could argue that transfer happens simultaneously,

$$\begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_1 = \begin{pmatrix} 1-t_{MB}-t_{MH} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ t_{MH} & \mathbf{1} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} \\ t_{MB} & \mathbf{0} & \mathbf{0} & 1 \end{pmatrix} \begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_0 \equiv T_{MBH} \begin{pmatrix} M \\ H \\ L \\ B \end{pmatrix}_0 . \quad (10.199)$$

This is not equal to the first nor the second approach. While the third approach seems intuitively right, there is the danger of $t_{MB}+t_{MH}$ being greater than one, resulting in a negative amount of *Salmonella*. This is ultimately the consequence of transfer rates collected from different experiments. In the work of Mylius *et al.* 2007 an extra correction is added to the transfer rates (a term $-2t_{MB}t_{MH}$) in matrix entry (1,1). However, given that transfer rates are typically in the order of hundredths or thousandths, this risk is negligible.

In the second step, the board and hands can contaminate the RTE food, if eaten, and if the hands and board are not properly sanitized. Now, a consumer handles the board or hands unsafely according to a Bernoulli (binomial with one trial) distribution, $X_b \sim B(1, p_B)$ and $X_H \sim B(1, p_H)$, respectively. Furthermore, preparation of a salad is described by $X_s \sim B(1, p_S)$. Thus, to be explicit, the X's are random variables taking the values:

$$X_B = \begin{cases} 1 & \text{if unsafe board handling} \\ 0 & \text{if safe board handling} \end{cases}, \quad (10.200)$$

$$X_H = \begin{cases} 1 & \text{if hands not washed} \\ 0 & \text{if hands washed} \end{cases}, \quad (10.201)$$

$$X_S = \begin{cases} 1 & \text{if salad is eaten} \\ 0 & \text{if salad is not eaten} \end{cases}. \quad (10.202)$$

Note that we assume that hand washing removes all *Salmonella*. The above considerations result in the following expression for describing salad preparation,

$$\begin{bmatrix} M \\ H \\ L \\ B \end{bmatrix}_2 = \{(1-X_s)+X_s((1-X_H)I+X_H T_{HL})((1-X_B)I+X_B T_{BL})\} \begin{bmatrix} M \\ H \\ L \\ B \end{bmatrix}_1, \quad (10.203)$$

where I indicates the identity matrix. Combining the previous relations, we obtain the random vector describing both minced meat and salad handling,

$$\begin{bmatrix} M \\ H \\ L \\ B \end{bmatrix}_2 = \{(1-X_s)+X_s((1-X_H)I+X_H T_{HL})((1-X_B)I+X_B T_{BL})\} T_{MBH} \begin{bmatrix} M \\ H \\ L \\ B \end{bmatrix}_0 . \quad (10.204)$$

Finally, we can derive expressions for M_2 and L_2. The *Salmonella* on the lettuce (L_2) will be directly ingested, while those remaining on the meat (M_2) will undergo an additional heat treatment.

$$M_2 = (1 - t_{MH} - t_{MB})M_0,$$
$$L_2 = X_S(X_H t_{MH} t_{HL} + X_B t_{MB} t_{BL})M_0. \quad (10.205)$$

Using the data from Table 10.4 and Table 10.5, we find for the expected values $m_2 = E(M_2)$ and $l_2 = E(L_2)$ the values $m_2 = 0.94m_0$ and $l_2 = 5.2 \cdot 10^{-4} m_0$ cfu. Thus, the meat is still potentially highly contaminated, while a moderate dose is directly ingested via the RTE product (e.g. lettuce).

10.3.4 Cooking of hamburger patties

With regard to the heating of minced meat, two data sources were found. Firstly, the mathematical model and measurements of, describing temperatures at various depths in a piece of meatloaf, cooked in a convection oven Holtz & Skjoeldebrand 1986. Secondly, measurements describing the internal and external temperature of minced beef in a conventional oven Hollywood, Varabioff *et al.* 1991. However, we are concerned with cooking of hamburger patties in a frying pan, having a completely different temperature profile.

The temperature difference between the outside and inside of hamburger patties (100g, thickness 1cm) has been described Juneja *et al.* 1997. The temperature difference ΔT in Fahrenheit as a function of time t in minutes during cooking was determined to be:

$$\log(\Delta T) = 1.95 - 0.16t. \quad (10.206)$$

This formula implies that inside temperature equals the outside temperature after approximately 12 minutes, after which the relation is no longer valid (the internal temperature would exceed the external temperature!). Also, the initial temperature difference is about 100 degrees. This model is rather simple, distinguishing only between an inner and outer part of the pattie.

We would like to model spatial temperature distribution in more detail, so previous attempts are not suitable for our purposes. We develop a new model, aiming to describe the temperature distribution, evolving over time, in more detail. Let us start with the basic physical processes acting when frying a minced meat product:

1. Conductive heat transfer from the frying pan to the oil, and to the product
2. Internal heat redistribution by diffusion

We will assume that the heat transfer from pan to oil to product is perfect, i.e. the bottom of the product is kept at the frying temperature at all times. There are other processes at work, such as the formation of a crust, inhibiting heat flow from the inside to the outside, and transport of water and oil components inside of the product. We will neglect the second process, but account for crust formation in a simplified manner. We mainly focus on internal diffusion.

10.3.5 Physical Cooking Model

Diffusion as a function of time and spatial coordinates is governed by the heat equation (see e.g. Hallström *et al.* 1988 or De Jong *et al.* 2005),

$$\frac{\partial T(x,y,z,t)}{\partial t} = \kappa \Delta T(x,y,zt). \quad (10.207)$$

In a Cartesian coordinate frame, the Laplacian is defined as

$$\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}. \quad (10.208)$$

The parameter κ [m^2/s] is known as the thermal diffusivity and represents the material properties of the product. It is defined as

$$\kappa = \frac{\lambda}{\rho c_p}, \quad (10.209)$$

with

- ρ, the density of the product [kg/m^3],
- c_p, the specific heat capacity [J/(kg K)],
- λ, the thermal conductivity [W/(mK)].

At the boundaries we need boundary conditions. The simplest boundary condition is at the bottom ($y = 0$), where the temperature is kept at the heating temperature T_H,

$$T(x,y,0) = T_H. \quad (10.210)$$

At the other boundaries, heat flow depends on the ambient temperature T_A. The description of the boundary condition is based on Newton's law of cooling[27]. This law states that the heat flux is proportional to the temperature difference, and is given by

$$\kappa \frac{\partial T(x,y,z,t)}{\partial n} = -\alpha [T(x,y,z,t) - T_A]. \quad (10.211)$$

Here n is the unit outward normal to the boundary and α is the convective surface heat transfer coefficient in [W/m^2K].

It is actually easier to work with the temperature difference $D = T - T_A$. This keeps the heat equation unchanged, and yields the boundary conditions

$$D(x,y,0) = T_H - T_A \equiv D_H, \quad (10.212)$$

$$\frac{\partial D(x,y,z,t)}{\partial n} = -\frac{\alpha}{\kappa} D(x,y,z,t). \quad (10.213)$$

We now consider a hamburger which has rotation symmetry, such that viewed from the side we have a rectangle of width W and height H, measured in meters. Figure10.2 schematically represents the situation as outlined above.

[27] Also known *as Fourier's Law*, or *The Law of Heat Conduction.*

10.3.6 Description of Cooking process

The model developed in the previous section allows us to model the cooking of the minced meat patty in some detail. We will model the following stages, after the recipe described by Bergsma, Fischer *et al.* 2007,

Looking of one side of the patty for one minute at high temperature. Cooking temperature and ambient temperature are fixed. Heat flows through the sides of the patty depending on the difference in temperature with the environment (ambient temperature).

The product is turned. It is assumed that a crust has formed at this point, at the cooked side. We set the conductivity of all sides to zero (i.e. a perfectly insulating patty). Setting all sides instead of only the top has only a small effect. The sides have a small area compared to the top and bottom, and the bottom is now directly heated and is subject to a boundary condition of the type (A10.245). Experiments have shown us that the difference between zero conductivity and low conductivity is very small in terms of the final result. The product is cooked for a few more minutes, at a lower temperature.

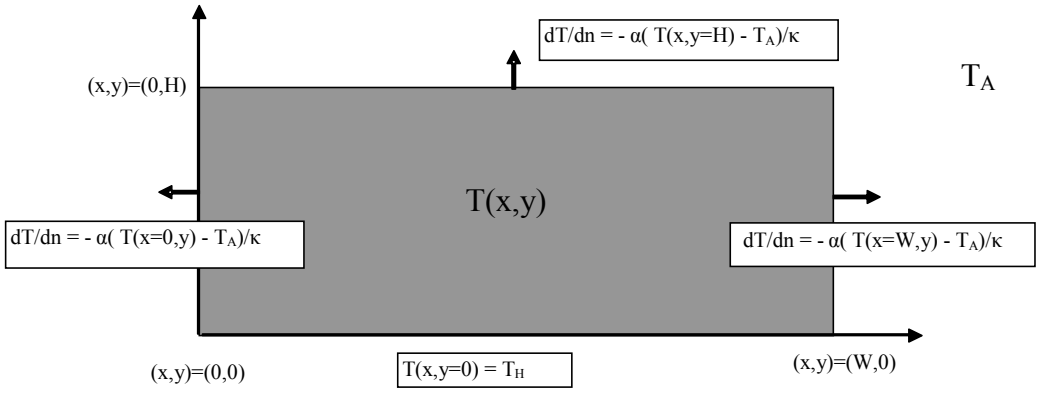

Figure 10.2: The geometry of the minced meat patty. Boundary conditions are boxed.

10.3.7 Finite difference approximation

The equations as stated are not accessible to the usual exact solution methods, such as separations of variables or Fourier techniques. Therefore, we construct a numerical solution. The simple description of the boundary (aligned with the coordinate axes), makes the problem suitable for approximation using finite differences. The mathematical description of the finite difference approximation is given in Appendix 10.5.

10.3.8 Estimation of parameters

Table 10.6 provides estimates for the parameters related to the heat equation.

The surface convective heat transfer coefficient could not be obtained from the literature. However, a rough approximation can be obtained by dividing the thermal conductivity by a typical length scale. Such a length scale would be the cubic root of the volume, which in turn is determined from the portion weight divided by the density. Portion sizes vary greatly per MS and per consumer, but using a typical value of 100g we arrive at a typical volume of 83cm^3. Taking the cubic root, a typical length scale would be 0.04m.

Table 10.6: Parameters used in the cooking process. For comparative purposes, the parameters for water have also been included.

Quantity	**Pork Minced Meat**	**Source**	**Water**
Density	1200 kg/m^3	Torstveit & Magnussen 1999	998 kg/m3
Specific heat capacity	3500 J/(kg K)	Hardarsson, 1998; Rimestad *et al.*, 1995	4186 J/(kg K)
Thermal conductivity	0.49 W/m K	Heldman & Lund, 1992	0.6
Thermal diffusivity	1.2e-7 m2/s	Calculated from the above	1.43e-7

Cooking times and temperatures are hard to obtain. Below we list a few sources,

- The USDA recommends cooking at 71 °C (internal temperature!) for 8 to 10 minutes.
- The Dutch 'Voedingscentrum' recommends cooking for 12 to 15 minutes per 100g, with no temperature indication.
- 'Voorlichtingsbureau Vlees' recommends 8 to 12 minutes per 125g.
- Information from hamburger labels, obtained at a local supermarket, give 9 minutes.
- Sunflower oil has its smoke point at 232 degrees

In the end, we have chosen to fry initially at 180 °C, turning the temperature down to 100 °C after crust formation. This setting reproduces an internal temperature of 70 °C after about eight minutes (Figure 10.3).

Official cooking times lie between 8 and 15 minutes. However, we would like to take into account possible disregard of the recommendations. We assume that 8-15 minutes is the 2.5% to 97.5% percentile of a normal distribution, with mean.

$$\mu_5 = 11.5 \text{ minutes} \qquad (10.214)$$

Then the standard deviation σ_5 is given (see e.g. Rice 1995 pg. 205) by

$$\sigma_5 = (15 - 8)/(2 * 1.96) \qquad (10.215)$$

and the cooking time distributed according to

$$T_5 = \Re(N(\mu_5, \sigma_5)) \quad (10.216)$$

Finally, D-values and z-values are needed in order to couple the cooking time and temperature to the inactivation of *Salmonella*. Unfortunately, D-, and z-values for ground pork could not be located in the literature. But, according to Murphy *et al.* 2002, the D-values and z-values in beef patties are

$$D_{60} = 4.8 \quad (10.217)$$

$$z = 9.14 \quad (10.218)$$

10.4 Results

Figure 10.3 shows the temperature distribution during cooking of the minced meat patty. The temperature starts at ambient temperature (panel 1), slowly penetrates into the interior (panel 2), heats the other side of the patty after turning (panel 3) and slowly cooks the rest of the patty after the heat is turned down (panel 4 to 12).

Perhaps easier interpreted is Figure 10.4, the mean temperature and core temperature during cooking. The mean temperature increases steadily, almost two-phase linearly (note the change in slope after 2 minutes). In contrast, the core temperature is far from linear, resembling a sigmoidal curve. Such curves were obtained before, one may compare our results with e.g. Hollywood *et al.* 1991 (Fig. 1), or Holtz & Skjoeldebrand 1986 (Fig 5).

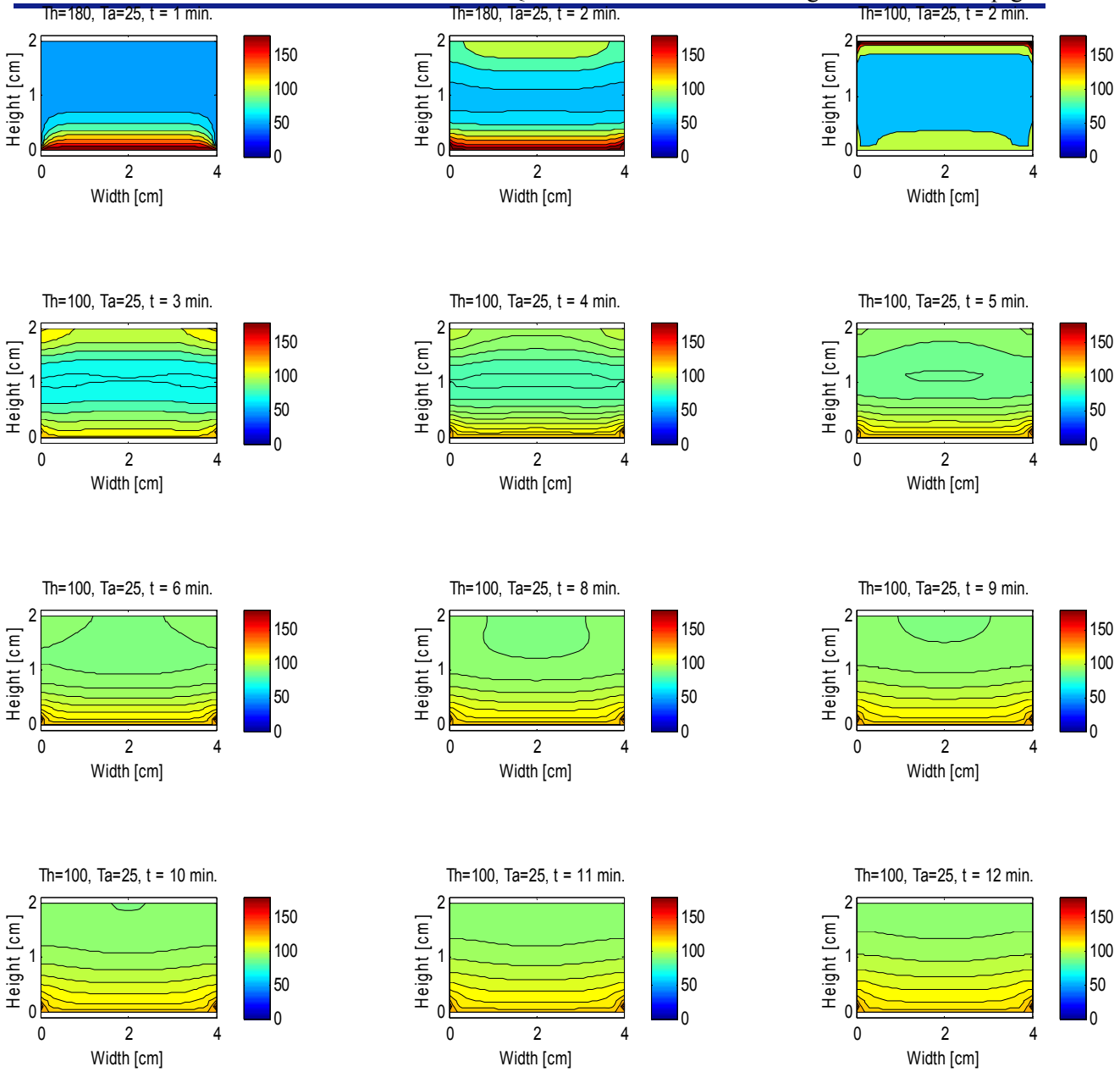

Figure 10.3: Temperature distribution in a pork patty, during cooking. Colours indicate the average temperature in a 1mm square, but contour lines have been smoothed. Note this is not in log-scale

Using the decimal reduction time and the z-value, the temperature fields could be used to obtain a contour plot depicting the survival of *Salmonella*. Firstly, 10^6 cfu were evenly distributed over the patty. Then, for 10 minutes, in each cell, the *Salmonella* was reduced according to the decimal reduction value at the temperature at that point. Figure shows the result of this procedure. The rapid elimination of *Salmonella* at the top and bottom is easily observed, but it is also striking that the greatest number of *Salmonella* persists just above the centre of the patty.

The total number of surviving *Salmonella* was also plotted, as a function of time. The result is shown in Figure 10.4. It turns out that the data from the simulation is very well approximated using a cubicinterpolant.

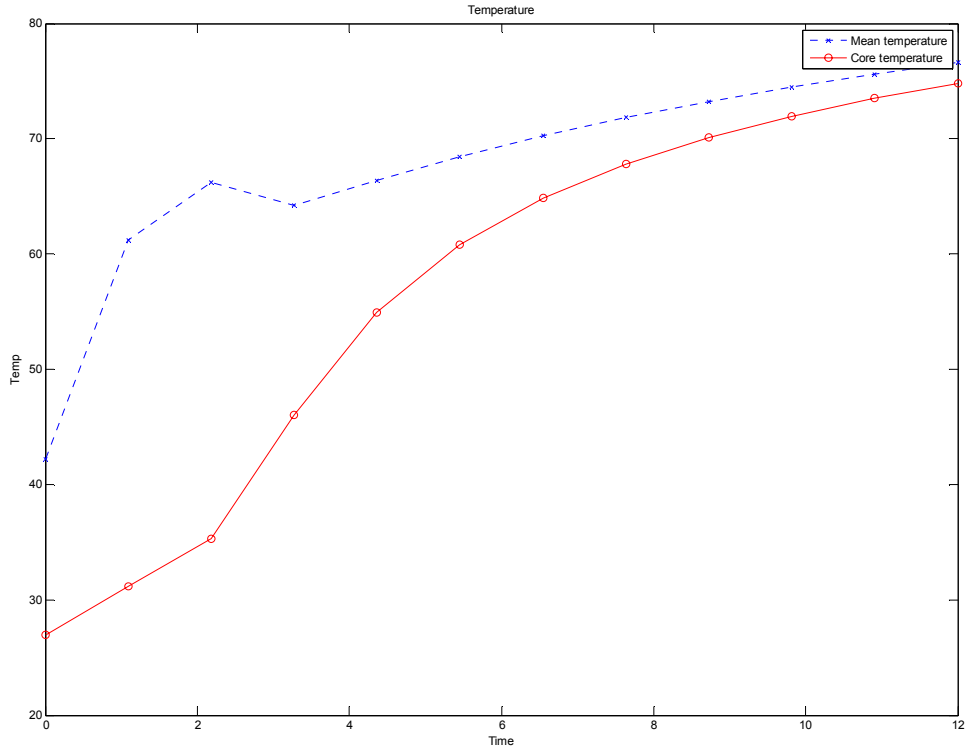

Figure 10.4: Mean temperature and core temperature during cooking of a pork patty.

$$N_5(t) = \log(N_4) + a_1 t + a_2^2 + a_3 t^3. \qquad (10.219)$$

In this formula, N_4 is the number of *Salmonella* in the minced meat after the cross-contamination phase. It was checked for several values of N_4 that the simple addition relation in equation (10.219) holds. In the model, the times t are realizations of the normal distribution (10.216). The coefficients of the cubic interpolant were determined to be

$$a_1 = -4.36 \times 10^{-3}, \qquad (10.220)$$

$$a_2 = -1.02 \times 10^{-2}, \qquad (10.221)$$

$$a_3 = +4.73 \times 10^{-2}. \qquad (10.222)$$

This formula can be considered the final result: the number of log reductions due to cooking of minced pork patties

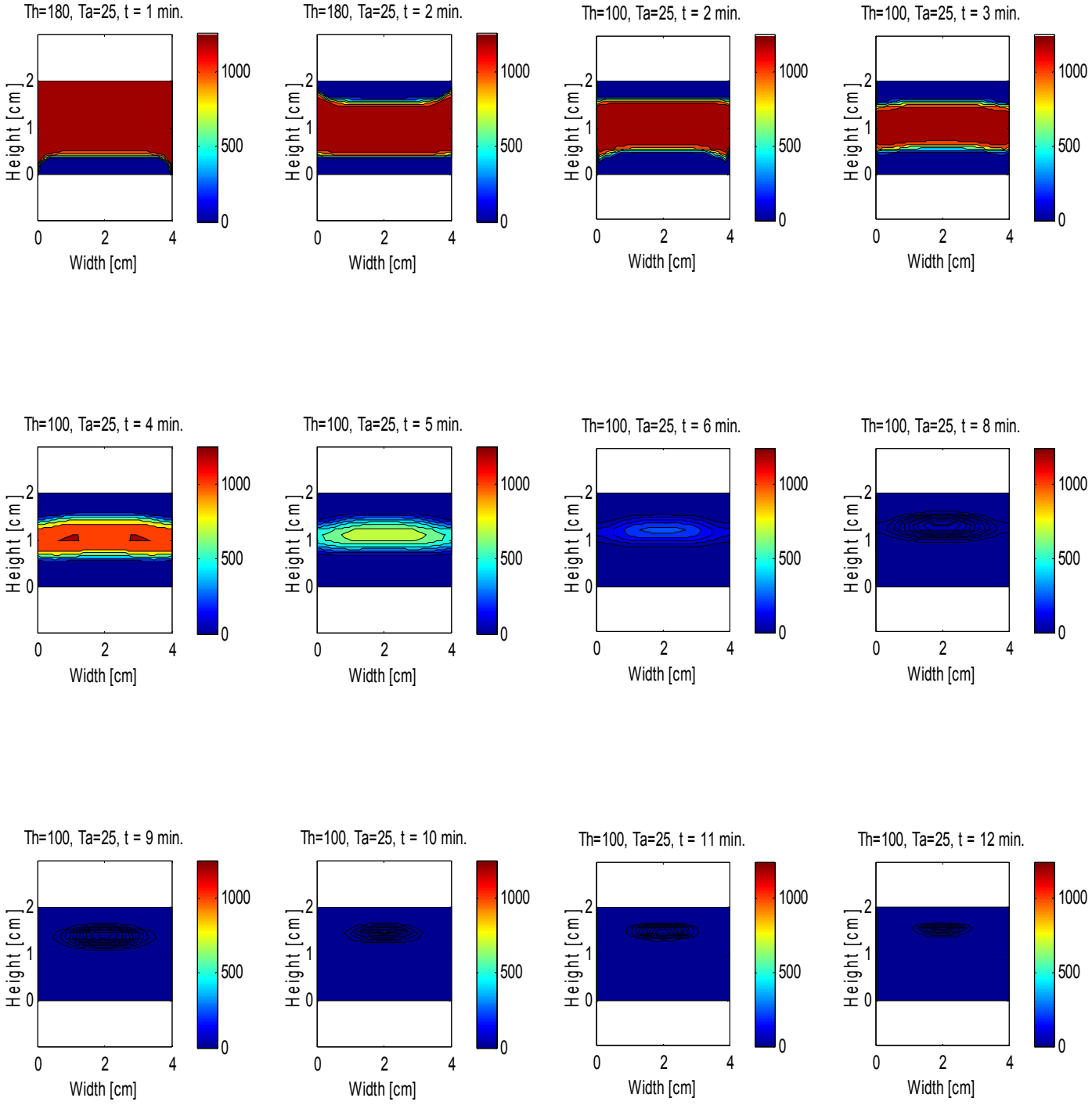

Figure 10.5: Remaining number of *Salmonella* during cooking, after simulated inoculation with 6 log cfu. Note this is not in log-scale.

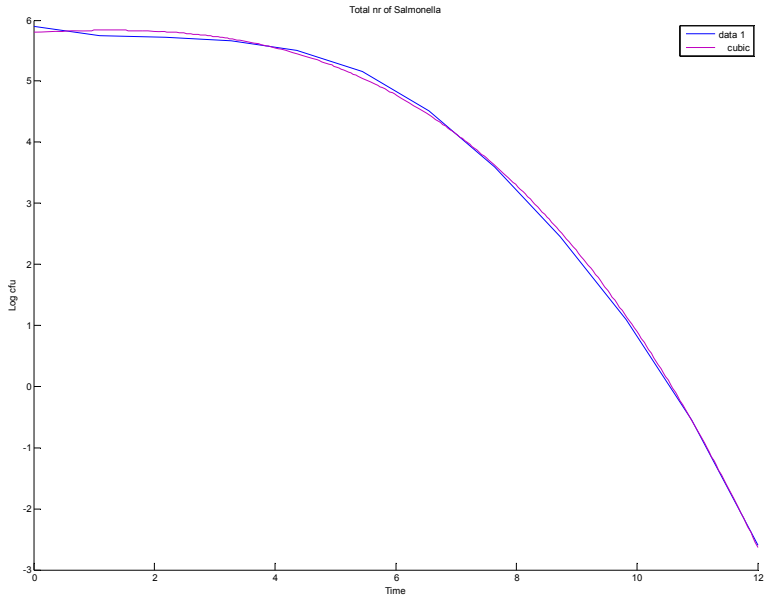

Figure 10.6: Remaining log cfu *Salmonella* during cooking. Overlayed is the cubic interpolant of the data.

10.5 Pork Cut Model

10.5.1 Preparation of pork cuts

We model the preparation of pork cuts mostly as a cross-contamination process. However, we will do so in more detail than the model used for cross-contamination when handling minced meat. We distinguish the following stages:

- Cutting, with transfer between pork cuts, knife, cutting board and hands.
- Washing of board, with cross-contamination between hands and tap
- Washing of knife, with cross-contamination between hands and tap
- Washing of hands, with cross-contamination between hands and tap
- Cutting of the salad

The following tables (Table 10.7-10.9) list the relevant parameters, these are dimensionless transfer coefficients. See the caption of Table 10.9 for references (a-g).

Table 10.7: Cross-contamination during cutting, for pork.

To / From	Pork cut	Knife	Cutting board	Hands
Pork cut		0.21e 0.0125 G=0.05	0.05d 0.013g E=0.032	0.042g 0.087c 0.25d
		0.011f		0.038f G=0.08
Knife	0.94e			
Cutting board				
Hands				

Table 10.8: Cross-contamination during cutting of the salad.

To / From	Salad	Knife	Cutting Board	Hands
Salad		0.21e		
Knife	0.65e 0.51b E=0.58			
Board	0.343g 0.079c 0.65d G=0.26			
	0.103f			
Hands	0.0207, 0.008c, 0.12d G=0.02			

Table 10.9: Survival rates during salad/hand/board/knife washing and cross-contamination between hands and tap.

To[28] / From	Tap	Hand	Salad	Board	Knife
Tap		0.023c			
Hand	0.002c	0.006c 0.035g 0.001a G=0.006	0.021g		
Salad			0.367g		
Board				0.046a 0.000g E=0.02	
Knife					0.000a

a	van Asselt *et al.* 2008, Table 2	e	Kusumaningrum *et al.* 2003, Tables 2 and 3
b	Moore *et al.* 2003, Table 1	f	Luber *et al.* 2006, Table 3
c	Chen *et al.* 2001, Table 4	g	Mylius *et al.* 2007, Table 1
d	Brynestad *et al.* 2008, Table 5		

When multiple entries are present we obtain one value using the arithmetic mean if values are comparable (indicated by E=... in the table), and in order not to make the smaller numbers insignificant, the geometric mean if values differ in orders of magnitude (indicated by G=... in the table).

We proceed with the cross-contamination model as performed before in Section 10.3.3. The transfer coefficients are labelled using the initial letters, e.g. t_{PH} for the transfer coefficient from pork cut to hands. Also the number of cfu on an object is given an obvious abbreviation (p for pork, k for knife, c for chopping board, h for hands, t for tap, s for salad). The vectors containing the numbers of cfu are subscripted with the stage in which they are considered.

$$\begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_1 = (I + T_{PK}T_{PH}T_{PB}) \begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_0 = \begin{pmatrix} 1-t_{PK}-t_{PC}-t_{PH} & t_{KP} & 0 & 0 & 0 & 0 \\ t_{PK} & 1-t_{KP} & 0 & 0 & 0 & 0 \\ t_{PC} & 0 & 1 & 0 & 0 & 0 \\ t_{PH} & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_0 \quad (10.223)$$

Next, the hands, board or knife are washed with a certain probability (See Table 10.5). Denote the events of unsafe behavior by X_H, X_B and X_K. When washing, a certain fraction of *Salmonella* on the hands will contaminate the tap. Afterwards, the hands are re-contaminated when closing the tap.

[28] Values on the diagonal are reduction rates.

In order for cross contamination to be relevant, a side dish must be prepared. Denote this event by X_S. The probabilities of the defined events occurring are binomially distributed, e.g. $X_H = \Re(B(1, p_H))$. Thus,

$$X_B = \begin{cases} 1 & \text{if unsafe board handling} \\ 0 & \text{if safe board handling} \end{cases}, \quad (10.224)$$

$$X_H = \begin{cases} 1 & \text{if hands not washed} \\ 0 & \text{if hands washed} \end{cases}, \quad (10.225)$$

$$X_K = \begin{cases} 1 & \text{if unsafe knife handling} \\ 0 & \text{if safe knife handling} \end{cases}, \quad (10.226)$$

$$X_S = \begin{cases} 1 & \text{if salad was prepared} \\ 0 & \text{if salad was not prepared} \end{cases}. \quad (10.227)$$

Let us assume that washing is in the order: board, knife, hands. Also, we only consider washing in the case that a side dish is prepared ($X_S = 1$). The washing of the board is then modelled using,

$$\begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_2 = \{(1 - X_S X_B) + X_S X_B T_{HT} T_{BB} T_{TH}\} \begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_1. \quad (10.228)$$

Then, the knife is washed,

$$\begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_3 = \{(1 - X_S X_K) + X_S X_K T_{HT} T_{KK} T_{TH}\} \begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_2. \quad (10.229)$$

And finally, the hands are washed,

$$\begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_4 = \{(1 - X_S X_H) + X_S X_H T_{HT} T_{HH} T_{HT}\} \begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_3. \quad (10.230)$$

The actual cutting of the salad is given by,

$$\begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_5 = \{(1 - X_S) + X_S T_{HS} T_{BS} T_{KS}\} \begin{pmatrix} p \\ k \\ c \\ h \\ t \\ s \end{pmatrix}_4. \quad (10.231)$$

Finally, we chain the matrices together to find p_5 and s_5, the final contamination on thee pork and salad, in terms of p_0, the initial contamination on the pork. In order to obtain a manageable expression we define

$$F = t_{HT} + t_{TH} - 2, \quad (10.232)$$

$$G = t_{TH} + t_{HH}(t_{HT} - 1). \quad (10.233)$$

Some algebra then shows that

$$\begin{aligned} s_5 = p_0 (& \\ & t_{BS} t_{PB} ((t_{BB} - 1) X_B X_S + 1) + \\ & t_{KS} t_{PK} ((t_{KK} - 1) X_K X_S + 1) + (\\ & \quad (t_{HT} - 1) X_H X_S + 1 + (\\ & \quad\quad (G - t_{HH}) X_H + \\ & \quad\quad F(1 + (G(F+1) - 1) X_H X_S) \\ & \quad\quad (X_K + X_B + F(2+F) X_K X_B X_S) \\ & \quad)t_{HT} X_S \\ &)t_{HS} t_{PH} \\ ) X_S & \end{aligned}, \quad (10.234)$$

$$p_5 = p_0(1 - t_{PB} - t_{PH} - t_{PK}) . \qquad (10.235)$$

We may simplify this a bit more by introducing $Y_B = X_B X_s$, and the same notation for the salad and hands. Also, we set $s_B = t_{BB} - 1$ and $t_{PBS} = t_{PB} t_{BS}$. Then

$$\begin{aligned} s_5 = p_0 (& \\ & (1 + s_B Y_B) t_{PBS} + \\ & (1 + s_K Y_K) t_{PKS} + \\ & (1 + s_H Y_H) t_{PHS} + (\\ & \quad (G - s_H - 1) Y_H + \\ & \quad F(1 + (G(F+1) - 1) Y_H) \\ & \quad (Y_K + Y_B + F(2+F) Y_K Y_B) \\ &) t_{HT} t_{PHS} \\ ) X_S & \end{aligned} . \qquad (10.236)$$

10.5.2 Cooking of pork cuts

With regards to the cooking of pork cuts, we assume no *Salmonella* survives the cooking process. The rationale for this claim is that the *Salmonella* are located at the pork cut surface, and thus all of them are directly heated.

10.6 Dry Cured Sausage

Following Lund *et al.* 2000, Chapter 19, we define a dry cured sausage as a sausage having its a_W reduced to at least 0.9 (corresponding to between 25% and 50% moisture loss (Anonymous 1997)) and having its pH reduced to at least 5.3, by means of a fermentation and drying process. See also Bacus 1986, (Chapter 4), for a overview from a microbiological perspective of the production of fermented sausages.

Sausages with an a_W between 0.9 and 0.95 are called semi-dry, and will not be considered here. We assume that the basis of the sausage consists of 80% of minced pork meat.

The preparation of a dry cured sausage can be divided into several stages, as described by Lund *et al.* 2000, (Fig. 19-2). Firstly, the raw ingredients are salted, the sausage is filled, and if applicable a starter culture is added (e.g. *P. acidilactici, P. pentosaceus* or *Micrococcus* strains (Bacus 1986)). Then, the actual fermentation takes place, lowering the pH. This may be done at temperatures between 25°C and 43°C (North-American Style), or <25°C (European Style) (ICMSF 2005). However, not all time-temperature combinations are safe. In Anonymous 1997 a degree-hour control is suggested, i.e. the product of the temperature in excess of 60 (in Fahrenheit) and time (in hours) should not exceed a certain predefined value. The model we propose for modelling log-reductions does not depend on the specific temperature during fermentation, but rather on the final pH obtained.

Finally, during an extended drying period the a_W is lowered. This phase is also called 'ripening' or 'ageing' and gives the product its typical flavour. If the resulting sausage is stored, the temperature may not exceed 25 degrees.

Table 10.10: Summary of the preparation of dry cured sausage. In the following table we summarise the relevant conditions of the preparation process.

Stage	Time	T[°C]	pH start	pH end	aW start	aW end
Salting	-	<5	5.5	5.5	0.96	0.96
Fermentation	2-4 days	25-43	5.5	4.6-5.3	0.96	0.96
Drying	>4 weeks	10-15	<5.3	<5.3	0.96	0.90
Storage		<25	<5.3	<5.3	0.90	0.90

Under such environmental circumstances, survival or even growth is possible. See for example Goepfert & Chung 1969, Smith *et al.* 1975, Baran & Stevenson 1975 or Masters *et al.* 1981.

Comparing this table to Table 10.2 one sees that the environmental factors during the preparation are close to the extremes of the theoretical growth limits. Another complication is that the growth limits are interdependent. For example, at low pH the minimum and maximum growth temperatures could be larger than those listed. Usually, this interdependence is modelled by a minimal convex polyhedron, the 'true' region in which growth is possible (see e.g. Koutsoumanis *et al.* 2006). Somewhere in this polyhedron will be the optimal point $T = T_{\mathrm{opt}}$, $pH = pH_{\mathrm{opt}}$, etc, where the growth rate is at its maximum value. Moving towards the boundary, the growth rate will diminish, reaching zero at the boundary. This leads to the following schematic figure given in Figure 10.7.

The same type of figure may be drawn for the lag phase parameter.

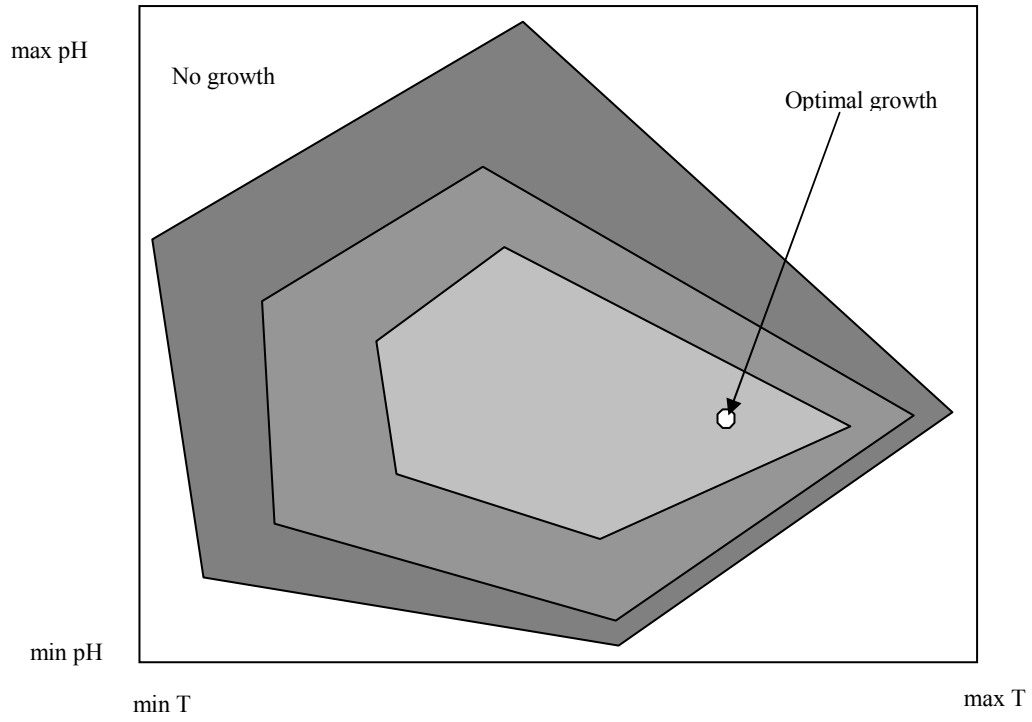

Figure 10.7: Growth, dependent on environmental factors.

In reality, other factors also play a role, most notably a_W (or NaCl) and availability of substrate. Also, previous environmental factors play an important role, a well known example being an extended lag phase in the case of temperature shocks (McKellar & Lu 2004).

In a dry fermented sausage, the environmental parameters are typically rather close to the borders of the growth/no growth region, which makes application of a growth model dubious. Furthermore, pH and water activity are not constant during the process.

Considering these complicating factors, we conclude that application of a primary and secondary growth model can not be justified in this case. Rather, we choose to base our model on measured data. We will use a previously developed model, as described in the next section.
This model was based on many data sets obtained from several fermentations. A complicating factor is that fermentations may also fail. Failure may be attributed to several events, e.g. failure to reach a low pH during fermentation, or failure to reach a high a_W during drying (Bacus 1986). These events may have several causes, e.g. improper sanitation, inadequate temperature or humidity control, low salt concentration (ICMSF 2005). Also, so-called DFD-meat has a higher pH value than normal pig meat, opening the possibility of insufficiently lowered pH (see e.g. Guàrdia *et al.* 2005 or Feiner 2006 (Chapters 4 and 16)

Modelling these events is not feasible, and data on percentages of failed fermentations are not available. Therefore, we propose an alternative route to estimate the occurrence and result of fermentation failure. The approach is based on the assumption that failed fermentation is associated to *Salmonella* outbreaks, while successful fermentation is associated to sporadic salmonellosis cases (incidental illness due to high *Salmonella* load, even though the fermentation was successful). Even subject to successful fermentation, a dry cured sausage may still cause illness, due to e.g. high initial contamination levels of the minced meat used. The following table (Table 10.11) summarises the *Salmonella* outbreaks due to fermented sausage over the past ten years.

From Eurostat we have approximately 4.7×10^9 kg of sausage consumed per year (not necessarily fermented). A batch is approximately 250 kg Alban, Olsen *et al.* 2002, which means that approximately 18.8 million batches per year are consumed. From the above table we find a number of outbreaks of about 1 per year in the EU, the probability of a batch causing an outbreak is $1/18.8 \times 10^6$.

A typical dry fermented sausage is 250g (Alban, Olsen *et al.* 2002), leading to 1.000 sausages per batch. From the above table we see that per batch, on average, approximately 1000 persons get ill. Thus the probability of getting ill from a sausage of a failed batch is about 1.

Thus, when eating a sausage, there is a $1/18.8 \times 10^6$ probability of becoming ill. The model runs 10,000 fermented sausages per iteration. Before encountering one illness, we expect around 1800 iterations (this takes about one hour).

Not only the running time of the model is prohibitive, also the numbers of illness are very low (1000/year) compared to the number of illnesses stemming for successful batches (approximately 18000/year, calculated from our model).

Table 10.11: Reported outbreaks in the EU.

MS (origin of product)	Data	Reported Cases	Estimated Illnesses	Source
NW	2006	54	2020	Emberland *et al.* 2006
SP	2006	10	374	Nygård *et al.* 2007
IT	2004	63	3509	Luzzi *et al.* 2007
DE	2004	525	11340	Gilsdorf *et al.* 2005
DE[29]	2001	192	4147	Bremer *et al.* 2004

For these reasons (lack of data on failed fermentation, prohibitive model running time and comparatively small number of cases), we choose not to model failed fermentations, but only successful fermentations instead. Appendix 10.6 gives some directions for modelling failed fermentation.

10.6.1 *Salmonella* Reduction Model

A model for successful fermentation, based on a polynomial fit of log reductions of *Salmonella*, dependent on pH, a_W and temperature, was proposed in Hwang *et al.* 2008. The authors also provide an extensive overview of previous measurements, and show good correspondence with those data. We reproduce their results in terms of log reductions during fermentation (F, [log cfu]), drying (D, [log cfu]) and storage (S, [log cfu/day]), dependent on pH at beginning of drying (a), a_W at end of drying (b) and temperature of storage (c)

$$F(a) = -90.5a + 8.9a^2 + 230.9, \quad (10.237)$$

$$D(a,b) = 5.6a + 21.4b - 11.7ab + 0.5a^2 + 8.1b^2 - 3.6, \quad (10.238)$$

$$S(a,b,c) = -6.0a - 72.4b + 0.14c + 7.0ab - 0.1bc + 20b^2 + 47. \quad (10.239)$$

We use the data listed in the paper to obtain distributions for the parameters. The temperature had a very flat histogram and is best described by a uniform distribution. The water activity and pH were assumed to be normally distributed (Figure 10.8), but the number of data points is low. The usage of a normal distribution does open the possibility for rare occurrences of extreme values. The fitted distributions are given by,

$$a = \Re(N(4.29, 0.07)), \quad (10.240)$$

$$b = \Re(N(0.87, 0.002)), \quad (10.241)$$

$$c = \Re(U(4, 25)). \quad (10.242)$$

[29] Only 4 days of fermentation of the implicated type of sausage.

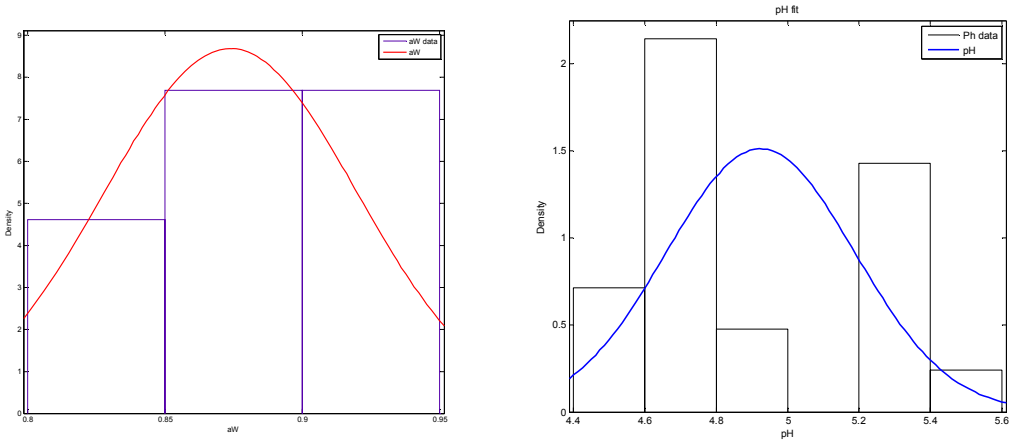

Figure 10.8: Water activity and pH, fit according to the experiments in Hwang *et al.* 2008.

Finally, we need the number of days of storage, which are not available from the paper. We do have a few numbers from other sources, summarised in Table 10.12.

Note that Table 10.12 contains a variety of dry fermented sausage products, hence the large variation in storage time. From this table we can conclude that there is a large range to the number of storage days, but we cannot deduce anything on the distribution of storage time. Thus, we describe storage time T using a uniform distribution

$$T = \Re(U(4, 90)) \tag{10.243}$$

Table 10.12: Reported drying periods of dry fermented sausage.

Source	Number of days
Ihnot *et al.* 1998	56
Krämer 2002, page 300	3-10
Feiner 2006, Chapter 17	80-90 4-5

10.7 Model Assumptions

Throughout the preceding Sections several assumptions were explicitly or implicitly made. In this Section we summarize the most important ones.

- Minced meat, pork cut and fermented sausage cover the pork spectrum in terms of microbial hazard.
- During transport and storage growth models are adequate for modelling *Salmonella* behaviour.
- For minced meat, temperature abuse during cooking is the most important factor. This is well modelled using a physical model taking only diffusion into account.
- For pork cuts, cross-contamination to utensils and a side dish is the most important factor. (But note that cross-contamination is also modelled for minced meat). This is well modelled using fixed transfer rates.
- For fermented sausage, the model of Hwang *et al.* 2008 for successful fermentation is adequate.

- Failed fermentation has a negligible effect in terms of cases of illness.
- We assume our parameter estimates are reasonable (to be verified using uncertainty analysis) and capture the variability.

10.8 Parameter Estimation & Identification of Key Data Gaps

In this Section we summarise the parameter estimations made throughout this report for easy reference (Table 10.13).

The most serious data gaps are the time temperature combinations for the transport and storage phases. We frequently had to resort to data from other MS. Also, transfer coefficients and probabilities of hazardous actions are parameterised with a little amount of data only.

10.9 Results

The results obtained during the Preparation & Consumption modules of the QMRA are dependent on the output of the previous modules (Farm, Transport & Lairage, Slaughter & Processing). Therefore, our discussion will be based on relative effects of phases within this module.

Figure 10.9 shows the results for each MS obtained from the Pork Cut module.

The figures show the average contamination of positive products (top panel), and the prevalences (lower panel), over the phases. The phase and unit under investigation is listed at the ticks of the x-axis. The vertical axis is in units of average 'log cfu'. Here the geometric average is taken over all products within one iteration (typically 10,000), and an arithmetic average over the iterations. The iterations induce variability in the results. This variability is represented by 'variability bars', having ticks at the 5th percentile, 50th percentile (median) and 95th percentile.

It should be kept in mind at all times that the prevalence graphs and bar chart should be considered as a whole. If a slight drop is observed in average log cfu contamination per positive product, it can very well be that there is also a tremendous drop in prevalence (in the case that the reduction was such that many products dropped to zero *Salmonella*, while the remaining products were not much affected). Thus, the decrease of *Salmonella* numbers might be higher than it seems when superficially considering the bar charts only.

The phases from transport to refrigeration show little increases in *Salmonella* numbers for MS1, MS2 and MS3. Although the possibility of growth exists, the effect is minimal. There is a larger increase for MS4, due to the different parameter estimates used (see Section 10.1.3). Since no inactivation or cross-contamination is possible, the prevalence within each MS remain constant, typically around 1×10^{-3} (MS1, MS3); 0.04 (MS2) or 5×10^{-3} (MS4).

Table 10.13: Parameter values used in the Consumer & Preparation phases. First index indicates cluster number (1=MS2, 2=MS1, 3=MS3, 4=MS4). When expressions for the distributions were too lengthy, references to the equations are listed instead.

Parameter	Value	Description	Reference
$T_{*,1}$	110	Transport time slaughterhouse to retail.	Derens *et al.* 2006
$\mathcal{T}_{*,1}$	See eq. (10.190)	Temperature during transport slaughterhouse to retail.	Peck *et al.* 2006
$T_{1,2} = T_{2,2} = T_{3,2}$	See eq. (10.191)	Transport time from retail to the domestic home	Appendix 10.1
$T_{4,2}$	$\Re(BP(10,45,300))$	Transport time from retail to the domestic home	Appendix 10.1
$\mathcal{T}_{1,2} = \mathcal{T}_{2,2} = \mathcal{T}_{3,2}$	See eq. (10.194)	Temperature during transport from retail to the domestic home	Appendix 10.1
$\mathcal{T}_{4,2}$	$\Re(BP(6,10,20))$	Temperature during transport from retail to the domestic home	Appendix 10.1
$T_{*,3}$	See eq. (10.193)	Storage time in the refrigerator	Appendix 10.1
$\mathcal{T}_{*,3}$	See eq. (10.196)	Temperature in the refrigerator	Appendix 10.1
Growth rates/lag phases for each product			Appendix 10.1
Cross-contamination transfer parameters for minced meat			Appendix 10.1
Probabilities of hazardous actions in the domestic kitchen			Appendix 10.1
ρ	1200 kg/m^3	Density of minced meat	Torstveit & Magnussen 1999
c_p	3500 J/(kg K)	Specific heat capacity of minced meat	Hardarsson, 1998; Rimestad *et al.*, 1995
λ	0.49 W/m K	Thermal conductivity of minced meat	Heldman & Lund, 1992
T_H	180°C (initial) 100°C (later)	Heating temperature of minced meat	Estimated from several recommendations
T_5	See eq. (10.216)	Cooking time	Estimated from several recommendations
D_{60}	4.8	D-value in beef patty	Murphy *et al.* 2002
z	9.14	z-value in beef patty	Murphy *et al.* 2002
Cross-contamination transfer parameters for pork chop (cutting pork)			Appendix 10.1
Cross-contamination transfer parameters for pork chop (cutting salad)			Appendix 10.1
Survival rates during washing of hands/salad/board/knife			Table 10.9
a	$\Re(N(4.29,0.07))$	pH at beginning of drying (fermentation process)	Hwang *et al.* 2008
b	$\Re(N(0.87,0.002))$	a_W after drying (fermentation process)	Hwang *et al.* 2008
c	$\Re(U(4,25))$	Temperature during storage (fermentation process)	Hwang *et al.* 2008
T	$\Re(U(4,90))$	Duration of storage (fermentation process)	Table 10.12

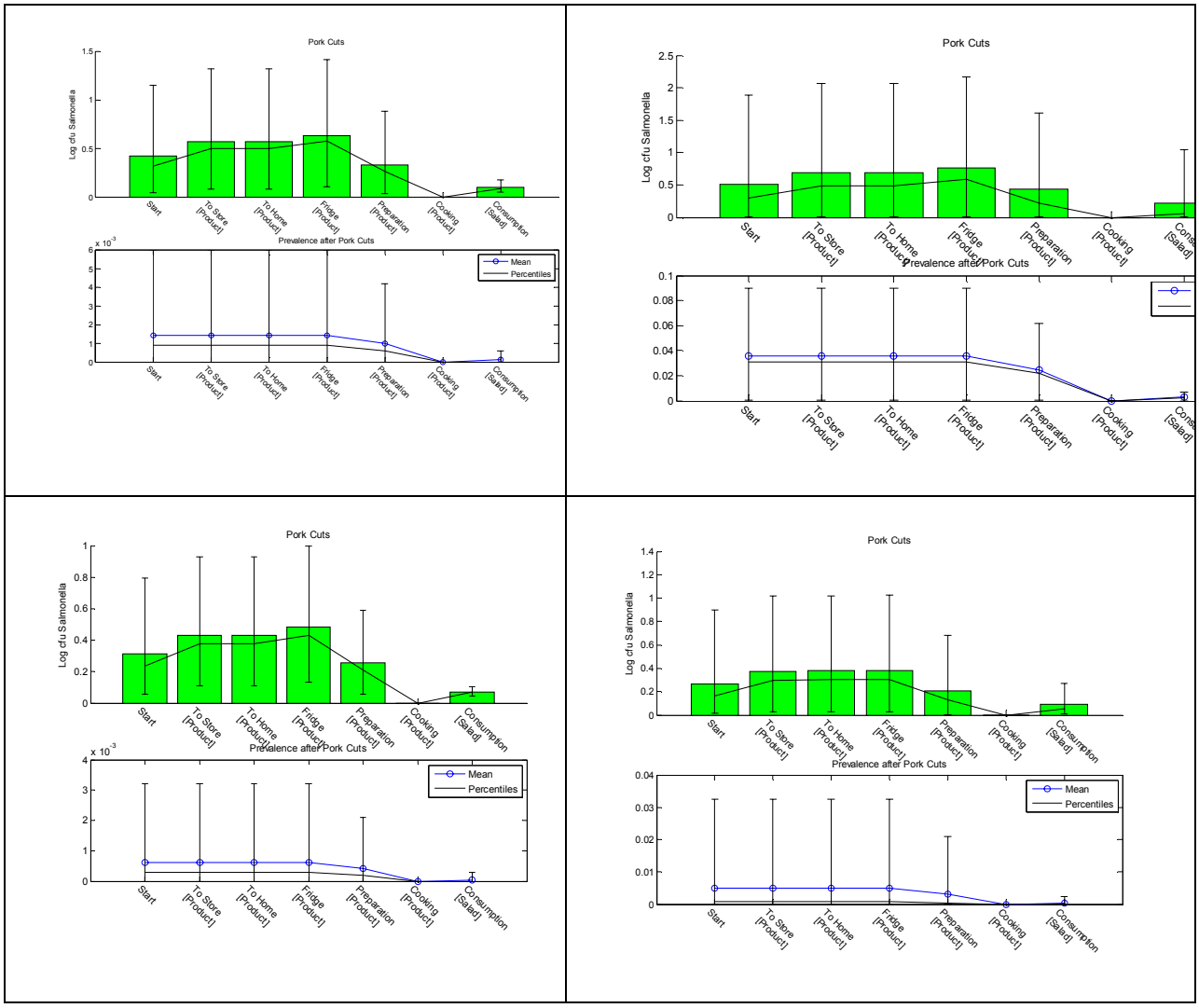

Figure 10.9: *Salmonella* numbers (top panel) and prevalence (bottom panel) for pork cuts during stages of the Preparation & Consumption module for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right).

Note that with prevalence's this low, the log cfu numbers are based on a few products only, out of the 10,000 products per iteration.

The next phase is preparation, where cross-contamination and inactivation play important roles. We observe a decrease in both prevalence and numbers, which is due to *Salmonella* ending up on the salad (which is not included in the total number at this point) and due to *Salmonella* transferred to hands, knife or board being inactivated.

After preparation follows cooking, which we assume kills all pathogens on the product. However, *Salmonella* still reside on the salad, which is finally consumed.

We now consider the Minced Meat Module, which contains the same phases as the pork cut module, shown in Figure 10.10.

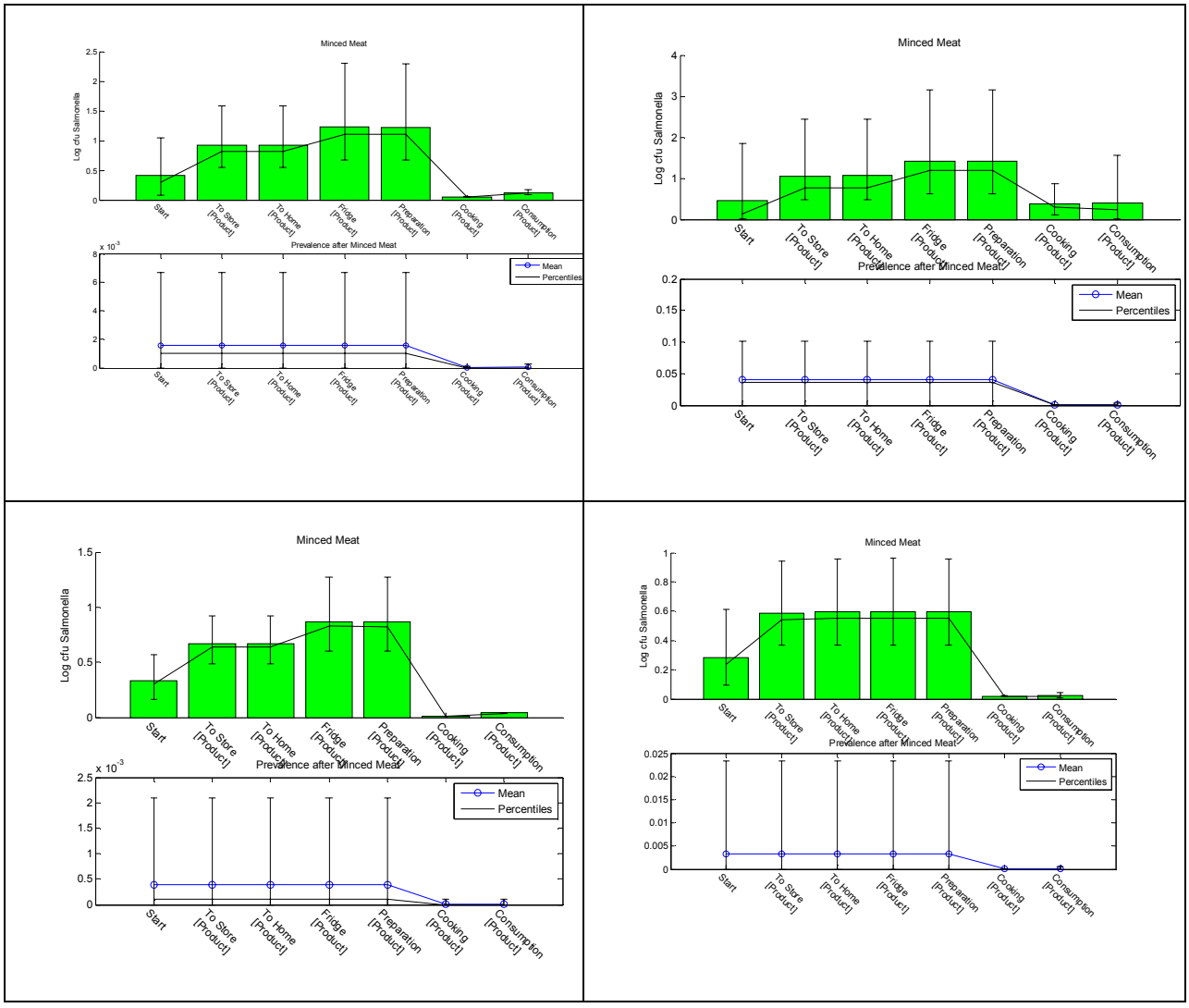

Figure 10.10: *Salmonella* numbers (top panel) and prevalence (bottom panel) for minced meat during stages of the Preparation & Consumption module for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right)

As compared to pork cuts, more growth is observed during transport to the store and in the fridge. This must be attributed to growth factors of minced meat, since travel times and temperatures are the same as used in the pork cuts module.

Next, the preparation phase seems to have little effect on the bacterial numbers. Of course, the preparation phase is very different from that of pork cuts (minced meat is not cut).

The cooking phase is not fully efficient. Although the prevalence drops dramatically, there are still some *Salmonella* remaining on contaminated products. Finally, during the consumption stage, the numbers on the salad are added to those on the patty. This increases the numbers, but also the prevalence (since salads may be contaminated while the associated patty is not).

Finally, we present the results for fermented sausages in Figure 10.11.

For all four case study MSs we observe a continuous decrease in prevalence, as expected since we do not explicitly model failed fermentation. The bacterial numbers go down during curing and drying, but seem to go up slightly at storage. The model does allow for growth, which is most pronounced in the storage phase, because it is the phase where previous pH or a_W deficiencies have most opportunity to result in favourable growth conditions. When pH or a_W allow for growth, additionally time and temperature combinations during storage do in principle allow for growth.

For each of these three products, the averages presented in Figure 10.12-10.14 are averages of distributions of *Salmonella* numbers of the products. It is interesting to consider those distributions at consumption, since they drive the risk by means of the dose-response relation.

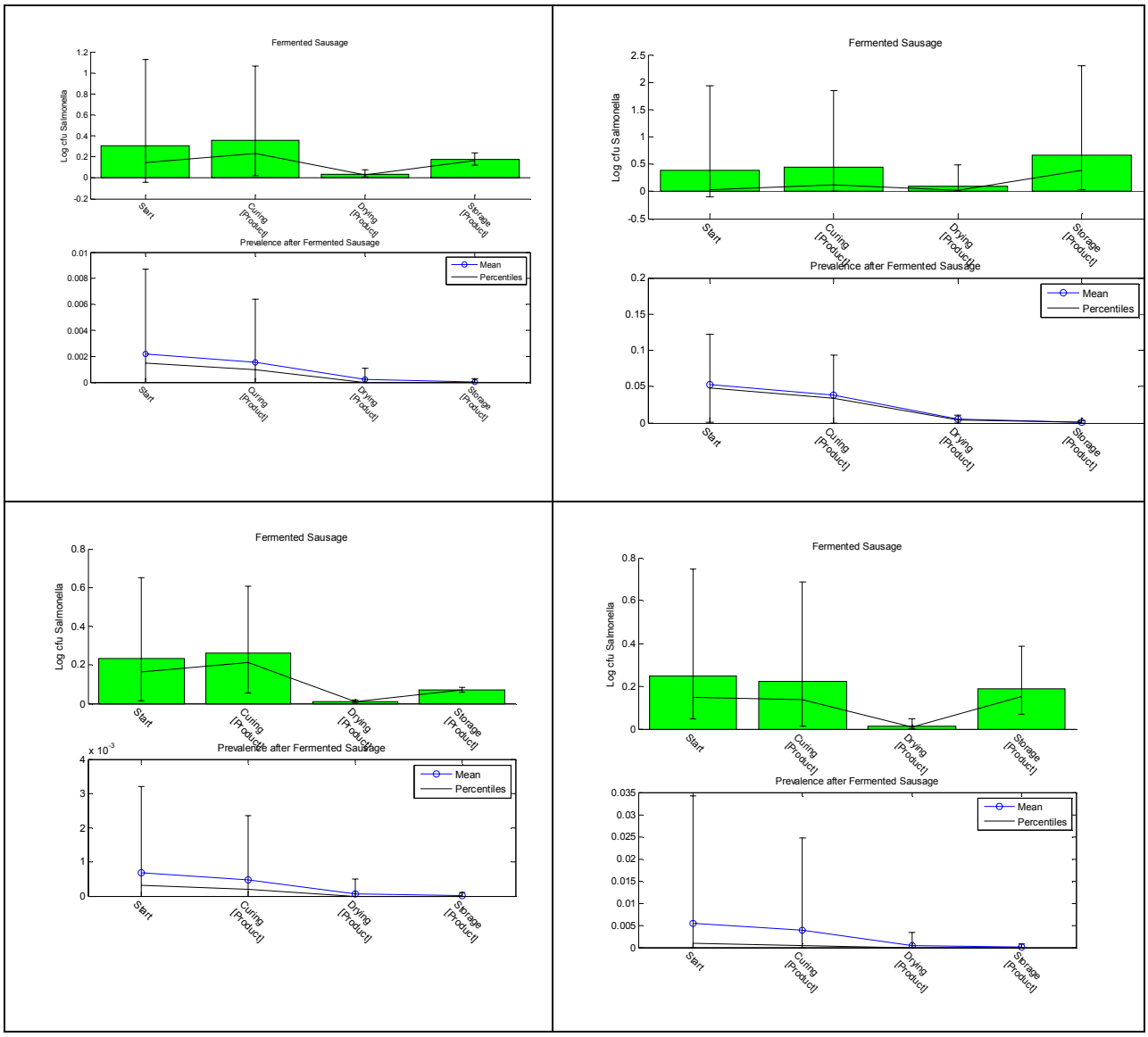

Figure 10.11: *Salmonella* numbers (top panel) and prevalence (bottom panel) for fermented sausage during stages of the Preparation & Consumption module for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right)

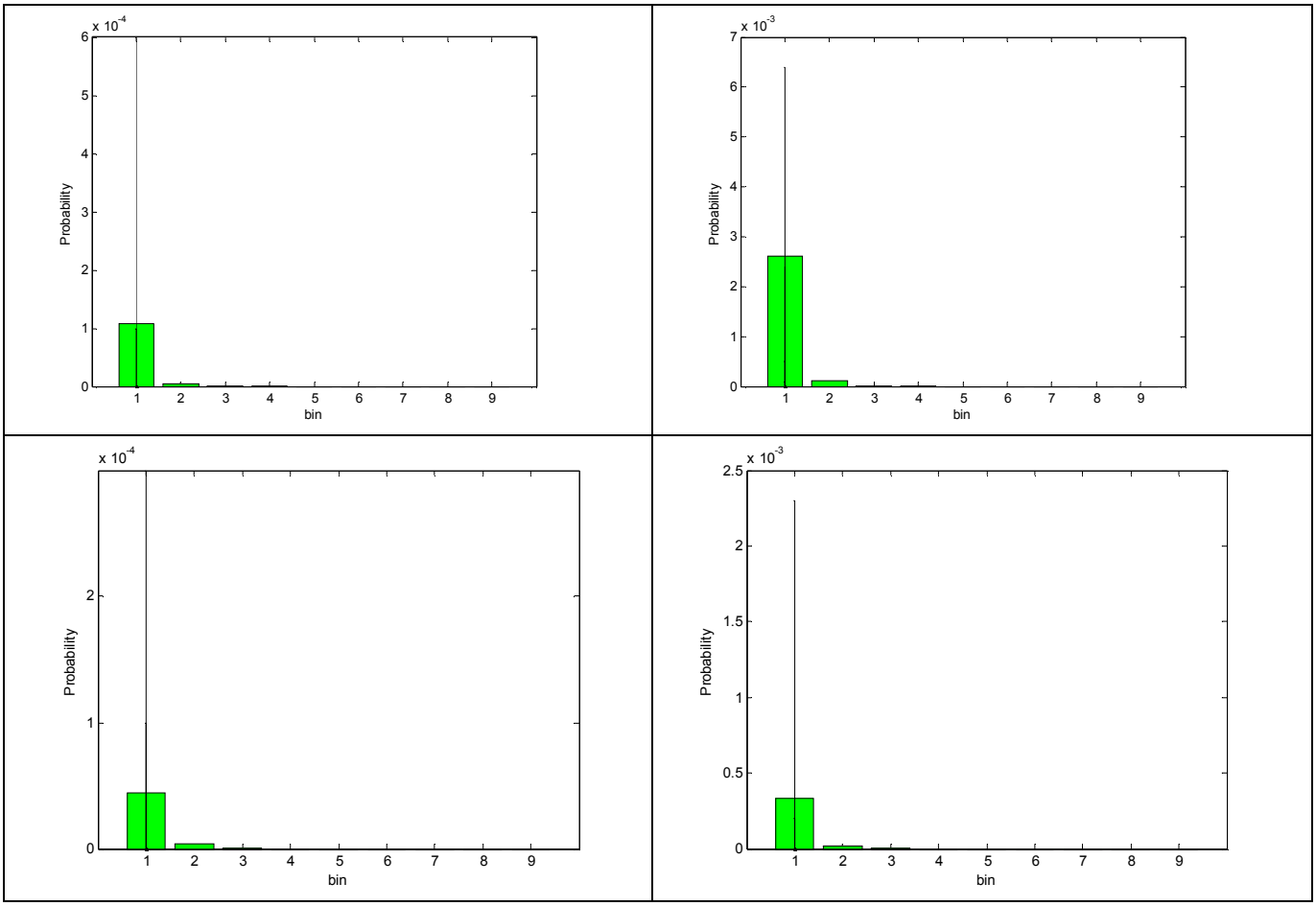

Figure 10.12: Distribution of *Salmonella* dose for pork cuts for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right). Vertical axis shows probability of the dose, horizontal axis is the dose category (1 stands for 1-10 *Salmonella*, 2 stands for 10-100 *Salmonella*, etc.)

The distributions are presented in Figure 10.12 for pork cuts; Figure 10.13 for minced meat and Figure 10.14 for fermented sausage. The bins are categories, bin 1 represents 10^0 to 10^1 *Salmonella*, bin 2 represents 10^1 to 10^2 *Salmonella*, etc. On the vertical axis is the probability of a product having such a *Salmonella* load. The products show similar behaviour of the contaminated products (uncontaminated products are not shown), the probability decreases approximately exponentially. Although the probability of an extreme dose, like 10^6 *Salmonella*, is low, it is not zero. Such behaviour is also found in other risk assessments, for example Nauta *et al.* 2007, (Fig. 3).

For pork cuts (Figure 10.12) it can be seen that the dose is often very small at less than 10 *Salmonella*. However, as remarked above, this dose does vary and pork cuts in MS2, in particular, were predicted to be highly contaminated.

For minced meat (Figure 10.13) the doses are higher than for pork cuts. Most of the ingested doses are less than 10 *Salmonella* for all MSs.

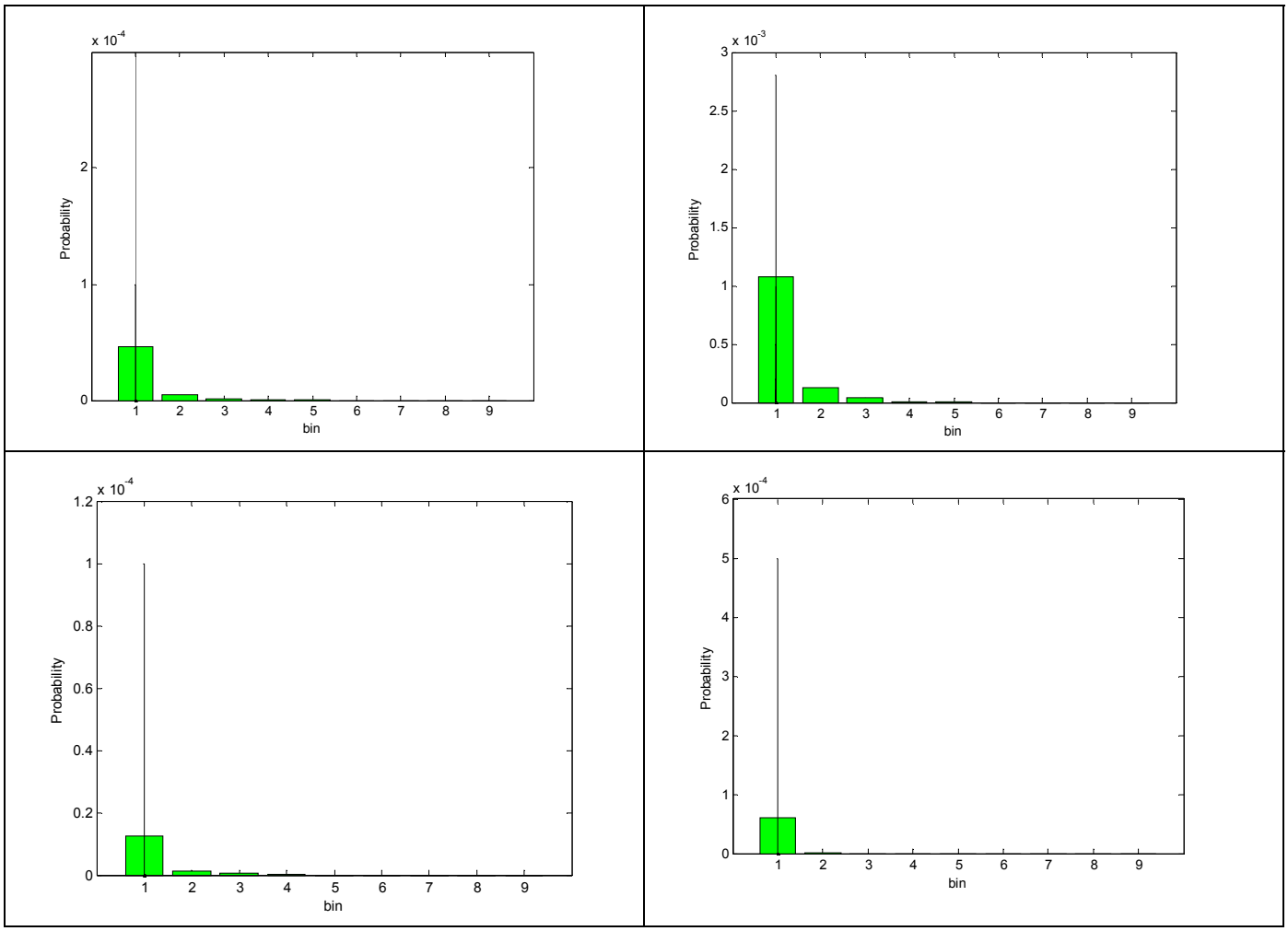

Figure 10.13: Distribution of *Salmonella* dose for minced meat for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right). Vertical axis shows probability of the dose, horizontal axis is the dose category (1 stands for 1-10 *Salmonella*, 2 stands for 10-100 *Salmonella*, etc.)

Finally, Figure 10.14 shows the distribution of *Salmonella* doses for fermented sausage. From this figure, it can be seen that, as before, most ingested contaminated servings of fermented sausage contain less than 10 *Salmonella*.

10.10 Sensitivity Analysis

For the Preparation & Consumption module we have three response variables we conduct three sensitivity analyses, one for each product type; pork cuts, minced meat and fermented sausage. This is because many of the parameters in the module specifically affect one product type only, or have different parameter estimates depending on the product type.

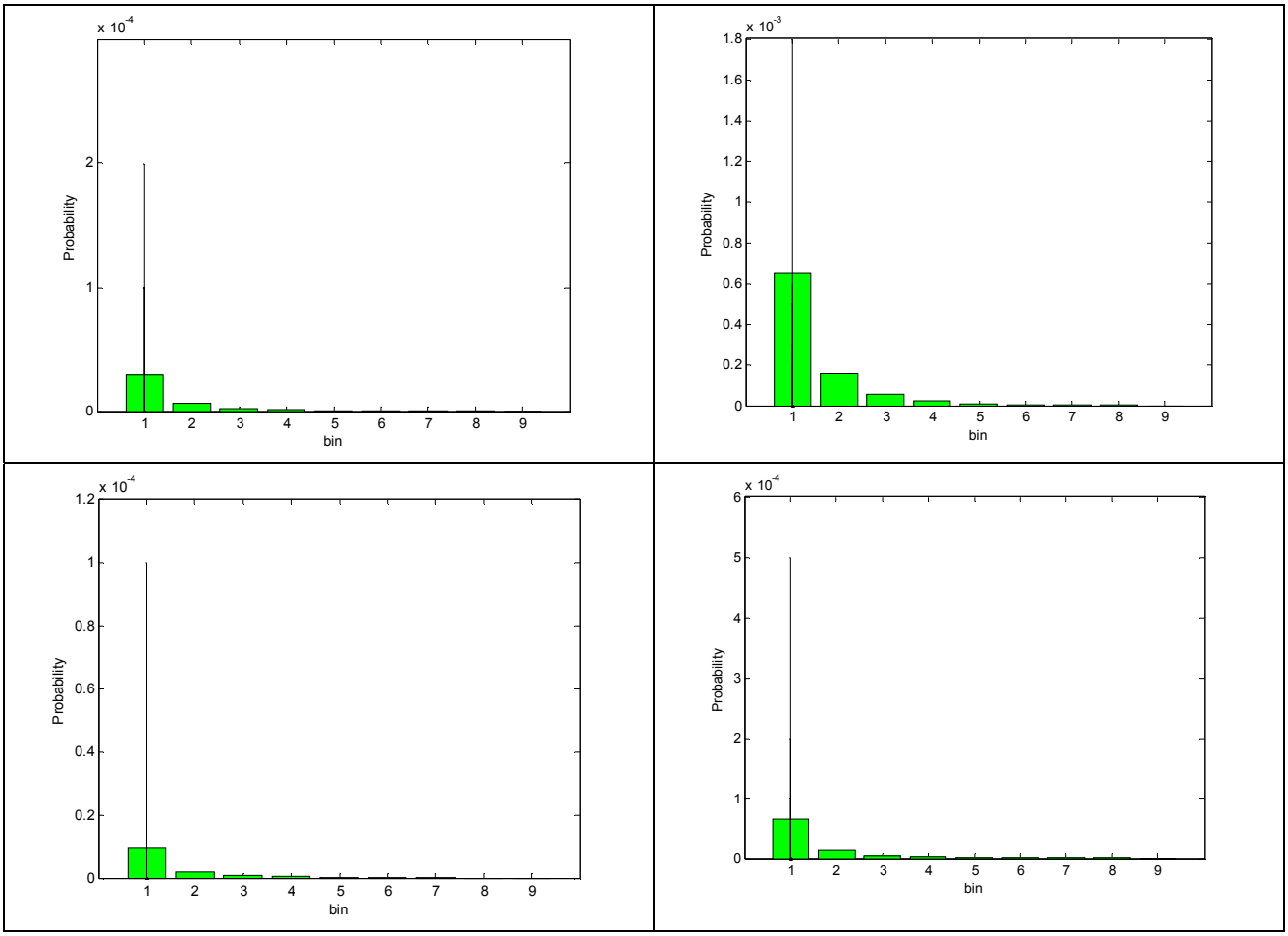

Figure 10.14: Distribution of *Salmonella* dose for fermented sausage for the 4 case study MSs. MS1 (top left); MS2 (top right); MS3 (bottom left); MS4 (bottom right). Vertical axis shows probability of the dose, horizontal axis is the dose category (1 stands for 1-10 *Salmonella*, 2 stands for 10-100 *Salmonella*, etc.)

10.10.1 Pork cuts

For pork cuts we use the number of *Salmonella* on the pork cut at the point of consumption as the response variable. The results for the pork cuts sensitivity analysis are shown in Figure 10.15 to Figure 10.18. Here we can see a difference between member states. For MS2 (the MS with the highest prevalence at the point of consumption and higher variation of doses) the consumption of salad is the most significant factor, while for MS1 it is the knife cleaning and for MS3 it is the fridge temperature.

10.10.2 Minced meat

For the Preparation & Consumption minced meat module we use the number of *Salmonella* on the minced meat portions at the point of consumption as the response variable. The results are shown in Figure 10.19 to Figure 10.22. There seems to be variation between MSs as to the most significant parameters for minced meat, but board cleaning, salad consumption, fridge temperature and fridge time seem significant for all MSs. The magnitude of the difference in values between these parameters is generally small, suggesting that there may not be a large amount of difference in their importance.

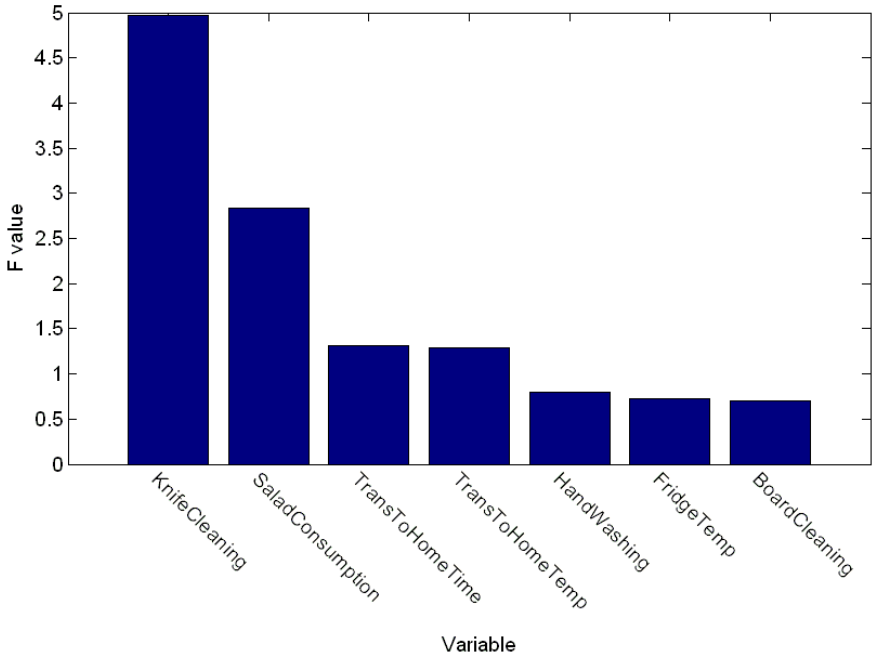

Figure 10.15: Preparation & Consumption: Pork cuts sensitivity analysis for MS1

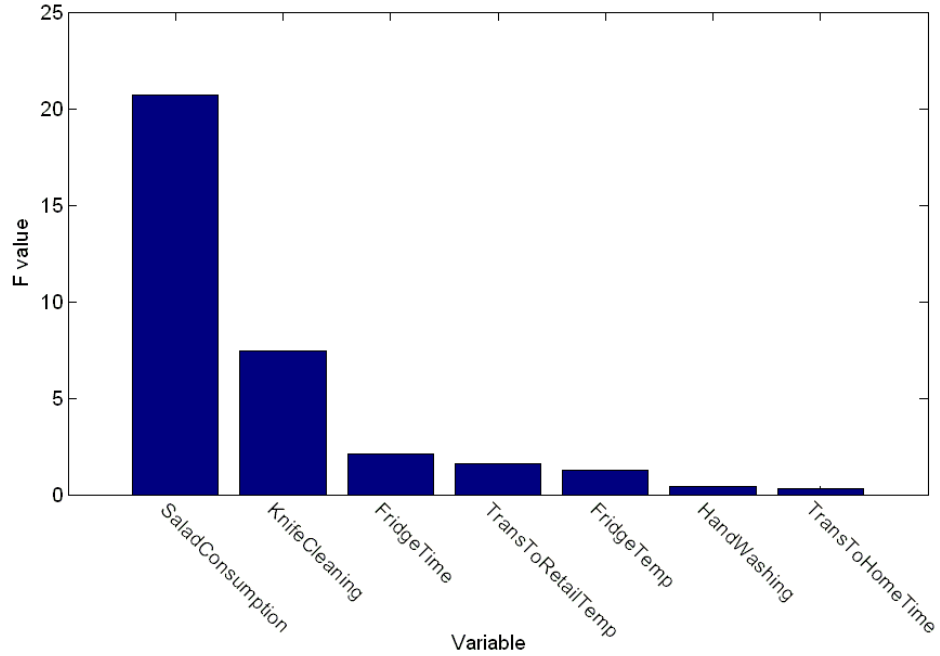

Figure 10.16: Preparation & Consumption: Pork cuts sensitivity analysis for MS2

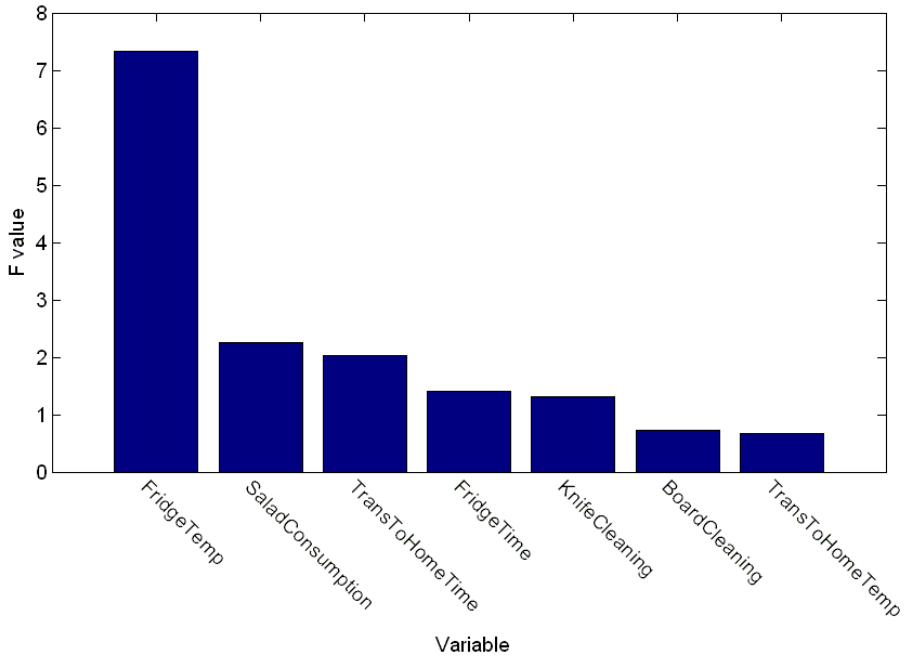

Figure 10.17: Preparation & Consumption: Pork cuts sensitivity analysis for MS3

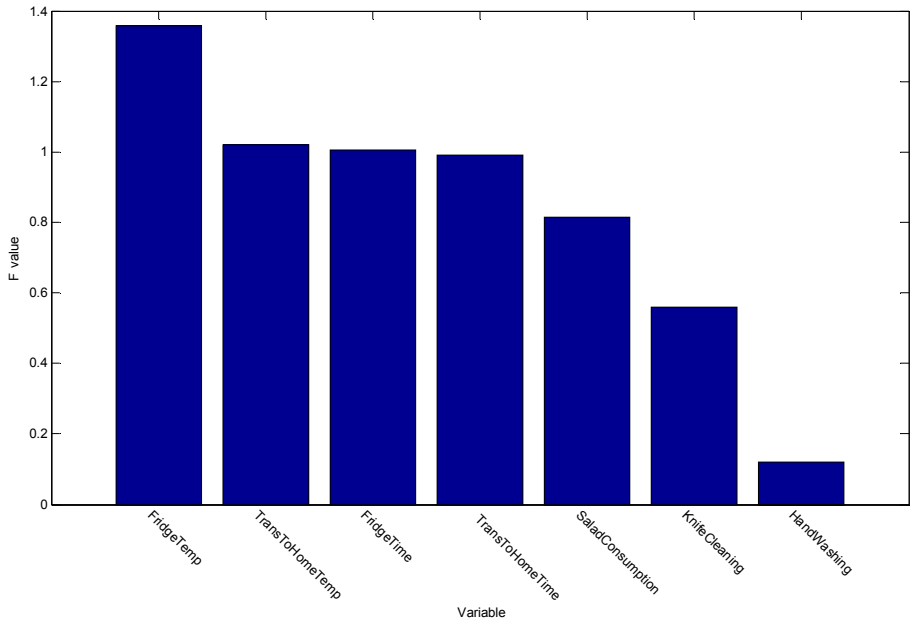

Figure 10.18: Preparation & Consumption: Pork Cuts sensitivity analysis for MS4

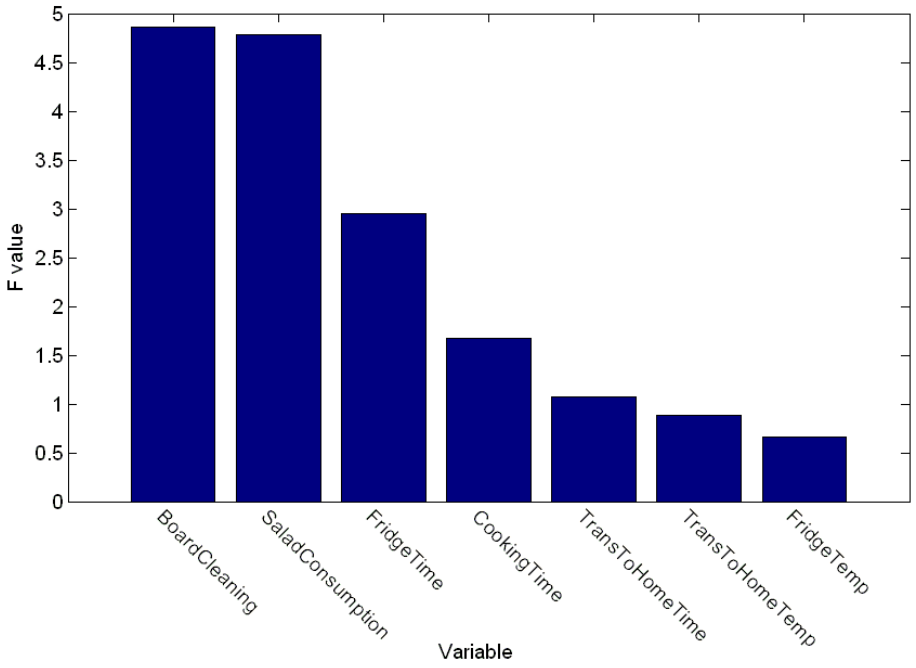

Figure 10.19: Preparation & consumption: Minced meat sensitivity analysis for MS1

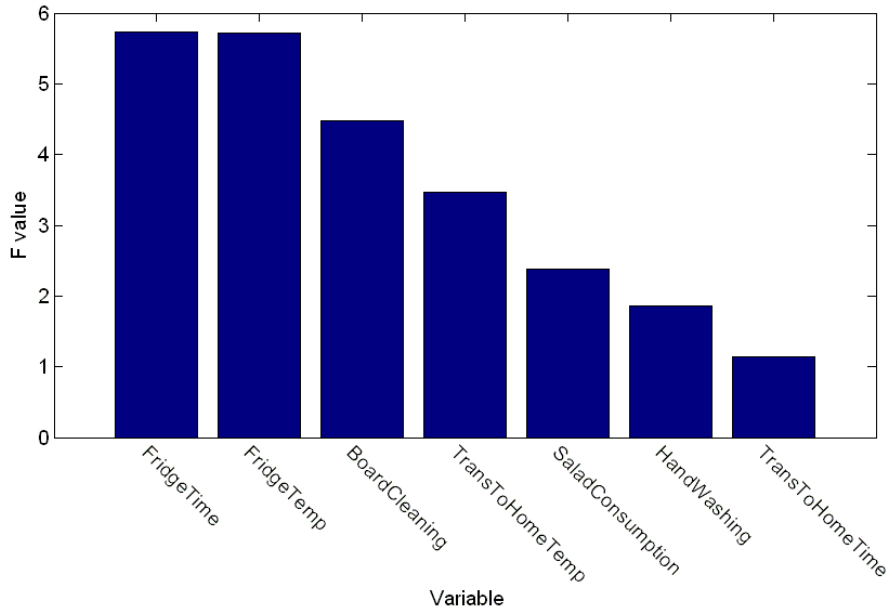

Figure 10.20: Preparation & Consumption: Minced meat sensitivity analysis for MS2

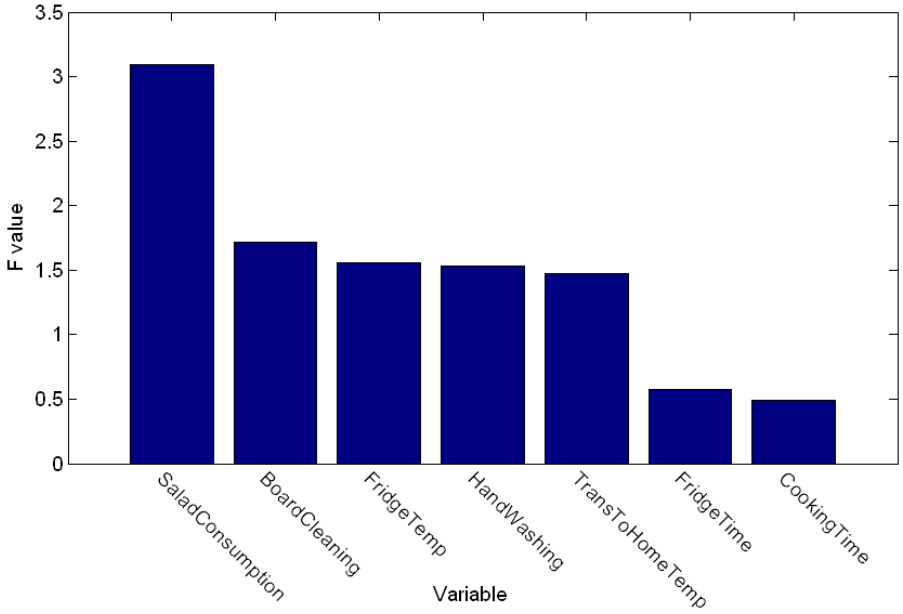

Figure 10.21: Preparation and consumption: Minced meat sensitivity analysis for MS3

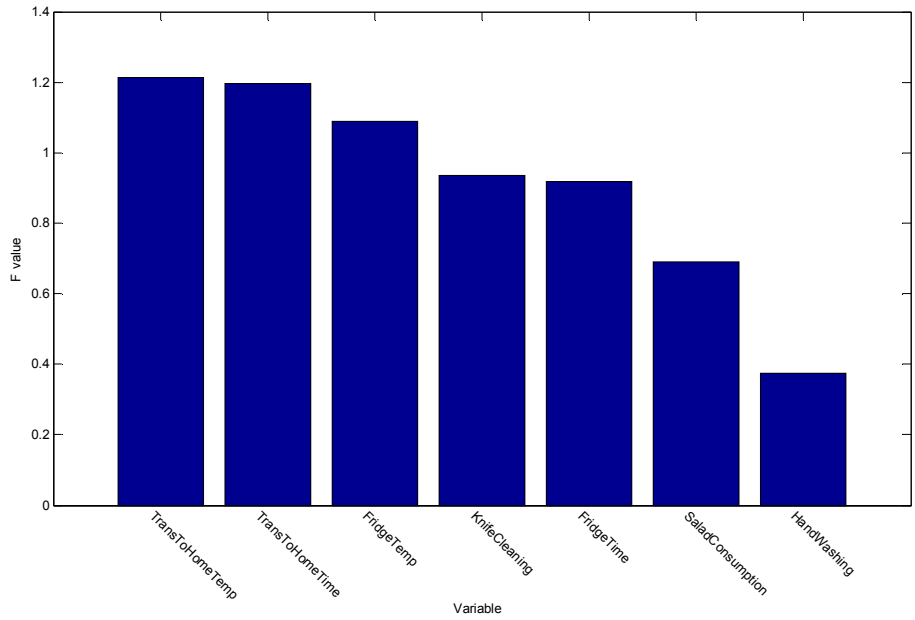

Figure 10.22: Preparation & Consumption: Minced meat sensitivity analysis for MS4

10.10.3 Fermented Sausage

For the Preparation & Consumption fermented sausage module (note this also includes the manufacture of the fermented sausages) we used the number of *Salmonella* on the fermented sausage portions at the point of consumption as the response variable. From this analysis we concluded that there is little difference in the significance of the factors for any of the MSs (and therefore no graphs are presented here). The F values were all quite low suggesting that none of the variability in any of the parameters has a particularly significant effect on the response variable.

10.11 Appendix 10.1: Data tables per MS

Data obtained from literature searches for the Preparation & Consumption module. The abbreviations for the EU MSs are give in Appendix A9.4.

Table A10.14: Travel or storage times in the food pathway, for each MS. (* = recommendation)

Time / **MS**	**Transport Store to Home**	**Refrigerator**	
		Pork cut	Minced Meat
MS2	**Table A10.**	$DG([16,72,104,29,13,3,0,11,0,0,0,0,3,0],[1/4.1/2,1,2,3,4,5,6,7,8,9,10,12,14])$ Evans 1998, Question 8	
NL		2 days Voedingscentrum *	1 day Voedingscentrum *
DE		$U(4,7)$, EFSA 2008a	
FR	66m, Peck, Goodburn *et al.* 2006		
IE	Table 10.2		
FI	$BP(10,45,300)$, EFSA, 2008a		
SE		See Table A10.	
Other	USA, New Zealand. See **Table A10.**	New Zealand, See Table A10.	

Table A10.15: Travel times from retail to home, for the USA (FAO 2002) , New Zealand (Gilbert *et al.* 2007), IE(Bolton *et al.* 2005) , and the UK (Evans & Stanton 1991).

Freq. / **Time.**	**USA**	**New Zealand**	**IE**	**MS2**
-	0.000			
15	0.005	0.398		
30	0.050	0.358	0.58	0.96
45	0.180	0.133		
60	0.250	0.056		0.02
75	0.220	0.019		
90	0.160	0.009	0.35	
105	0.070	0.006		
120	0.030	0.012		0.02
180		0.003	0.07	
240	0.035	0.003		
300		0.003		

Table A10.16: Storage times of meat in refrigerator, New Zealand, (Gilbert *et al.* 2007) and Sweden (Marklinder *et al.* 2004)

Days	**NZ**		**SE**
	Fresh meat	Minced beef	Minced Meat
0-1			0.75 [30]
1-2	72.5	73.7	0.21
2.5-4	22.2	21.2	0.04
4.5-7	4.9	4.8	
7-14	0.3	0.3	

[30] We added 5% to the category '1 day' and 5% to the category '1-2 days'.

Table 10.17: Temperatures in the consumer food pathway, for each MS.
(* = recommendation)

Temp. / MS	Transport Store to Home	Refrigerator
MS2		Table A10.19 min=-0.9, mean=6, max=11.4, (James and Evans 1992)
BE		7.0, (Devriese *et al.* 2004)
FR	Table A10.	(-,avg,+) =(1.1, 5.2, 10.8), (Rosset *et al.* 2004) $N(6.6, 2.3)$, (Laguerre *et al.* 2002), Table A10.19
DE	7, (EFSA 2008a)	
IE		Table A10.19 $N(6.52, 2.63)$, (Flynn *et al.* 1992)
NL	7.9, sd = 5.9 (Voedingscentrum 1999)	7 Voedingscentrum *, Table A10.19
FI	$BP(6, 10, 20)$, (EFSA 2008a)	
SE		(-,mean,+,sd)=(0.8, 6.2, 11.3,2.3) (Marklinder *et al.* 2004) Table A10.19
EL		Table A10.19

Table A10.18: Temperatures during domestic transport (France), (Derens *et al.* 2006)/

T	Fraction
<0	0.003
0-2	0.023
2-4	0.135
4-6	0.242
6-8	0.253
>8	0.344

Table 10.19: Refrigerator temperatures for IE (Kennedy *et al.* 2005), UK1 (Evans & Stanton 1991), UK2, FR1,FR2,FR3, EL, (Nauta *et al.* 2003) , PT Azevedo *et al.* 2005 and NL (Notermans *et al.* 1997).

T / Frequency	IE	FR1	FR2	FR3	PT	EL	UK1	UK2	NL
0-1	0.00						0.01		
1-2	0.04				0.00		0.02		
2-3	0.06						0.05		
3-4	0.05				0.13		0.09		
4-5	0.12			0.2			0.11	0.18	0.30
5-6	0.11	0.3	0.48		0.16		0.17	0.12	
6-7	0.25						0.21	0.14	0.416
7-8	0.12	0.29			0.31	0.45	0.15	0.31	
8-9	0.13						0.12	0.17	0.26
9-10	0.05		0.34	0.74	0.27		0.04	0.04	
10-11	0.01					0.35	0.01	0.03	0.016
11-12	0.04				0.10		0.01		
12-13	0.02	0.41	0.18	0.06	0.02	0.25	0.00		0.016

Table A10.20: Probabilities of hazardous actions in the domestic kitchen, per MS/country.

MS / Prob.	BE	NL	NZ	IE	MS2	DK	DE,	Australia	(N-)USA
P_h don't wash hands	0.14n	0.2a 0.51h	0.27b	0.35c	0.02d 0.93-1m		0.2	0.47j	0.29k 0.29-0.57m 0.2m
P_k unsafe knife handling			0.41b	0.03c	0.34d 0.23-0.61m		0.5	0.34	
P_b unsafe board handling	0.06n	0.73h	0.28b	0.04c 0.18e	0.36d 0.08 g 0.66-0.75m	0.19f	0.5	0.3j	
P_s prepare a salad							0.3		

a	Mylius *et al.* 2007	g	Worsfold & Griffith 1997
b	Gilbert *et al.* 2007	h	de Vries-Pels 1999
c	Kennedy *et al.* 2005, Bolton, *et al.* 2005	i	Brynestad *et al.* 2008
d	Parry, *et al.* 2002, questionnaire	j	Jay *et al.* 1999
e	FSA Ireland, 1998, see Kusumaningrum *et al.* 2004	k	Mistak 2001
f	Christensen *et al.* 2005	m	Redmond & Griffith 2003
		n	Devriese *et al.* 2004

10.12 Appendix 10.2: Literature Survey of Growth Models

Table 10.21: Some primary and secondary models which have been fit to data. 'Prev' indicates that a previous state has been considered. Plusses and minuses roughly indicate the measured importance of the factor.

A few models for which data have been Collected	Primary	Data on		Medium	Factors			Note
		μ	λ		T	pH	NaCl	
Gibson, Bratchell *et al.* 1988	Gompertz	polynomial	polynomial	Minced Pork and lab. media	+		-	Could not reproduce results. But usable minced pork parameters
Mann, Smith *et al.* 2004	(Only raw data)	-	-	Minced pork, Pork chops	+			
Mackey and Kerridge 1998	Gompertz	Ratkowsky	Ratkowsky	Minced beef	++			
Oscar 1999b	Two-phase linear	polynomial	polynomial	Brain Heart Infusion Broth	+	++ Prev-		
Oscar 1999a	Two-phase linear	polynomial	polynomial	Sterile Chicken Breast	++ Prev--[31]			
Oscar 1999c	Two-phase linear	polynomial	polynomial		+		Prev-	
Oscar 2002	Two-phase linear	CTM, Ratkowsky	hyperbola, Ratkowsky, nonlinear Arrhenius	Cooked Chicken	+			
Oscar 2005	Modified Logistic[32]	hyperbola with cutoff	hyperbola	Sterile chicken + BHI	++			
Oscar 2006	Logistic	Logistic (non-standard model)			++			Low initial concentration

[31] Those previous temperatures are not freezer temperatures, which do have a significant effect.
[32] Three phase linear, logistic and modified Gompertz were also fitted, but not reported. According to the author, the modified logistic model was superior.

10.13 Appendix 10.3: Growth Models

For a general introduction into the field of predictive microbiology, see van Gerwen & Zwietering 1998, Whiting 1995, or McKellar & Lu 2004.

In the following we will add a superscript 'min' or 'max' to indicate minima and maxima (e.g. $T_{\max}$ is the maximal temperature). A superscript indicates optimal values. In the following we will assume that each of the parameters $\phi \in \{T, pH, a_W\}$ lies within the interval between its minimum and maximum, i.e.

$$\phi \in [\phi_{\min}, \phi_{\max}], \text{ for } \phi \in \{T, pH, a_W, N\}. \qquad \text{(A10.244)}$$

A10.3.1 Primary Models

Primary models relate the number of bacteria at a certain time to the specific growth rate μ, the lag phase parameter λ and the initial or final numbers $N_{\min}$ or $N_{\max}$. Any environment specific factors (pH, temperature, water activity, etc.) are not considered in a primary model.

The simplest model is the exponential model,

$$N(t) = N_{\min} \max(e^{\mu(t-\lambda)}, 1), \qquad \text{(A10.245)}$$

possibly truncated at a maximum value,

$$N(t) = \min(N_{\min} \max(e^{\mu(t-\lambda)}, 1), N_{\max}). \qquad \text{(A10.246)}$$

Confusingly, Oscar 1999b calls (A10.245) a 'two-phase linear model'.

Two more advanced models, featuring an inflection point, are the modified logistic model,

$$\ln(\frac{N(t)}{N_{\min}}) = \ln(\frac{N_{\max}}{N_{\min}}) / (1 + \exp(\frac{4\mu_{\max}}{\ln(N_{\max}/N_{\min})}(\lambda - t) + 2)), \text{ for } t \geq 0. \text{(A10.247)}$$

and the modified Gompertz model,

$$\ln(\frac{N(t)}{N_{\min}}) = \ln(\frac{N_{\max}}{N_{\min}}) \exp(-\exp(1 + \frac{e\mu_{\max}}{\ln(N_{\max}/N_{\min})}(\lambda - t))), \text{ for } t \geq 0, \text{(A10.248)}$$

with $e = \exp(1)$. Gibson, Bratchell *et al.* 1987 compared these two models. Note that, since the graph of the logarithm of the concentration is no longer linear, the specific growth rate is no longer constant. This is the motivation for the introduction of $\mu_{\max}$, the maximum specific growth rate, attained at the inflection point.

Both models have the undesirable feature that $N(0) \neq N_{\min}$.

Finally, we discuss the Baranyi-Roberts model (or Baranyi model). This model is not an empirical model but rather a mechanistic model, developed in a series of papers, Baranyi, Roberts *et al.* 1993, Baranyi and Roberts 1994 and Baranyi and Roberts 1995. In fact, the model is given by a system of differential equations, allowing also for varying temperatures. For a fixed temperature an analytic solution can be written,

$$\ln(N(t)) = \ln(N_{\min}) + \mu_{\max} A(t) - \ln(1 + \frac{N_{\min}}{N_{\max}}(e^{\mu_{\max} A(t)} - 1)), \text{ (A10.249)}$$

$$A(t) = t + \frac{1}{\mu\max}\ln\left(\frac{e^{-\mu\max t} + q_0}{1 + q_0}\right). \qquad \text{(A10.250)}$$

The parameter q_0 is an initial state of a limiting substrate, which is generally unknown. However, it can be related to a lag phase-like parameter:

$$\lambda \approx \frac{\ln(1 + 1/q_0)}{\mu\max}. \qquad \text{(A10.251)}$$

The above formulas are valid only for a constant environment (e.g. temperature). In all other cases, differential equations need to be solved.

Growth experiments with varying temperature were performed by Ingham, Wadhera *et al.* 2005 for the surface of chicken and ground beef. Those authors also note a good correspondence between the predictions from PMP 7.0[33], which uses the Baranyi model, and their experimental findings.

A10.3.2 Choosing a primary model

In choosing the model there are a number of factors to consider. Is the model accurate? Are growth parameters for *Salmonella* available? Is the model flexible?

The Gompertz and Baranyi models are known to provide the highest quality fits over large times. The exponential model is too simple to capture the bacterial dynamics.

Growth parameters for *Salmonella*, in several environments have been published. However, care must be taken in using parameters estimated for a certain primary model in another primary model. They do not represent exactly the same quantity!

The Baranyi model is certainly the most flexible model, it can handle varying temperature profiles. This is a useful feature in modelling cooking or defrosting.

The most important drawbacks of the models are the following. The logistic and Gompertz models do not reproduce $N_{\min}$ at time zero. The Baranyi model needs either an unknown q_0, or a parameter which only resembles the lag parameter.

Below we present two figures, based on estimates from Oscar 1999c, using the estimated lag time and specific growth rates estimated in this paper, at two temperatures.

[33] Pathogen Modeling Program 7.0, http://pmp.arserrc.gov/PMPOnline.aspx

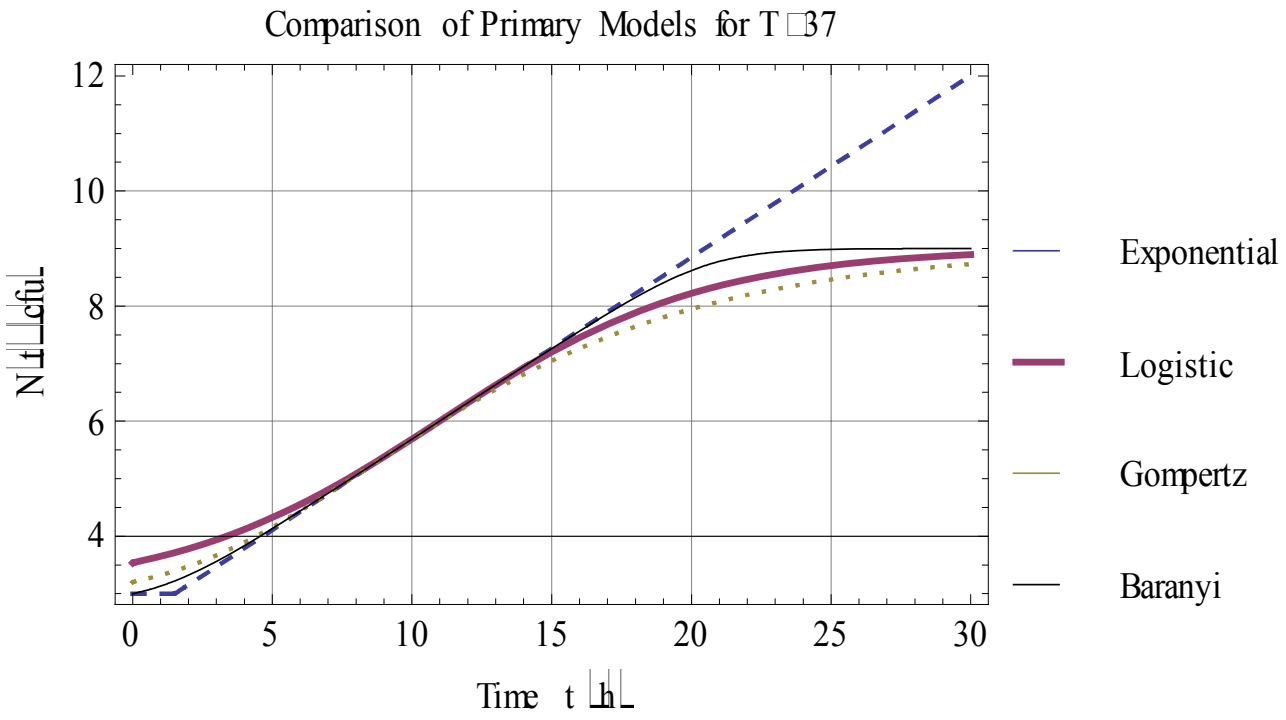

Figure A10.23: A comparison of several primary growth models, for T=37°C.

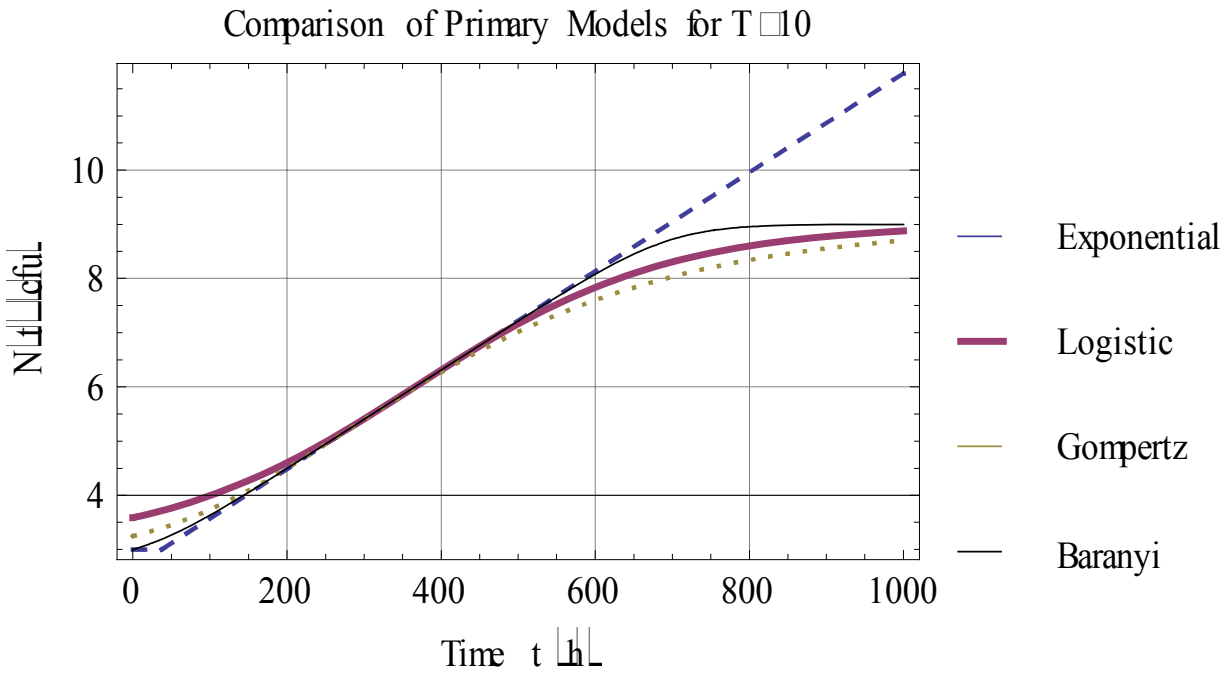

Figure A10.24: A comparison of several primary growth models, for T=10°C.

We find that the logistic and Gompertz curves over-estimate the initial concentration, as expected, while the Baranyi curve reproduces the initial concentration correctly. Also, the Baranyi curve is closest to the 'true' growth curve (not provided). This suggests using the Baranyi model.

Estimation of specific growth rates and lag rates is done using secondary models, which are discussed in the next section.

A10.3.3 Secondary Models

Secondary models consider the growth rate μ and/or the lag parameter λ as a function of environmental factors.

Square root models

A popular second order model is the square root model

$$\mu = b^2(T - T^-)^2(a_W - a_{W_{\min}})(pH - pH_{\min}), \qquad \text{(A10.252)}$$

where b is a parameter of the model. Square root models are also termed Ratkowsky or Bélehrádek models and were firstly introduced in Ratkowsky *et al.* 1982. Sometimes extensions, incorporating $T_{\max}$, $pH_{\max}$ and $a_{W\max}$, of the following form are used:

$$\mu = b^2(T - T_{\min})^2(pH - pH_{\min})(1 - e^{c_T(T-T\max)})^2(1 - e^{c_{pH}(pH-pH\max)})$$
$$(1 - e^{c_{a_W}(a_W - a_W\max)}), \qquad \text{(A10.253)}$$

see Ratkowsky *et al.* 1983. Wijtzes *et al.* 1995 suggested a model like (A10.252) with an extra factor $(pH - pH_{\max})$ and in Wijtzes, McClure *et al.* 1993 a model like (A10.253) but without the exponential factor for the maximum pH.

The gamma concept

The gamma concept was firstly introduced by Zwietering *et al.* 1992. An often used particular type is the square root gamma model,

$$\mu(T, pH, a_w) = \mu^*\gamma(T; T_{\min}, T_{\text{opt}}, T_{\min})\gamma(pH; pH_{\min}, pH_{\text{opt}}, pH_{\max})$$
$$\gamma(a_W; a_{W_{\min}}, a_{W_{\text{opt}}}, aW_{\max}), \qquad \text{(A10.254)}$$

with

$$\gamma(\phi; \phi_{\min}, \phi_{\text{opt}}, \phi_{\max}) =$$
$$\left|\left(\frac{\phi - \phi_{\min}}{\phi_{\text{opt}} - \phi_{\min}}\right)\left(\frac{\phi_{\max} - \phi}{\phi_{\max} - \phi_{\text{opt}}}\right)\right|, \text{ for } \phi_{\max} \neq \phi_{\text{opt}}, \qquad \text{A(10.255)}$$

$$\gamma(\phi; \phi_{\min}, \phi_{\text{opt}}, \phi_{\max}) = \left|\left(\frac{\phi - \phi_{\min}}{\phi_{\text{opt}} - \phi_{\min}}\right)\right| \text{ for } \phi_{\max} = \phi_{\text{opt}}, \qquad \text{(A10.256)}$$

and

$$\mu_{\text{opt}} = \mu(T_{\text{opt}}, pH_{\text{opt}}, a_{W_{\text{opt}}}). \qquad \text{(A10.257)}$$

This function has the important property $\gamma(\phi_{\min}) = \gamma(\phi_{\max}) = 0$ and $\gamma(\phi_{\text{opt}}) = 1$.

Note how the gamma has deviating parameters for temperature in the equation (no $T_{\max}$). For the optimum and maximum water activity the value one is usually taken.

Other types have been proposed, e.g. with a Ratkowsky-type exponential

$$\gamma(\phi) = [\frac{\phi - \phi_{\min}}{\phi_{\text{opt}} - \phi_{\min}} \frac{1 - e^{c(\phi - \phi^+)}}{1 - e^{c(\phi^* - \phi^+)}}]^2. \qquad \text{(A10.258)}$$

Cardinal models

In the previous models, the parameters are considered to be not directly correlated to biological phenomena. For example $T_{\min}$ is not the 'real' minimum growth temperature for the micro-organism. Rather, it is a model extrapolation, and thus determined from data, mostly measured far away from $T_{\min}$. In cardinal models however, the parameters are considered to be directly biologically interpretable.

A cardinal temperature model (CTM) with inflection was introduced by Rosso *et al.* 1993 and later used in Rosso *et al.* 1995, it reads

$$\gamma(T) = (T - T_{\max})(T - T_{\min})^2\{(T_{\text{opt}} - T_{\min})[(T_{\text{opt}} - T_{\min})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max})(T_{\text{opt}} + T_{\min} - 2T)]\}^{-1}. \qquad \text{(A10.259)}$$

Spices and salt

The salt content may be directly related to the water activity. Spices and herbs may also have a large impact on the growth factor Koutsoumanis, Lambropoulou *et al.* 1999 but are hard to quantify.

Secondary lag time model

If needed, secondary models for the lag time can be used in the same way as above, in which case λ^* becomes a parameter. The difficulty is that the occurrence (and length) of a lag time is dependent on previous environmental factors. One needs to take into account e.g. temperature shock.

A popular choice is Baranyi & Roberts 1994

$$\lambda = \frac{\ln(\alpha_0)}{\mu}, \qquad \text{(A10.260)}$$

which relates the lag-time to the growth-rate, using an 'initial state parameter' α_0. For $\alpha_0 = 1$ there is no lag, while for $\alpha_0 = 0$ the lag phase is infinitely long.

Another possibility Oscar 2002, called a hyperbola model, takes temperature explicitly into account,

$$\lambda = (\frac{p}{T - q})^m. \qquad \text{(A10.261)}$$

Here p, q are parameters with biological meaning and m is an exponent to be estimated. Furthermore, a Ratkowsky model is often used, Ratkowsky *et al.* 1983,

$$\lambda = \frac{1}{b(T - T^{-})^2}. \qquad \text{(A10.262)}$$

Finally, we mention the nonlinear Arrhenius model of Davey 1989,

$$\lambda = A + B/T + C/T^2, \qquad \text{(A10.263)}$$

where A, B, C are fitted parameters without biological meaning.

Tertiary models

Tertiary models combine first order and second order models, and often incorporate variability. A user-friendly computer implementation of combined first and second order models is also thought of as being a tertiary model.

10.14 Appendix 10.4: Inactivation, D-values and Z-values

Let $N(t)$ be the number of bacteria at time t, and let $M(t) = \log(N(t))$ be the log-number of bacteria. At a temperature T (measured in degrees centigrade), exponential inactivation may be modelled using the decimal reduction value D_T,

$$M(t) = M(0) - \frac{t}{D_T}, \quad \text{(A10.264)}$$

$$N(t) = N(0)10^{-\frac{t}{D_T}}. \quad \text{(A10.265)}$$

The unit of D_T is log-reductions per minute, and therefore time is also measured in minutes. Usually, only a reference value at a certain temperature is known, e.g. D_{60}. The decimal reduction time at other temperatures can be found from the Z-value (measured in degrees centigrade), which is the temperature needed to lower the decimal reduction time by one log. Thus, D_T is assumed to be linear in T,

$$\log(D_{T_2}) = \log(D_{T_1}) - \frac{T_2 - T_1}{Z}, \quad \text{(A10.266)}$$

$$D_{T_2} = D_{T_1}10^{-\frac{T_2-T_1}{Z}}. \quad \text{(A10.267)}$$

For example, for a reference value at $T = 60$, having the value D_{60} and $Z = 5$, the equation becomes

$$D_T = D_{60}10^{-\frac{T-60}{5}}, \quad \text{(A10.268)}$$

and $D_{65} = D_{60}/10$. The decimal reduction time is lowered with increasing temperature. Inserting the equation involving the Z-value into the inactivation equations gives the final result,

$$M(t, T; T_1) = M(0; T; T_1) - \frac{t}{D_{T_1}}10^{\frac{T-T_1}{Z}}, \quad \text{(A10.269)}$$

$$N(t, T; T_1) = N(0, T; T_1)10^{-\frac{t}{D_{T_1}}10^{\frac{T-T_1}{Z}}}. \quad \text{(A10.270)}$$

10.15 Appendix 10.5: Numerical solution of the heat equation

A10.5.1 Finite Differences

Let us first discretise the function in the x and y directions, using n and m, respectively, grid points. Then the resolutions (grid-spacings) are

$$h_x = W/(n-1). \quad \text{(A10.271)}$$

$$h_y = H/(m-1). \quad \text{(A10.272)}$$

In order to greatly simplify the process, we assume that $h_x = h_y = h$, i.e. equal grid spacing. This does set a constraint on the allowed dimensions of the domain. Also, set $x_i = hi$ and $y_j = hj$ for $0 \leq i < n$ and $0 \leq j < m$. Furthermore, we abbreviate $D(x_i, y_j, t) = D_{ij}(t)$. Often, we will suppress time dependence, $D_{ij}(t) = D_{ij}$, when there is no risk of confusion. The usual 2-point difference approximations to the second derivatives are then

$$\frac{\partial^2 D_{ij}}{\partial x^2} = \frac{D_{i-1,j} - 2D_{ij} + D_{i+1,j}}{h^2}, \text{ for } 0 < i < n-1, \quad \text{(A10.273)}$$

$$\frac{\partial^2 D_{ij}}{\partial y^2} = \frac{D_{i,j-1} - 2D_{ij} + D_{i,j+1}}{h^2}, \text{for } 0 < j < m-1. \quad \text{(A10.274)}$$

Combining these, we find the 5-point difference method for the Laplacian,

$$\Delta D_{ij} = \frac{1}{h^2}(D_{i-1,j} + D_{i,j-1} - 4D_{ij} + D_{i+1,j} + D_{i,j+1}). \quad \text{(A10.275)}$$

At the bottom boundary, $j = m-1$, the boundary condition is,

$$D_{i,m-1} = D_H, \text{ for } 0 < i < n-1. \quad \text{(A10.276)}$$

At the leftmost boundary, $i = 0$, where the outward normal is equal to minus the x-derivative, the boundary condition becomes,

$$\frac{\partial D(x_0, y_j, t)}{\partial x} = \frac{\alpha}{\kappa} D_{0,j}(t), \text{ for } 0 < j < m-1. \quad \text{(A10.277)}$$

Plugging in the usual second order accurate difference approximation to the first derivate,

$$\frac{\partial D(x_0, y_j, t)}{\partial x} = \frac{D_{1,j} - D_{-1,j}}{2h} = \frac{\alpha}{\kappa} D_{0,j}(t), \quad \text{(10.278)}$$

presents us with a difficulty, since we have to evaluate the temperature outside of the grid. Luckily, this grid point drops out of the equation when we consider that the differential equation also has to hold at the boundary,

$$\Delta D_{0j} = \frac{1}{h^2}(D_{-1,j} + D_{0,j-1} - 4D_{0,j} + D_{1,j} + D_{0,j+1}), \quad \text{(A10.279)}$$

and plug in the boundary condition,

$$\Delta D_{0j} = \frac{1}{h^2}(0 + D_{0,j-1} - (4 + \frac{2h\alpha}{\kappa})D_{0,j} + 2D_{1,j} + D_{0,j+1}). \quad \text{(A10.280)}$$

The result is an asymmetrical 4-point difference, without any offending points outside of the domain. At the rightmost boundary, $i = n - 1$, we follow the same strategy. Here, the normal derivative is in the positive x-direction, and given by

$$\frac{\partial D(x_{n-1}, y_j, t)}{\partial x} = \frac{D_{n,j} - D_{n-2,j}}{2h} = -\frac{\alpha}{\kappa} D_{n-1,j}. \quad \text{(A10.281)}$$

This yields the following Laplacian,

$$\Delta D_{n-1,j} = \frac{1}{h^2}(2D_{n-2,j} + D_{n-1,j-1} - (4 + \frac{2h\alpha}{\kappa})D_{n-1,j} + 0 + D_{n-1,j+1}). \quad \text{(A10.282)}$$

The top boundary, at $j = 0$ has the normal in the negative direction. Thus,

$$\frac{\partial D(x_i, y_0, t)}{\partial y} = \frac{D_{i,1} - D_{i,-1}}{2h} = \frac{\alpha}{\kappa} D_{i,0}. \quad \text{(A10.283)}$$

This gives the Laplacian

$$\Delta D_{i0} = \frac{1}{h^2}(D_{i-1,0} + 0 - (4 + \frac{2h\alpha}{\kappa})D_{i0} + D_{i+1,0} + 2D_{i,1}). \quad \text{(10.284)}$$

The only points left out of the discussion so far are the corner points. At these points we have two boundary conditions to take care of. Essentially, the calculation is the same as the previous calculations, and we only give the results,

$$\Delta D_{0,0} = \frac{1}{h^2}(0 + 0 - (4 + \frac{4h\alpha}{\kappa})D_{00} + 2D_{10} + 2D_{01}), \quad \text{(A10.285)}$$

$$\Delta D_{n-1,0} = \frac{1}{h^2}(2D_{n-2,0} + 0 - (4 + \frac{4h\alpha}{\gamma})D_{n-1,0} + 0 + 2D_{n-1,1}). \quad \text{(A10.286)}$$

The boundary condition at the bottom can also be nicely incorporated in the Laplacian at $j = m - 2$,

$$\Delta D_{i,m-2} = \frac{1}{h^2}(D_{i-1,m-2} + D_{i,m-2} - 4D_{i,m-2} + D_{i+1,m-2} + 0 + D_H). \quad \text{(A10.287)}$$

At the boundaries $i = 0, n - 1$, this needs the usual modification,

$$\Delta D_{0,m-2} = \frac{1}{h^2}(0 + D_{i,m-2} - (4 + \frac{2h\alpha}{\kappa})D_{0,m-2} + 2D_{1,m-2} + 0 + D_H). \quad \text{(A10.288)}$$

$$\Delta D_{n-1,m-2} = \frac{1}{h^2}(2D_{n-2,m-2} + D_{n-1,m-2} - (4 + \frac{2h\alpha}{\kappa})D_{n-1,m-2} + 0 + 0 + D_H) \quad \text{(A10.289)}$$

From this point onward the variables with coordinate $j = m - 1$ can be removed from the system. All the above conditions can be succinctly written using stencils. We write for the stencil S_{ij},

$$S_{ij} = \{a, b, c, d, e, f\}, \quad \text{(A10.290)}$$

when

$$\Delta D_{ij} = (aD_{i-1,j} + bD_{i,j-1} + cD_{ij} + dD_{i+1,j} + eD_{i,j+1} + f). \quad \text{(A10.291)}$$

Using this notation, and the abbreviation $g = 2h\alpha/\kappa$, we summarize the above as

$$S_{ij} = h^{-2}\begin{cases} 1 & 1 & -4 & 1 & 1 & 0 & 0 < i < n-1 & 0 < j < m-1 \\ 0 & 1 & -(4+g) & 2 & 1 & 0 & i = 0 & 0 < j < m-1 \\ 0 & 0 & -(4+2g) & 2 & 2 & 0 & i = 0 & j = 0 \\ 2 & 0 & -(4+2g) & 0 & 2 & 0 & i = n-1 & j = 0 \\ 0 & 1 & -(4+g) & 2 & 0 & D_H, \text{ for} & i = 0 & \text{, and } j = m-2 \\ 1 & 1 & -4 & 1 & 0 & D_H & 0 < i < n-1 & j = m-2 \\ 2 & 1 & -(4+g) & 0 & 0 & D_H & i = n-1 & j = m-2 \\ 2 & 1 & -(4+g) & 0 & 1 & 0 & i = n-1 & 0 < j < m-1 \\ 1 & 0 & -(4+g) & 1 & 2 & 0 & 0 < i < n-1 & j = 0 \end{cases} \quad \text{(A10.292)}$$

We now proceed by putting all unknown data points into one vector u. This is accomplished by numbering the grid points in a row-by-row basis, such that entry k in u belongs to grid point $i + jn$. We introduce the mapping $\phi_{ij} = i + jn$ to perform this mapping. Our aim is now to write the heat equation in the form

$$\frac{du}{dt} = \kappa Au + r, \quad \text{(A10.293)}$$

with a suitable matrix A containing the discretization of Δ. As we have seen above, this is accomplished using simple difference equations. For a certain (i, j) we have an entry $u_{\phi_{ij}}$ and an equation consisting of the vector product between row ϕ_{ij} of A with $u_{\phi_{ij}}$, plus or minus D_H. For example, in the interior,

$$A_{\phi_{ij},\phi_{i-1,j}} = h^{-2}, \quad \text{(A10.294)}$$

$$A_{\phi_{ij},\phi_{i,j-1}} = h^{-2}, \quad \text{(A10.295)}$$

$$A_{\phi_{ij},\phi_{i,j}} = -4h^{-2}, \text{ etc...} \quad \text{(A10.296)}$$

At the boundary extra terms $r_{\phi_{ij}} = D_H/h^2$ appear.

A10.5.2 Method of lines

The method of lines is a technique where all but one variable in a partial differential equation is discretised, and the remaining variable explicitly solved. This is what we will describe in this section.

Firstly, we perform an eigenvalue decomposition of the matrix A,

$$A = V^T \Lambda V, \tag{A10.297}$$

with eigenvalues $\Lambda = \mathrm{diag}(\lambda_0, \ldots, \lambda_n)$, and eigenvectors in the columns of V. Note that $V^T V = I$, by orthogonality of the eigenvectors. Insert the eigenvalue decomposition into the differential equation,

$$\frac{du}{dt} = \kappa(V^T \Lambda V u + r) \rightarrow \frac{dVu}{dt} = \kappa(\Lambda V u + V r). \tag{A10.298}$$

Introduce $w = Vu$ and $s = Vr$ then the equation can be written,

$$w'(t) = \kappa(\Lambda w + s). \tag{A10.299}$$

Thus, for each component of w we have,

$$w_i'(t) = \kappa(\lambda_i w(t) + s_i). \tag{A10.300}$$

Given the initial condition $u(0) = 0 \rightarrow w(0) = Vu(0) = 0$, we can solve each of those equations,

$$w_i(t) = \frac{s_i}{\lambda_i} e^{\kappa \lambda_i t} - \frac{s_i}{\lambda_i} = \frac{s_i}{\lambda_i}\left[e^{\kappa \lambda_i t} - 1\right]. \tag{A10.301}$$

In terms of the original variables, using component-wise division and multiplication,

$$u(t) = V^T (Vr) \lambda^{-1} (e^{\kappa \lambda t} - 1). \tag{A10.302}$$

Not only do we need a zero initial condition, but also an arbitrary initial condition, used when flipping the minced meat patty. In this case $w(0) = Vu(0) = w_0$ and the differential equation is solved by the more general expression

$$u(t) = V^T \{(Vr)\lambda^{-1}[e^{\kappa \lambda t}(1 + (Vu_0)\lambda(Vr)^{-1}) - 1]\}. \tag{A10.303}$$

10.16 Appendix 10.6: Some models for failed fermentation

This section presents a thought experiment on a model for failed fermentation. In the end, the model will prove to be inadequate. However, it is educational and highlights the results of improper assumptions.

We suppose that an outbreak occurs only if fermentation fails. In this case we assume that every contaminated sausage causes illness. The number of illnesses is therefore dependent on the sausage prevalence within a batch. This we can estimate using our model.

Suppose the baseline prevalence of sausages is p_B, if we also interpret this number as the probability that a sausage is contaminated, we can model the number of contaminated sausages in a batch using a Poisson distribution,

P(k out of n sausages contaminated | p_B) = Poisson(k; p_Bn). (A10.304)

Here n=10,000 is the number of sausages in a batch. We note that the number of illnesses per outbreak is roughly between 1000 and 10,000. The probability of an outbreak of this type is

P(outbreak) =

P(between 1000 and 10,000 sausages contaminated) ×

P(fermentation fails and failure is unnoticed).

The first probability (call it P_B) is easily calculated,

$$P_B = e^{-p_B n} \sum_{i=1000}^{10.000} \frac{(p_B n)^i}{i!} . \qquad \text{(A10.305)}$$

If we apply an intervention, in the form of a log-reduction of *Salmonella*, just before the fermentation phase, we can now assess the impact. After application of a m-log increase, call the resulting probability P_m. Note that a -m log increase is a m log decrease and that $P_B=P_0$.

When the probability of failed fermentation remains constant, the relative increase in the probability of an outbreak rm is given by $r_m=P_B/P_m$.

Now, there is a problem with equation (A10.305). For every reasonable value of p_B, the result P_B is astronomically small and can never be used for outbreak estimation. For example, from the model we find p_B is approximately 2/1000, yielding P_B approximately 10^{-1250}.

The crucial unrealistic modelling assumption is "interpret p_B as the probability that a sausage is contaminated". In reality, the basic material (minced meat) will be obtained from one (or a few) sources. If one portion is contaminated, it is very likely that other portions are also contaminated. There is no single p_B that can act as a probability of contamination, there is significant clustering. In the next section however, we will see that for a given batch size and contamination of the basic material, we can make some statements on the outbreak size.

A10.6.1 An alternative fermentation failure model

As discussed before, simulation and modelling of failed fermentation is challenging. However, on a theoretical basis we can still obtain some results on the expected reduction in outbreak sizes dependent on the concentration of *Salmonella*.

Suppose we have a batch of n sausages, and the basic material contained m *Salmonella*. When dividing m *Salmonella* over $n < m$ sausages, we are interested in the event X that $k \leq n$ sausages are contaminated (a prevalence of k/n). Denote this probability by $P(X = k) = f(n, m, k)$. Note that when $n \geq m$ the following analysis does not hold, but an alternative approach is possible, which will not be explored here since this situation is unlikely to result in an outbreak. Finding an expression for the probability $P(X = k)$ is not as easy as it appears on first sight. From Torabi 2009 we find a closed form expression for f,

$$f(n, m, k) = n^{-m}\binom{n}{k}k!S(m.k). \qquad \text{(A10.306)}$$

Here $S(m, k)$ is the Stirling number of the second kind, which has the interpretation of "the number of ways to partition a set of m elements into k nonempty subsets". The other factors have obvious interpretations. The Stirling number of the second kind can be defined in many ways (Abramowitz & Stegun 1972). An explicit expression is

$$S(m, k) = \frac{1}{k!}\sum_{j=0}^{k}(-1)^{k-j}\binom{k}{j}j^m \qquad \text{(A10.307)}$$

However, this fact was not recognized by Torabi 2009 who used the right-hand side of (AA10.307). Using a symbolic computing language, we can now calculate probabilities and expectations, or draw graphs of the distribution for selected values of n and m. An example is shown in Figure A10.25.

Note that for large n and m we have a pronounced peak: the average value is close to the most probable value. Also, one suspects that a Poisson distribution will be a good approximation for such large values, a conjecture which we will not explore further here.

The expected value of the number of contaminated sausages, which depends on both n and m is given by

$$E(X) = \sum_{i=0}^{n} if(n, m, k) \qquad \text{(A10.308)}$$

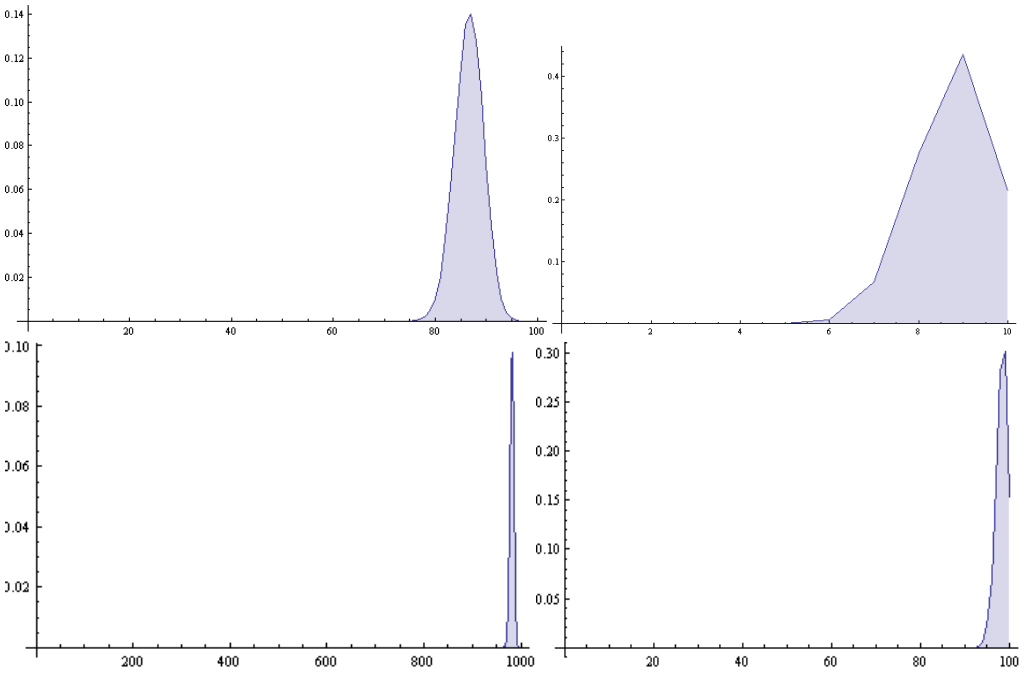

Figure A10.25. Probabilities of k sausages out of m are contaminated. Top left (n,m)=(10,20), Top right (n,m)=(100,200), Bottom left (n,m)=(100,400), Bottom right (n,m)=(1000,4000).

The following table lists some numerical results,

Table A10.22 Expectation of the number of contaminated sausages, for various m and n.

	m=2n	**m=3n**	**m=4n**	**m=5n**	**m=6n**
n=10	8.78423	9.57609	9.85219	9.94846	9.98203
n=100	86.602	95.0959	98.2049	99.343	99.7595
n=1000	864.8	950.288	981.721	993.279	997.529

We are interested in the effect of an intervention on the size of an outbreak. In other words, the effect of a reduction in m on $E(X)$. The following table lists the reduction of the expected number of contaminated sausages. We calculate the expectation $E(X|m_2)$ with $m_2 = m/10$ and divide by expectation $E(X|m_1)$ with $m_1 = m$. We use the same range of values for m as in the above table and obtain

Table A10.23 Reduction in prevalence upon reduction of the contamination by one log unit.

	m1=2n, m2=2n/10	**m1=3n m2=3n/10**	**m1=4n m2=4n/10**	**m1=5n m2=5n/10**	**m1=6n m2=6n/10**
n=10	0.216297	0.282997	0.349059	0.411631	0.469403
n=100	0.210264	0.273723	0.337079	0.397606	0.453935
n=1000	0.209703	0.272857	0.335955	0.396285	0.452471

For example, in a batch of 1000 sausages, bringing down the initial concentration from 4000 to 400 decreases the prevalence by a factor 0.336. For higher values of n the calculation becomes unfeasible. But, the percentages seem to stabilize, and depend only on m.

The results for a 2-log reduction are,

Table A10.24 Reduction in prevalence upon reduction of the contamination by two log units.

	m1=2n, m2=2n/100	**m1=3n m2=3n/100**	**m1=4n m2=4n/100**	**m1=5n m2=5n/100**	**m1=6n m2=6n/100**
n=100	0.0229787	0.0312327	0.0401242	0.0493341	0.0586609
n=1000	0.0229083	0.0311159	0.0399602	0.0491245	0.0584081

As before we see that the higher the initial contamination, the less impact log reductions have on the prevalence reduction.

Finally, we present the results of the log-reductions in a figure, drawn with $n = 1000$.

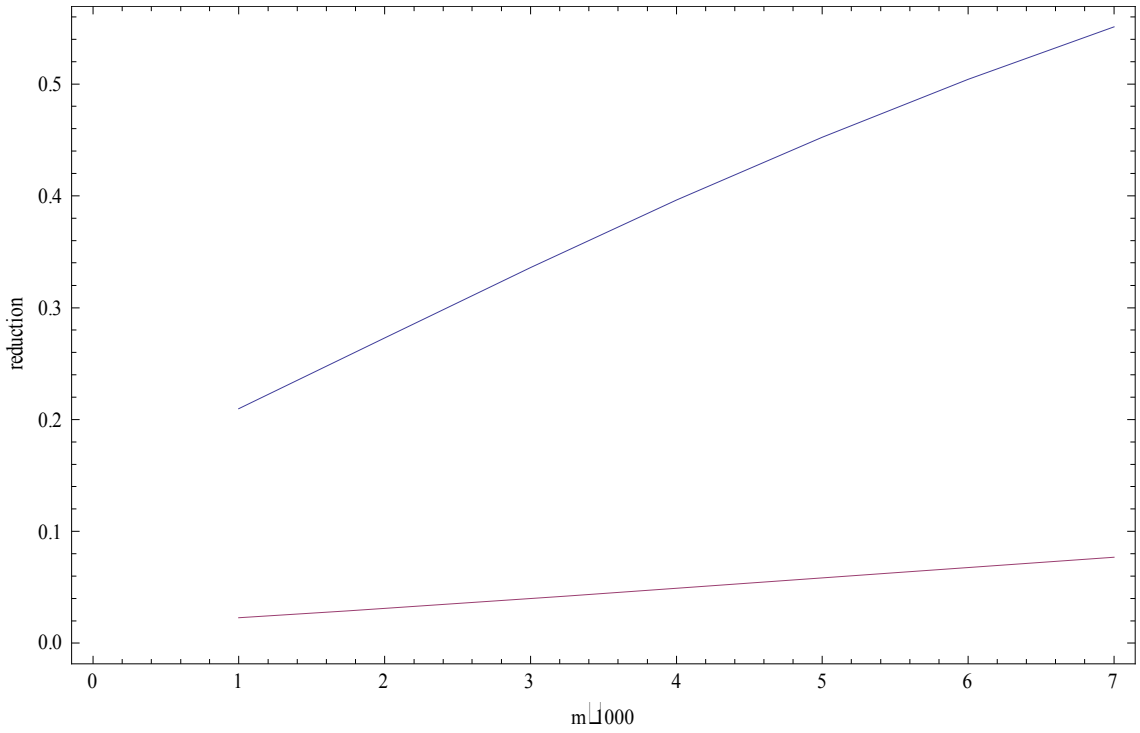

Figure A10.26. Reduction factors for the prevalence as a function of m. Drawn for n=1000. Blue line: one log decrease. Purple line: two log decrease.

Figure A10.29 should be read as follows. When e.g. m/1000 = 4, or m=4000, we have four times as many *Salmonella* as sausages. The result of a one log reduction (top curve) is that the new prevalence is approximately 38% of the old prevalence. The result of a two log reduction (bottom curve) is that the new prevalence is approximately 4% of the old prevalence. We would like to remind the reader that we associate prevalence directly to outbreak size, thus a 38% percent reduction in prevalence directly translates to a 38% reduction in outbreak size. We make no claims on the total number of outbreaks.

Note that the top curve (one log reduction) is not a straight line, nor is the bottom curve (two log reduction). But, the dependence of the prevalence reduction on the initial contamination is almost linear. Also, the dependence of the prevalence reduction on the log reductions is not exactly linear, dividing the points of the top curve by the points of the bottom curve would not exactly give a horizontal line, but it would be close.

As a conclusion, the result of a one log reduction can greatly influence the prevalence of contaminated sausages in a batch. Therefore, if the probability of fermentation failure remains a constant, such a reduction can have a significant beneficial effect on the outbreak sizes. The result of a two log reduction is, of course, an even lower prevalence, although not exactly a factor two. Relatively speaking the gain gets slightly lower for higher log-reductions.

We refer the interested reader to Nauta 2005 for more details on partitioning and mixing techniques similar to the issues discussed in this section.

11 A Dose-Response model for *Salmonella* in pig-meat products

11.1 Dose-response models in enteric infectious diseases

Enteric disease as a consequence of infection with pathogenic organisms can be expected to be related to the probability of infection due to the consumption (exposure) of organisms and the probability of the infection producing clinical illness. In earlier work the concept of "a minimal infective dose" was common, i.e. it was supposed that a certain number of pathogenic organisms are necessary to produce infection/illness (Untermann 1998) and at doses below this threshold infection will not take place. A more realistic scenario, which is now the accepted concept in the field of MRA, is that there is no threshold dose and that even the consumption of a single organism poses a (albeit small) definitive risk of infection.

A necessary condition for disease is the uptake of at least one infective organism (a "single hit"). If the host is able to kill or inactivate the organism, infection, multiplication and the formation of a clone to infect the host is prevented. There is, however, a (small) probability r that the organism will succeed in infecting the host (Teunis & Havelaar 2000).

If the inoculum contains n organisms the probability of at least one organism succeeds is the complement of the probability of absence of infection

$$P_{inf}(n;r) = 1-(1-r)^{n}. \qquad (11.1)$$

In reality the number n is not known, but the expected number of organisms in a random sample (D) can be characterized by a Poisson uncertainty. The probability of a least one infectious organism being taken up is a function of the expected number (Teunis & Havelaar 2000).

$$Pinf(D;r) = 1-e-r*D \qquad (11.2)$$

This is the exponential dose-response relation for a single-hit model with a fixed probability of infection r. This distribution has only one parameter r which is assumed to be the same for all of the organisms in the inoculum. The exponential dose-response model seems to work well with some intestinal parasitic pathogens e.g. *Giardia* and *Cryptosporidium* (Teunis *et al.* 1996). However, it can be assumed that in reality there will in most cases be variability in the interaction between the individual pathogenic organisms and hosts. This variability can be expressed as a beta distribution with two parameters α,β (Haas *etal.* 1998). Including this variability in equation (11.2) leads to

$$P_{inf}(D;\alpha,\beta) = 1-{}_1F_1(\alpha,\alpha+\beta,-D) \qquad (11.3)$$

In which ${}_1F_1$ is the Kummer confluent hypergeometric function (Abramowitz & Stegun 1984). This formula is mathematically cumbersome and can be replaced by the approximation developed by Furumoto and Mickey (Furumoto & Mickey 1967) on the condition that $\beta>>1$ and $\alpha<<\beta$.

$$P_{inf}(D;\alpha,\beta) = 1-(1+D/\beta)^{-\alpha} \qquad (11.4)$$

This is the most widely used model in microbiological risk assessment, referred to as the Beta-Poisson dose-response model.

As the ID_{50} (the dose needed to achieve a 50% probability of infection) is dependant on α,β it follows(Chen *et al. 2006*).

$$ID_{50(\alpha,\beta)} = \beta(2^{1/\alpha} - 1) \quad (11.5)$$

Equation (11.4) can be rewritten in a form without the β parameter (Haas *et al.* 1993):

$$P_{inf} = 1 - [1+D/ID_{50}(2^{1/\alpha} - 1)]^{-\alpha} \quad (11.6)$$

Another widely used dose-response model is the beta-binomial model. Fazil (1999) states that the beta-binomial is a modified form of the beta-poisson that incorporates the variability in the pathogen-host probability of infection given a certain, variable dose, rather than the average dose considered in the standard Beta Poisson model.

$$P_{inf}(n;r) = 1-(1-Beta(\alpha,\beta))^{n} \quad (11.7)$$

The beta-binomial model can be used in a MCMC environment, where sampling from the Beta-distribution with parameters α,β can be performed (Nauta *et al.* 2007).

Mean values for this probability can be calculated as

$$P_{inf}(n;r) = 1-(\Gamma(\alpha + \beta)\Gamma(\beta + n))/(\Gamma(\beta)\Gamma(\alpha + \beta + n)) \quad (11.8)$$

where Γ is the gamma function (Haas 2002).

Therefore, as expected, the, mean values obtained by MCMC simulation for the $P_{inf}(n;r)$ for the beta-binomial model are almost identical to $P_{inf}(D;\alpha,\beta)$ for the beta-Poisson model.

A reservation has been stated regarding the (simplified) Beta-Poisson model, as the confidence interval can be very wide in the case of very low doses (Teunis & Havelaar 1996). In these cases the model can predict a risk of infection that is higher than the risk of exposure, which obviously is not plausible (FAO/WHO 2003). When there is reason to expect problems of this kind the version using the confluent hypergeometric function can be used (Teunis & Havelaar 1996).

As described above, the probability of human illness is dependent on two probabilities – the probability of infection given exposure and the probability of illness given infection (i.e. $P_{(Illness|Infection)}$.). Data on $P_{(Illness|Infection)}$ is very scarce as in most outbreaks only the number of ill patients is known and no information of infected persons who do not become ill is available. However, feeding trial data can often provide information on this probability e.g. (McCullough & Eisele 1952 and Bemrah *et al.* 2003).

When describing $P_{(Illness|Infection)}$, probabilities from feeding trials are often used as the proportion of ill persons out of infected persons ignoring a dose-effect. For *Salmonella* a probability of 0.10 has been suggested (Bemrah *et al.* 2003) and for *Campylobacter* 0.33 (Nauta *et al.* 2007). Another possibility is applying a hazard function for the probability of illness given infection (Teunis *et al.* 1999). In some cases (e.g. *Campylobacter*) this leads to a decreasing probability of illness with increasing dose, which is not biologically convincing

11.1.1 Determination of model parameters and uncertainty analysis

The model parameters (r for the exponential model or α,β for the hypergeometric/beta-poisson/beta-binomal models) can be estimated from feeding experiments in volunteers (Black *et al.* 1988 and McCullough & Eisele 1952) experimental animals or from outbreak data (Teunis *et al.* 2004), if it is possible to obtain quantitative data for the inoculum ingested. In both outbreak data and data from feeding experiments with volunteers the representativity of the cases can be questioned, as there may be overrepresentation of e.g. young healthy males in volunteer studies and young children or elderly persons in outbreak data. In some occasions surrogate pathogens are used instead of the pathogen of interest (USDA-FSIS 1998).

Fitting the model to experimental or outbreak data can be performed by optimizing the log-likehood function by maximum-likelihood techniques (Teunis *et al.* 1996) or by Bayesian methods using Markov Chain Monte Carlo (Metropolis-Hastings algorithm) implemented e.g. in Mathematica (Teunis *et al.* 2008) or WinBUGS (Chen *et al.* 2006).

Confidence limits (e.g. 95%) for the dose-response curve can be obtained by bootstrapping (Medema *et al.* 1996) or by MCMC methods (Chen *et al.* 2006).

11.1.2 Bacterial dose-response models

When different dose-response models are compared in connection with bacterial enteric infections, the Beta-Poisson model is usually preferred. For *Salmonella enterica* v. meleagridis and *Campylobacter jejuni* the fit of the Beta-Poisson model was significantly better than the fit of the exponential model (Teunis *et al.* 1999). Also for other *Salmonella enterica* serovars e.g. *anatum* and *enteritidis,* , the Beta-Poisson model provides the best fit (Teunis *et al.* 1996 and USDA-FSIS 1998). Also Shigellosis is usually modelled by the Beta-Poisson model (USDA-FSIS 1998 and Crockett *et al.* 1996).

11.2 *Salmonella* dose-response models

The FAO/WHO *Salmonella* risk assessment (FAO/WHO 2002) refers to three previously published *Salmonella* dose-response models:

- The first (Fazil 1996) is the beta-Poisson model (Haas 1983) fitted to the human feeding trial data for *Salmonella* infection (McCullough & Eisele 1951).

- The second model was proposed in the US *Salmonella* Enteritidis Risk Assessment (USDA-FSIS 1998) and was based on the use of a surrogate pathogen (*Shigella*) to describe the dose-response relationship.

- The third model was introduced in a *Salmonella* Enteritidis risk assessment done by Health Canada (Health Canada, 2000, unpublished), which was based on a Weibull dose-response relationship that was updated to reflect selected outbreak information using Bayesian techniques.

The model chosen by the FAO/WHO *Salmonella* risk assessment (FAO/WHO 2002) was a beta-poisson model based on data from 23 *Salmonella* outbreaks shown in Table 11.1. It was concluded that the outbreak model (beta-Poisson model) should be preferred to the previous models. The following drawbacks of the previous models were mentioned:

Naive human feeding trial data (beta-Poisson model)
The model suffers from the nature of the feeding trial data (i.e. the subjects used were healthy male volunteers) and may not reflect the population at large. The model also tends to greatly underestimate the probability of illness as observed in the outbreak data.

US SE RA (beta-Poisson model)
The model uses human feeding trial data for *Shigella dysenteriae* as a surrogate pathogen, with illness as the measured endpoint in the data. The appropriateness of using *Shigella* as a surrogate for *Salmonella* is questionable given the nature of the organisms in relation to infectivity and disease.

Health Canada *Salmonella* Enteritidis (Weibull-Gamma model)
To date, this model has not been fully documented and lacks transparency. The model uses data from many different bacterial-pathogen-feeding trials and combines this information with key *Salmonella* outbreak data using Bayesian techniques. Using data from many bacterial-feeding trials and the current lack of transparency regarding their influence is a point of caution.

Table 11.1. Summary of outbreak data. (From FAO/WHO *Salmonella* risk assessment, 2002)

Case no.	Serovar	Food	Popn.(1)	Dose(2) Log CFU	Attack Rate(2)(%)	Reference(s)
1	*S. Typhimurium*	Water	N	2.31	10.63%	Boring, Martin and Elliott, 1971
	S. Typhimurium	Water	S	2.31	18.91%	
2	*S. Heidelberg*	Cheddar cheese	N	2.22	32.76%	Fontaine et al., 1980
3	*S. Cubana*	Carmine dye	S	4.57	70.93%	Lang et al., 1967
4	*S. Infantis*	Ham	N	6.46	100.00%	Angelotti et al., 1961
5	*S. Typhimurium*	Imitation ice cream	N	3.79	55.00%	Armstrong et al.,1970
7	*S. Newport*	Hamburger	N	1.23	1.07%	Fazil., 1996 Fontaine et al., 1978
11	*S. Enteritidis*	Hollandaise sauce	N	4.74	100.00%	Levy et al., 1996; USDA-FSIS., 1998
12	*S. Enteritidis*	Ice cream	N	2.09	6.80%	Vought and Tatini, 1998; Hennessy et al., 1996
13	*S. Typhimurium*	Ice cream	N	8.70	100%	Taylor et al., 1984
	S. Typhimurium	Ice cream	S	8.00	100%	
18	*S. Enteritidis*	Roasted beef	N	5.41	60.00%	Ministry of Health and Welfare, Japan, 1999
19	*S. Enteritidis*	Grated yam with soup	N	6.31	93.93%	
20	*S. Enteritidis*	Beef and bean sprouts	N	2.97	26.86%	Ministry of Health and Welfare, Japan, 1999
22	*S. Enteritidis*	Scallop with egg yolk	N	6.30	56.01%	
23	*S. Enteritidis*	Cake	N	5.80	84.62%	
24	*S. Enteritidis*	Peanut sauce	N	1.72	16.41%	
25	*S. Enteritidis*	Chicken and egg	N	3.63	18.75%	
25	*S. Enteritidis*	Chicken and egg	S	3.63	42.74%	
30	*S. Enteritidis*	Cooked egg	N	3.80	64.18%	
31	*S. Enteritidis*	Cake	N	2.65	27.33%	
32	*S. Enteritidis*	Egg salad	S	1.40	26.92%	
33	*S. Oranienburg*	Grated yam with soup	N	9.90	100%	

(1) Popn. = population exposed, where N = Normal population and S = Susceptible population.
(2) Expected value based on defined uncertainty ranges and distributions.

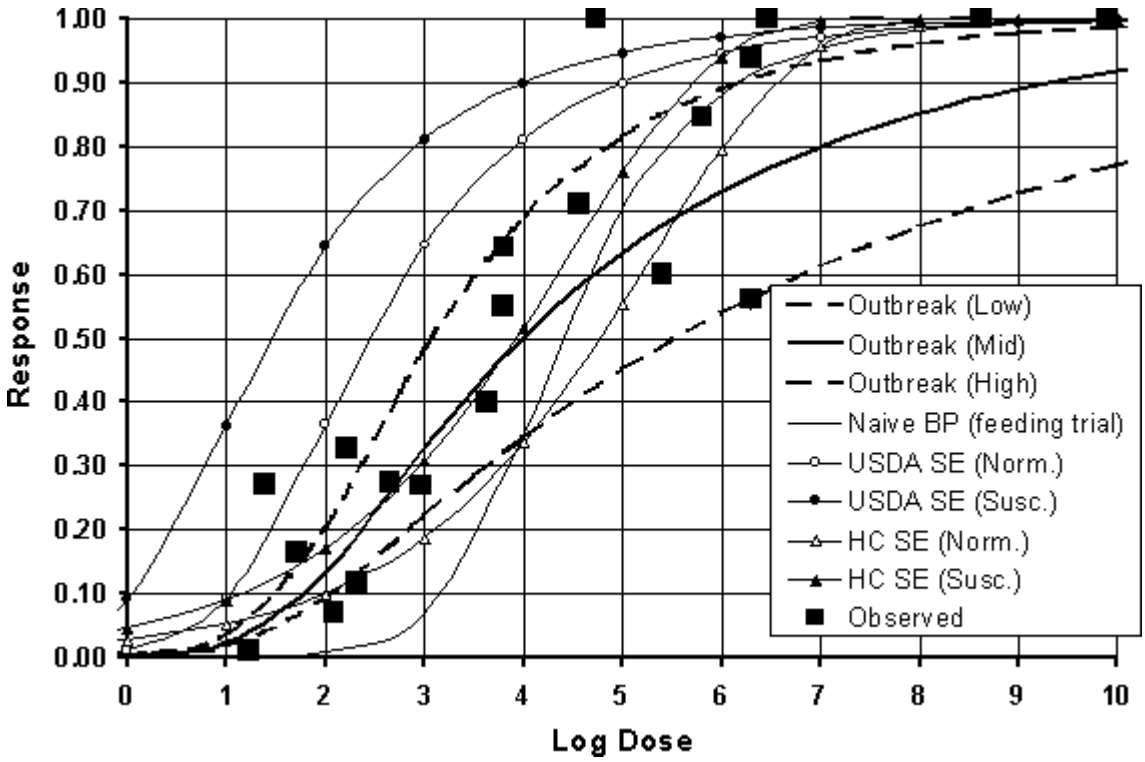

Figure 11.1 Comparison of all dose-response models with reported outbreak data. (FAO/WHO 2002)

Outbreak: Beta-Poisson curves estimated from outbreaks (marked Observed) in Table 11.1
Naïve BP: Beta-Poisson curve based on *Salmonella* feeding trial
USDA SE: Beta-Poisson curves based on *Shigella* feeding trial
HC SE: Weibull-gamma curves based on *Salmonella* feeding trial combined with outbreak data

Table 11.2. Beta-Poisson dose-response parameters that generate the approximate bounds shown in Figure 2 . (FAO/WHO 2002)

	Alpha	Beta
Expected Value	0.1324	51.45
Lower Bound	0.0763	38.49
2.5th Percentile	0.0940	43.75
97.5th Percentile	0.1817	56.39
Upper Bounc	0.2274	57.96

The alpha and beta parameters were estimated by bootstrapping from the original data set, creating 5000 datasets and fitting beta-Poisson curves to each set.

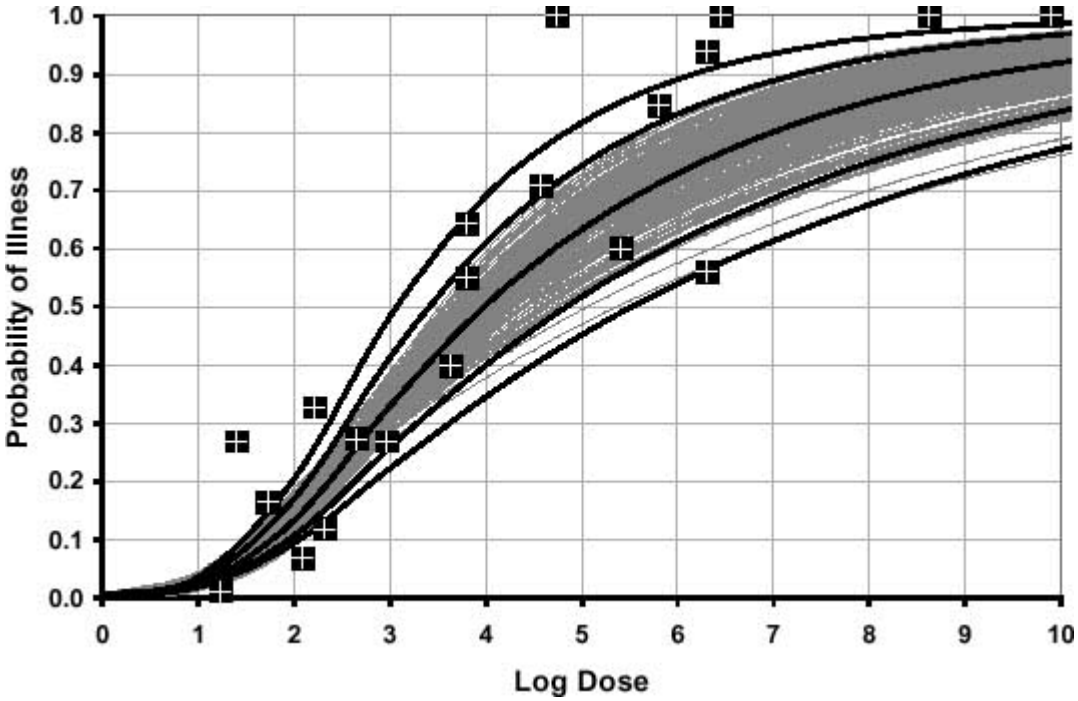

Figure 11.2 Uncertainty bounds for dose-response curves, compared with expected value for the outbreak data (FAO/WHO 2002)

Since the FAO/WHO *Salmonella* risk assessment relatively few *Salmonella* dose-response models have been published. Bemrah (Bemrah *et al.* 2003) has adopted the Beta-Poisson model for modelling probability of *Salmonella* infection from a turkey product. The parameters α and β were set to 0.33 and 139.9 (Rose & Gerba 1991) and the probability of illness given infection was estimated to be 10% based on data from the *Salmonella* feeding trial of McCullough & Eisele 1951.

Oscar (2004) has published a dose-response model based on human feeding trials (McCullough & Eisele 1951) with 13 different *Salmonella* strains using a three-phase linear model (minimal illness dose, median illness dose and maximum illness dose) with subsequent use in Pert distributions in a computer simulation model. Due to the scarcity of data for some strains and the feeding trial background of the data the model probably is not valid for universal use.

Recently a new methodology (Bollaerts *et al.* 2008) has been applied to the outbreak data used for the FAO/WHO *Salmonella* risk assessment. A generalized mixed model approach with serovar and food-matrix-specific random effects has been used. The usual conventional polynomials for continuous variables have been replaced by modified fractional polynomials and a two-stage bootstrapping procedure accounts for both stochastic variability and for data uncertainty. The method lacks the biological explanation of the parameters afforded by the beta-Poisson model, but may be appropriate when the effect and interaction of serovars and food-matrix is the topic of interest.

11.3 Choice of dose-response model for the EFSA *Salmonella* in Pigs QMRA

As the model will be applied to individual doses a Beta-Binomial version of the Beta-Poisson model will be used with the same α- and β- parameters (0.1324 and 51.45) as the FAO/WHO *Salmonella* risk assessment dose-response model. These parameters are estimated from outbreaks with several serovars and are deemed to be more appropriate on the EU-scale than parameters from feeding trials with single serovars.

As the outcome of the FAO/WHO *Salmonella* risk assessment dose-response (beta-Poisson) model is the probability of illness (attack rates) it should be noted that there is no need for a model step calculating $P_{(Illness|Infection)}$.

11.4 References

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12 Risk Characterisation

12.1 Baseline Results

12.1.1 Methods

The principal output of the model is the probability of *Salmonella* illness in each case study MS ($P_{illness}$) due to the consumption of pork cuts, minced meat and fermented sausages. The baseline model is run with the (baseline) values described in the previous chapters. These values represent the best estimates for the model parameters at the current time. For each parameter, efforts were made to use MS-specific values. However, if these were not available surrogate estimates were applied from a MS that had data for that parameter; first choice was from a MS that were grouped to the same cluster, and second choice from any MS within or outside the same cluster.

The model was separately run for the four case-studies: MS1, MS2, MS3 and MS4. More detail can be found on the running of the model in the technical chapters (Chapters 4-11), but briefly we give an overview. The Farm module was run for 500 days over 1,000 iterations (each iteration represents a different farm) for both small and large farms. The number of iterations and days was set to ensure convergence and to allow the variation between the different farm management set-ups to be represented for each MS. The output matrix from the Farm module serves as the input for the second phase of the exposure assessment (Transport – Consumption). This phase of the model is run for 10,000 iterations (where each iteration represents a different day in the slaughterhouse), to ensure model convergence. Within each iteration, 10,000 independent servings were simulated, and the numbers of CFUs per serving were input into a dose-response relationship estimating the probability of illness for each individual serving. This probability was used in a binomial trial predicting whether the serving resulted in a consumer's illness or not. This is done 10,000 times per product type, per iteration (i.e. there are 100,000,000 servings each of pork cuts, minced meat and fermented sausage considered within a MS model). The proportion of illness in the 10,000 servings was interpreted as the probability of infection ($P_{illness}$) in each specific iteration. Ten thousands iterations were run, estimating a new probability of infection within each iteration. The overall mean of the iteration specific probability of illness is the output of the model. Figure 4.1 in Chapter 4 summarises the model framework.

Based on the estimated probability of illness, obtained at the end of the dose response module, we estimate the annual number of cases for each member state, $N(G,H)$, where G={MS1, MS2, MS3, MS4}, and H={PC, MM, FS}, to represent pork cuts, minced meat and fermented sausage respectively.

$$N(G,H) = \frac{frequency_{consumption}(G,H)}{portion_{size}(G,H)} * P_{illness}(G,H) * Population_{size}(G) * 365 \qquad (12.1)$$

where $frequency_{consumption}(G, H)$ is the amount of product H consumed in G (grams per day per person), $portion_{size}(G,H)$ is the size of one portion of product H in G (grams) and $Population_{size}(G)$ is the population of G. Therefore, data on population size, frequency of

consumption and portion size of each product was collected for each MS and used to estimate the number of *Salmonella* cases in each MS, for each product. The estimates for the parameters $frequency_{consumption}$, $portion_{size}$ and $Population_{size}$ are given in Table 12.1. As described above, $P_{illness}$ is the output of the QMRA. In case of data unavailability, information from another MS country was utilised.

Appendix 12.1 provides a summary of EU consumption data that was collected as part of this project.

12.1.2 Baseline results

The running means of $P_{illness}$ for each product, after each iteration, are displayed in Appendix 12.2. For all products, convergence of the model was obtained after around 4,000 iterations (the average $P_{illness}$ stabilised). The final estimate of $P_{illness}$ is the mean after 10,000 iterations, for the three different types of products (Table 12.2).

Table 12.1: Consumption and demographic data used to calculate the number of cases of salmonellosis attributed to pork cuts (PC), minced meat (MM) and fermented sausage (FS).

	Freq. of consumption [g/day/person] ($frequency_{consumption}$)	Portion size [g] ($portion_{size}$)	Population ($Population_{size}$)
MS1	PC - 33 *	PC – 146 (5) **	8.2 million *****
	MM – 2.55 *	MM – 125 (6) ****	
	FS – 10 *	FS – 150 (6) ****	
MS2	PC – 3.5 *	PC – 146 (5) **	60.2 million *****
	MM – 2.83 (1) *	MM – 125 (6) ****	
	FS – 0.69 *	FS – 150 (6) ****	
MS3	PC – 43 *	PC – 200 (7) ***	38.1 million *****
	MM – 4.34 (2) *	MM – 77 (7) ***	
	FS – 2.25 *	FS – 110 (7) ***	
MS4	PC – 28.6(3) **	PC – 200 (7) ***	10.2 million *****
	MM – 4.48 (2) ***	MM – 77 (7) ***	
	FS – 8.56 (4) *	FS – 110 (7) ***	

(1) Data from Belgium; (2) Data from Slovenia; (3) Data from Luxembourg; (4) Data from Finland; (5) Data from Ireland; (6) Data from Sweden; (7) Data from Czech Rep.
* DAFNE project, ** Anon., 2008, *** Anon., 2004, **** Anon, 2009, ***** Anon., 2009a

Table 12.2: Baseline results from the model: mean probability of illness for member states, due to consuming one serving of pork cuts, minced meat or fermented sausage.

Mean probability of illness (one serving)	MS1	MS2	MS3	MS4
Pork Cuts	7.65×10^{-7}	1.86×10^{-5}	3.88×10^{-7}	2.55×10^{-6}
Minced Meat	8.84×10^{-7}	2.24×10^{-5}	2.32×10^{-7}	2.58×10^{-7}
Fermented Sausage	1.87×10^{-6}	4.25×10^{-5}	5.78×10^{-7}	4.29×10^{-6}

For all product types, the estimated value of $P_{illness}$ varies with a factor of about 100 between the countries. The average rate of illness is between 1 in 100,000 and 1 in 10 million servings of a particular product type. For all MSs, the product with the highest probability of illness, per serving is fermented sausage. The lowest risk, per serving, is associated with pork cuts (MS1, MS2) or minced meat (MS3,MS4). Across all products, MS2 and MS4 are predicted to have a higher probability of illness.

Table 12.3 provides the model predicted number of cases per year per MS, attributed to each product type. The predicted number of cases per MS, from the consumption of the 3 products, is estimated to be 949 (12 cases per 100,000 inhabitants) (MS1), 25,248 (42 cases per 100,000 inhabitants) (MS2), 1,509 (4 cases per 100,000 inhabitants) (MS3) and 2686 (26 cases per 100,000 inhabitants) (MS4). In addition to the effect of the estimated risk of illness, the consumption patterns also influenced the estimated number of cases. For example, although, per serving, the highest risk of illness for MS2 was fermented sausage, the number of predicted cases is the lowest as this product type isn't consumed as often as minced meat products or pork cuts.

12.2 Validation of the Model

12.2.1 Methods used for validation of the model

The validity of the model was assessed by comparing predicted results from the model with observed (epidemiological) results in the populations of interest. The comparison was done by relating the magnitude of the predicted and observed value, and qualitatively assessing the degree of agreement/disagreement.

Table 12.3: Number of cases, per year, attributed to pork cuts (PC), minced meat (MM) and fermented sausage (FS), for the four case- study Member States.

Number of predicted cases (per year)	MS1	MS2	MS3	MS4
Pork Cuts	520	9802	1162	1384
Minced Meat	125	11148	182	56
Fermented Sausage	375	4298	165	1246
Total	949	25248	1509	2686

In this project we compared the output at three different points in the model: 1) prevalence of lymph-node positive pigs post-lairage (comparable to the lymph-node positive prevalence from the EFSA baseline survey (EFSA 2008b)); 2) prevalence and concentration of contaminated portions at retail and 3) number of human cases. For each validation, the baseline model was used.

The validation of the prevalence post-lairage (i.e. before entering the slaughterhouse) allows an evaluation of the Farm and Transport & Lairage module. The validation at retail level permits an assessment of Slaughter & Processing module, which includes the cutting plant. The validation of the number of human cases assesses the Preparation & Consumption module. The validation of human cases is also a useful measure for the validity of the model as a whole.

Note that the observed (epidemiological) data to which the estimated parameters are being compared includes uncertainty due to sampling error (statistical uncertainty) and imperfect test sensitivity and/or specificity. In addition, the samples whereupon the epidemiological data are based do not match the units in which we work within the model. For example, tests used to detect *Salmonella* at slaughterhouse or retail will not be 100% sensitive and human epidemiological data will be subject to under-reporting. Therefore, the comparison of predicted and observed values was done on a qualitative basis with focus on whether the model was predicting the same trends that were indicated by the observed data.

12.2.2 Results of the validation

Validation at post-lairage level

Table 8.7 shows the changes in prevalence of infection during the stages of Transport & Lairage for large and small farms combined. The output at the end-point of the Transport & Lairage module (i.e. post-lairage) is the prevalence of lymph node positive pigs at slaughter, which is comparable to the observed prevalence in the EFSA baseline study (EFSA 2008b). The predicted and observed values are presented in Table 12.4. This table also includes the 2.5-97.5 percentiles of the 10,000 simulated prevalences of pigs with *Salmonella* (variability), and the 95% confidence interval for the observed prevalence (which indicates the precision of the observed prevalence - uncertainty).

Table 12.4: Prevalence at post-lairage predicted by the model, and the correspondent results reported to EFSA, in a baseline study (EFSA 2008b). Both estimates of prevalence refer to the lymph node.

MS	Model: prevalence of pigs with *Salmonella* (%) (mean), [2.5th – 97.5th] percentiles (%))*	EFSA Baseline results: prevalence of pigs with *Salmonella* (%) (mean), [95% CI]**
MS1	1, [0.83 – 3.66]	2, [1.1 – 3.6]
MS2	20, [15 – 55]	21.2, [17.8 – 25]
MS3	0.7, [0.64 – 2.8]	5.1, [3.7 – 6.9]
MS4	3.5, [3.5 – 17.5]	5.8, [3.8 – 8.9]

*[2.5th – 97.5th] percentiles describing the variability; ** [95% CI] describes the uncertainty

Concerning post-lairage, the results from the model match quite well to the results obtained in the EFSA survey, particularly for MS1, MS2 and MS4. For MS3, there is a discrepancy

between the prevalence observed in the survey and the predicted prevalence in the model. In particular, the model gives a lower average prevalence compared to what was observed in the survey. The predicted prevalence is also lower than the 5th percentile of the baseline prevalence for MS3 (3.7%). Investigation of the model suggests that this discrepancy likely comes from the generic model structure not capturing a specific aspect of production in MS3 at the farm, and particularly within the small farm model, given there is a relatively high percentage of small farms in MS3.

Validation at retail level

Table 12.5 shows the predicted and observed (whenever data were available) prevalence at retail level for the 4 MSs and for both pork cuts and minced meat. Also, the predicted prevalence for fermented sausages at storage is presented. The predicted mean value for the microbial load at the same stage is also presented in the table.

For MS1, comparing with data reported to EFSA, the model underestimates the prevalence for both pork cuts and minced meat. However, for both the predicted and reported data, minced meat has a higher prevalence than the pork cuts. Looking at the results for MS2, following the trend already seen in the post-lairage results, MS2 has a higher prevalence of *Salmonella* at retail than MS1 for each product type. For pork cuts in MS2, the model predicts a higher prevalence in pork cuts when compared to the prevalence observed by Little *et al.* (2008). There are many possible reasons for this divergence including that the QMRA will define a product as positive if it has 1 or more

Table 12.5: Predicted and observed (whenever data was available) prevalence at retail level for pork cuts (PC) and minced meat (MM) and fermented sausage (FS); Predicted microbial load at retail level also for the three product types (in *Salmonella* log cfu).

Member State	Product type	Prevalence predicted (%)	Predicted average microbial load (log CFU per portion)	Observed prevalence (%)	Source of data
MS1	PC	0.18	0.57	1 [(1)]	EFSA, 2009a
	MM	0.20	0.92	1.6 [(2)]	
	FS	0.004	0.17		
MS2	PC	4	0.69	1.9	Little et al. 2008
	MM	5	1.06		
	FS	0.09	0.66		
MS3	PC	0.07	0.44		
	MM	0.05	0.67		
	FS	0.001	0.06		
MS4	PC	0.5	0.37		
	MM	0.3	0.58		
	FS	0.01	0.17		

[(1)] Samples: 10/25 g; [(2)] Samples: 10 g;

Salmonella present; however a microbiological test would not be able to detect *Salmonella* at such low numbers. In addition the effects of between-slaughterhouse and between butchering stages may not have been captured sufficiently within the model.

It was not possible to get reported data on *Salmonella* prevalence or microbial load for MS3 and MS4. However, data from the EFSA trends and sources report (EFSA., 2009a) give ranges for the prevalence in pork cuts of 0%-6.1%, for minced meat 1.3% - 5.9% and for ready-to-eat minced meat/minced meat products (which includes fermented sausages) of 0%-3.3%. The results obtained in the model are in the same order of magnitude, with the results from all case studies falling within or slightly below these observed intervals. Concerning the concentration of *Salmonella* in contaminated cuts at retail; across a number of EU MSs, studies show that contamination on retail cuts is comparatively low (scaling up to the unit of a serving commonly less than 10 CFU/portion) (Prendergast *et al.,* 2009, Delhalle *et al.,* 2009). The average number of *Salmonella* contaminating the three product types was predicted in the simulations to range from 1-11CFU/portion for all MS/product-type combinations.

Overall it can be concluded that the model is producing realistic enough results at the point of retail to differentiate between MSs and provide a baseline from which to conduct intervention analysis.

Validation of the final output: number of cases

To validate the final output of the model, the total number of predicted cases per year (see Table 12.3) attributed to pork cuts, minced meat and fermented sausages was compared

with the total number of cases of salmonellosis (from all sources) reported by EFSA, 2009a (Table 12.6). In addition, information is provided on the predicted and observed number of cases per 100,000 habitants.

The model predicts that MS2 will have the highest incidence of salmonellosis, and the most cases of salmonellosis, due to consumption of the 3 pig meat products considered here, followed by MS4, MS1 and MS3. However, comparing this to the incidence estimated from the total number of reported cases to EFSA, MS4 has the highest number of cases per 100,000 inhabitants, followed by MS1, MS3 and MS2 (although MS2 and MS4 have similar numbers of reported cases).

Table 12.6: Total number of cases and cases per 100,000 habitants predicted (for pork cuts, minced meat and fermented sausage); total number of cases and cases per 100,000 habitants of salmonellosis (all sources); reported to EFSA, 2010

	Total no. cases predicted per year (PC+MM+FS)	Total no. reported cases of salmonellosis (EFSA 2010)
MS1	949 (12 cases per 100,000 habitants)	2310 (28 cases per 100,000 habitants)
MS2	25248 (42 cases per 100,000 habitants)	11511 (19 cases per 100,000 habitants)*
MS3	1509 (4 cases per 100,000 habitants)	9149 (24 cases per 100,000 habitants)
MS4	2686 (26 cases per 100,000 habitants)	10707 (105 cases per 100,000 habitants)

*Adjusting the MS2 number of reported cases for under-reporting of 3.2 [1.4-12.0] cases per reported case and 3.4-3.7% the estimated total number of *Salmonella* is between 548-5,110. This calculation was not possible to perform for the other MSs due to data gaps.

In summary, if we consider likely under-reporting ratios and the attributable fraction of cases to pig meat consumption, it is likely that (although the estimates of *Salmonella* prevalence look reasonable at the point of slaughter and retail) the QMRA is over-estimating the number of cases attributable to the 3 product types. However, a direct comparison between the numbers predicted and reported number of human cases is not straight-forward. The following possible issues have been identified:

- The number of cases of salmonellosis reported refer to cases from all sources, and not only pork. In Chapter 14, it is estimated that 10-20% of all *Salmonella* infections in EU are attributable to pork. Indeed, although not carried out for all 4 MSs, a source attribution study using MS2 *Salmonella* data estimated that between 3.4-3.7% of *Salmonella* were attributable to pigs/pork (Pires *et al.,* 2008, Pires, 2009).

- The reporting of cases itself is biased due to under reporting, caused by differences in health systems, by the fact that not all patients seek medical care, or that not all patients get tested. Several studies suggest multipliers of different values to get the real number of cases of salmonellosis. In England in the mid 1990s, investigators determined that for every laboratory-confirmed case of *Salmonella* reported to national surveillance, 3.8 cases occurred in the community (Wheeler *et al.,* 1999). In a study in US, this so called multiplier was estimated to be 38.6 (Voetsch *et al.,* 2004).

- The observed data of human cases or prevalences and concentration in the farm-to-consumption chain can originate from risk-based surveys, where data originated from the populations expected to have a relatively high prevalence of

Salmonella/salmonellosis, not reflecting the overall prevalence of *Salmonella*/salmonellosis.

- The QMRA was carried out for *all* types of *Salmonella.* Within the mandate, EFSA were asked "to consider all serovars in pigs that are of human health significance". EFSA, 2006 concluded that "all *Salmonella* serovars in pork are to be regarded as a hazard for public health" and recognised that there will be variability between strains in their behaviours across the food chain. It was therefore deemed acceptable by EFSA (as stated in the call for proposals) for the QMRA to consider all types similarly and hence that a QMRA for *Salmonella* spp. would be appropriate. However it is recognised that this assumption is not valid as, for example, many serovars are commonly seen in pigs but rarely observed in human infections (e.g. *S.* Derb*y)*, others are commonly detected in both pig populations and in human cases of salmonellosis (e.g. *S.* Typhimurium). This could be attributable to differences in the dose-response relationship (see bullet point below) or the possibility that *S.* Derby does not survive very well within the slaughter and processing environment.

- The validity of the predicted number of cases is dependent on the validity of the exposure assessment and the dose-response relationship. The exposure assessment has, to some extent, been validated at the point of retail. The Preparation & Consumption module is a necessary, but notoriously uncertain, module within the QMRA. In addition, the dose–response relationship is based on data from outbreaks, and as described above, we necessarily assume the same dose-response relationship for all strains of *Salmonella*, all meal types, and all ages/health status of consumers. This assumption could over-estimate the dose-response. For example, in many cases, an outbreak is caused by a high virulent strain. Therefore, the dose-response relationship used in this project is most valid for relatively high virulent strains, hence the use of a "high-virulent" dose-response curve will result in an overestimation of the predicted number of cases.

- It was decided not to include uncertainty in the model, although values of many of the parameters in the model were highly uncertain. The uncertainty analysis (section 12.3) reveals whether incorporating the uncertainty around these values in the model will influence the risk of illness significantly. If the analysis suggests that the uncertainty about a parameter estimate is significant, then if the estimate used in the baseline model was far from the true value, this will result in an over- or underestimation of the "true risk". Using the predicted risk in calculating the number of cases will then be different from the true number of cases.

As stated above, the purpose of looking to observed data to validate the results of the model is not to compare the absolute values, but instead analyse and evaluate the relative values and trends. A total concord in values of predicted and observed prevalence or number of cases is not expected due to different biases in both predicted and observed values. Indeed, many QMRAs overestimate the number of cases, for example Nauta *et al.,* 2001 and Nauta *et al.,* 2007.

The validation of the model suggests that a large majority of the important factors that determine the *Salmonella* contamination within the pig meat food chain are captured within the model, certainly at the point of slaughter and in the slaughterhouse. Clearly, factors important in determining human infection have not been captured, as the number of cases per year is over-predicted. However, we must assess the impact these missed factors have

on interpreting the effectiveness of interventions at a MS level. In our opinion, with due attention paid to the uncertainties identified (below and in the other chapters), the model can be used to assess the relative effect of interventions (e.g. percentage of reduction in the number of cases)[34].

12.3 Uncertainty Analysis

As highlighted above, many of the model parameter estimates are highly uncertain due to significant data gaps / deficiencies (see Chapters 7-11). The objective of the uncertainty analysis is to assess the effect that these parameters have on the model output and, in particular, the probability of illness therefore providing insight into the reliability of the results predicted by the model. The uncertainty analysis also indicates the degree to which key data gaps are contributing to the uncertainty about the probability of illness. Identification of important data gaps will be important in the prioritisation of future data collections in EFSA and the MSs with the aim to reduce the uncertainty in the model output.

All MS-specific parameters are inputs into the model and can be updated, if more recent or more appropriate data become available. Similarly, the MS-specific values of the parameters can be changed in accordance to other MS data, thereby allowing the prediction of risk of illness in other EU MSs.

12.3.1 Methods used in the uncertainty analysis

Due to the non-existence or unavailability of data needed for some of the parameters in the model, some of the values and distributions used are uncertain. In the case of missing or incomplete knowledge, proxy-data is used, for example data reported in other countries, or data referring to products other than pork. To assess the influence of these parameters, alternative scenarios of the model were run, where the uncertain parameters were changed to a minimum and a maximum value, respectively. These alternative values were subjectively identified, based on our opinion concerning what was the realistic minimum and maximum value of the parameter (therefore, the result from the uncertainty analysis is influenced by our subjective opinion of realistic values). The resulting probability of illness (for the three products) is compared with the baseline results. In cases where a parameter has a distribution associated with it, the alternative scenario is run with differently parameterised probability distribution.

The effect on the output from the different scenarios was assessed by the relative effect of each parameter on $P_{illness}$ for each product. The relative effect was quantified using Equation 12.2

$$\frac{Pillness_{alternative\ value}}{Pillness_{baseline\ results}} - 1 \qquad (12.2)$$

[34] The absolute value of risk is important in determining the relative effect of interventions, as the effect of changing a mechanism will be dependent on the initial value of the concentration/prevalence. However, given we are probably within an order of magnitude difference from the number of cases, and are within right order of magnitude at slaughter and retail prevalence, then we assume the relative effects can be reliably estimated with the current model.

Due to the long running time of each simulation, the uncertainty analysis was performed for MS1 and MS2 only. To reduce the duration of the simulations, the number of iterations used in the uncertainty analysis was reduced compared to the baseline simulation. For the Farm module, the alternative scenarios were run for 300 iterations and 500 days to obtain the revised Farm Matrix for each scenario investigated. This was then, followed by 10,000 simulations for the remainder of the model to obtain the alternative probability of illness.

For the non-farm parameters (i.e. those in the Transport & Lairage; Slaughter & Processing; Preparation & Consumption modules), the matrices from the baseline Farm module were used and then 10,000 simulations were run.

The parameters investigated in the uncertainty analysis are provided in Table 12.7, 12.8, 12.9, 12.10 representing the different parts of the model respectively.

The uncertainty analysis results are shown through clustered-bar plots that allow an easy visualisation of the relative effect of each parameter in the final output.

Table 12.7: List of the parameters and alternative values, from the Farm module, included in the uncertainty analysis.

Farm parameters	Original Value	Alternative value
Prevalence of infection within a herd	0.21	0.01 & 1
Probability of feed lot contamination	0.1	0.01 & 0.09
Cleaning coefficient for solid flooring	50	30
Decay constant for *Salmonella* inside farm environment	0.04	0.1
Max of faeces ingested by the piglets	20	100
Max of faeces ingested by finishers	100	400

Table 12.8: List of the parameters and alternative values, from the Transport & Lairage module, included in the uncertainty analysis.

Transport & Lairage Parameters	Original Values	Alternative Values
Probability of the pigs being stressed during transport	0.2	0.5 & 0.1
Concentration of *Salmonella* on skin (1log, 2logs, 3logs)	[0.3 0.5 0.2]	[0.5 0.3 0.2]
α parameter dose response	0.1766	0.01
β parameter dose response	20235	100235

Table 12.9: List of the parameters and alternative values, from the Slaughter & Processing module, included in the uncertainty analysis..

Slaughterhouse & Cutting Plant Parameters	Original Values	Alternative Values
Frequency of puncturing the gut (LS)	(0.012, 0.02)	(0, 0.0001) & (0, 0.1)
Frequency of puncturing the gut (SS)	(1/3000, 2/3000)	(0, 0.0001) & (0, 0.1)
Inactivation Singeing (SS)	2.37	1 & 10
Dehairing transfer machine to pig (LS)	(-1.5, 0)	(-5, 0) & (0,0)
Amount of faeces spilled while dehairing (LS)	10	1 & 50
Amount of faeces spilled at belly opening (LS)	(6.6, 20.3)	(6.6, 10) & (6.6, 50)
Scalding temperature (SS)	(55, 60, 65)	(60, 65, 70) & (65, 70, 75)

Table 12.10: List of the parameters and alternative values, from the Preparation & Consumption module, included in the uncertainty analysis.

Consumption Parameters	Original Values	Alternative Values
Time in consumer´s fridge for MM	[1/4,1/2,1,2,3,4,5,6,7,8 ,9,10,12,14]	Double and half the values
Time in consumer´s fridge for PC	[1/4,1/2,1,2,3,4,5,6,7,8 ,9,10,12,14]	Double the values
Portion size PC	0.146	0.03 & 0.3
Portion size MM	0.125	0.03 & 0.3
Portion size FS	0.150	0.03 & 0.3
Sausage pH	N(4.29 , 0.07)	N(4.29 , 0.5) & N(4.29 , 0.1)
Cross – contamination for PC	ths=0.02,tth=0.023, tpb=0.023, tbs=0.26 (1)	All 0.1 & 0.01

(1) For more information on these parameters, please refer to Chapter 10

12.3.2 Results

The relative effect of changing to alternative values of parameters in the Farm module in MS1 and MS2, respectively are presented in Figures 12.5 and 12.6. The relative effect of changing to alternative values of parameters in the Transport & Lairage module in MS1 and MS2, respectively are presented in Figures 12.7 and 12.8, and the relative effect of changing to alternative values of parameters in the Slaughter & Processing module in MS1 and MS2, respectively are presented in Figures 12.9 and 12.10, and the relative effect of changing to alternative values of parameters in the Preparation & Consumption module in MS1 and MS2, respectively are presented in Figures 12.11 and 12.12.

The parameters where the uncertainty values has large impact on the probability of infection are listed in Table 12.11

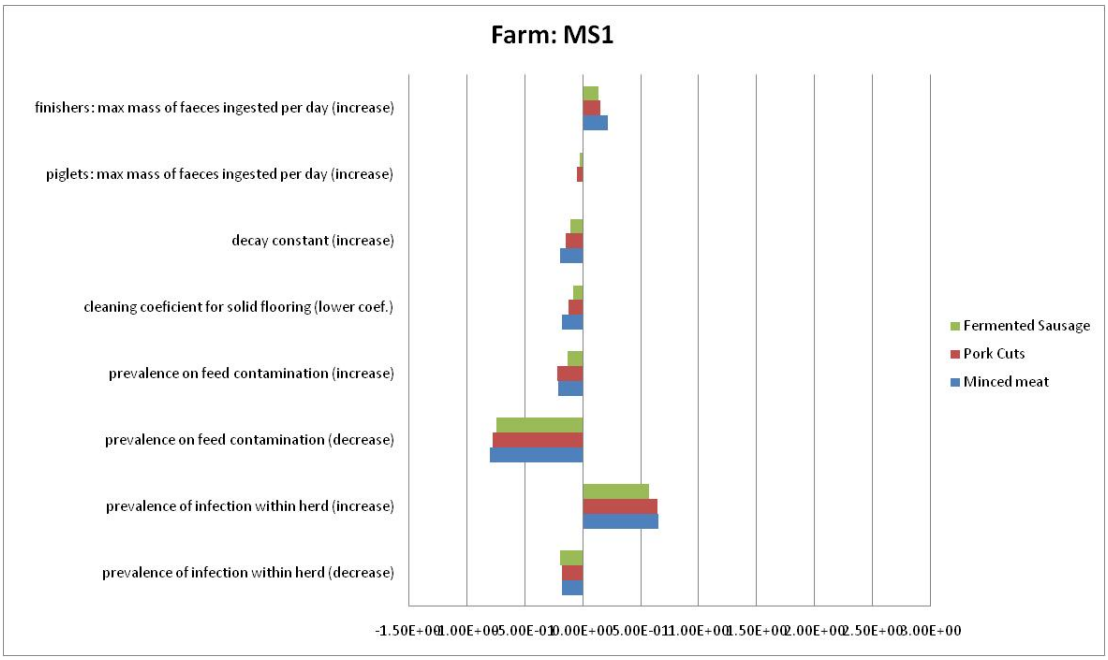

Figure 12.5 Results of the sensitivity analysis considering parameters in the Farm module of the model representing MS1. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the baseline model, i.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk.

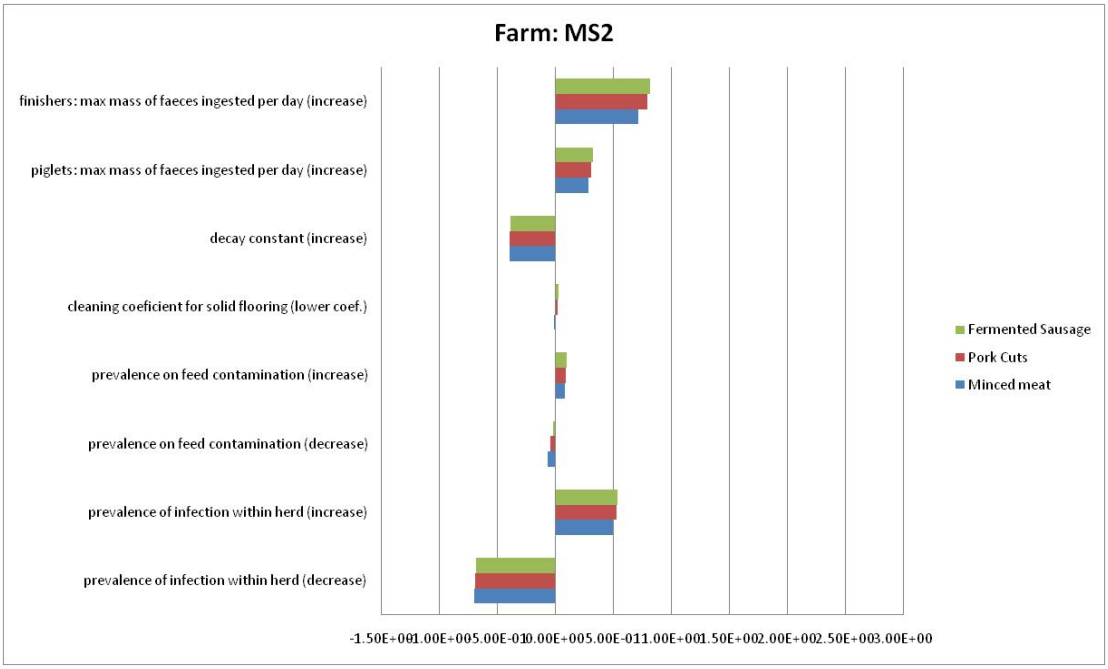

Figure 12.6 Results of the sensitivity analysis considering parameters in the Farm module of the model representing MS2. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the baseline model, i.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk.

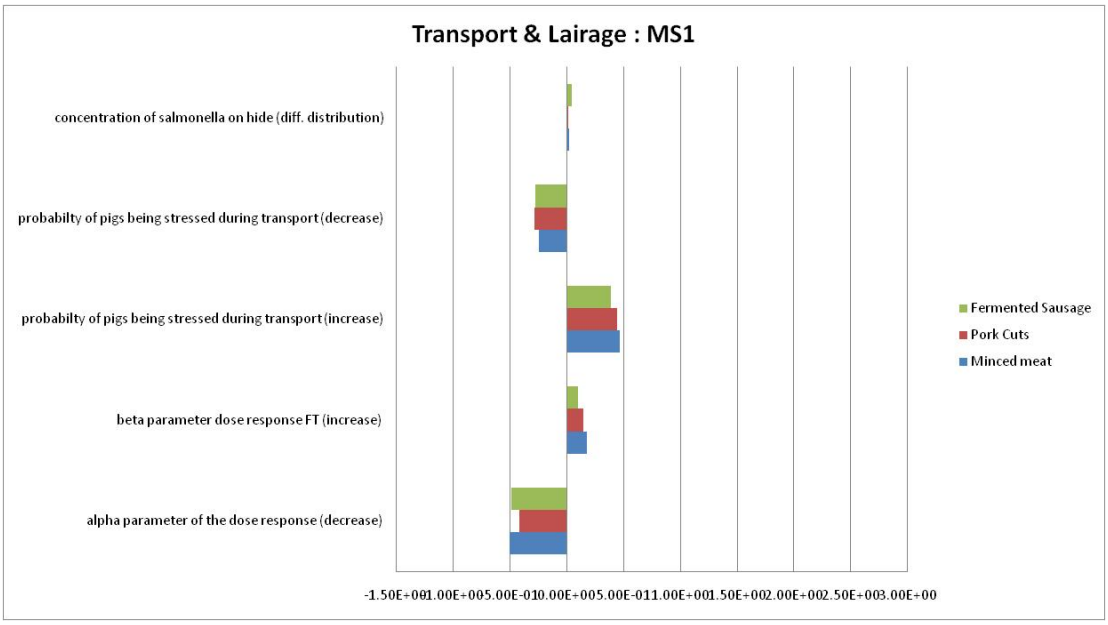

Figure 12.7 Results of the sensitivity analysis considering parameters in the Transport & Lairage module of the model representing MS1. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the baseline model, i.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk.

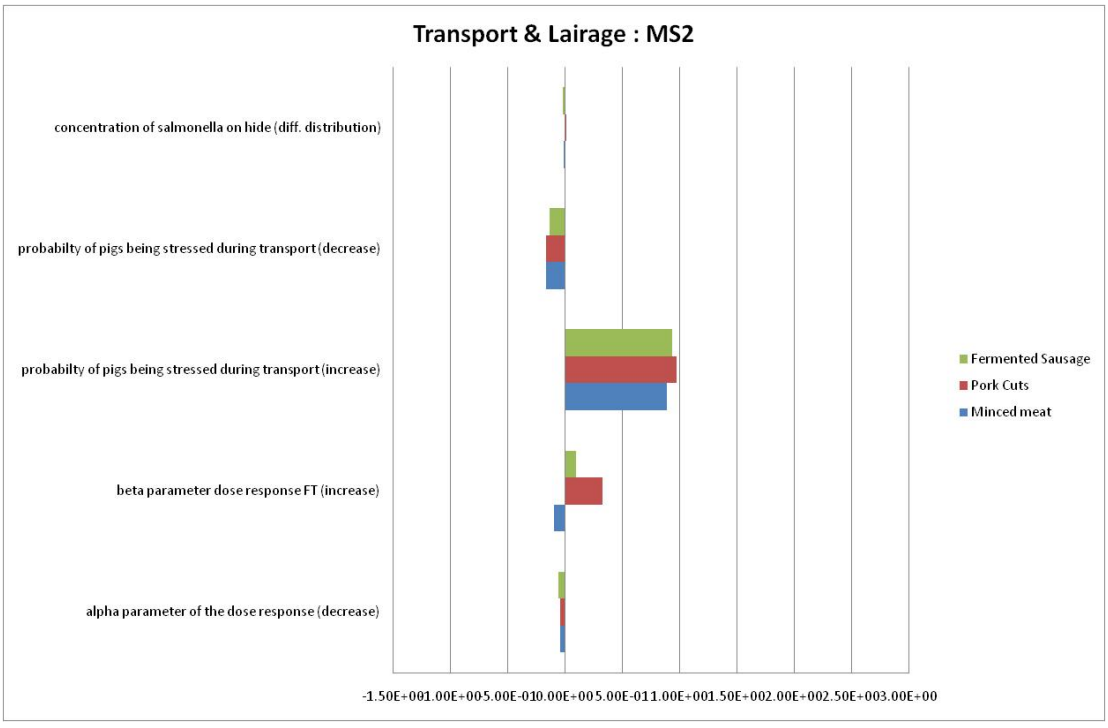

Figure 12.8 Results of the sensitivity analysis considering parameters in the Transport & Lairage module of the model representing MS2. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the baseline model, i.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk.

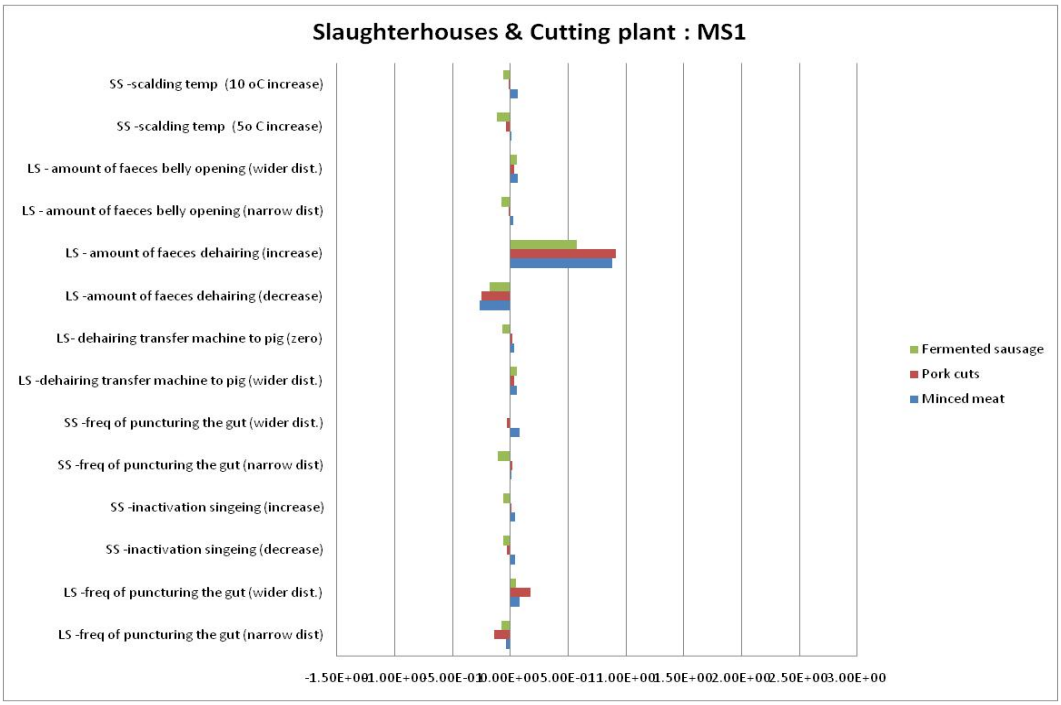

Figure 12.9 Results of the sensitivity analysis considering parameters in the Slaughter & Processing module representing MS1. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the baseline model. I.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk

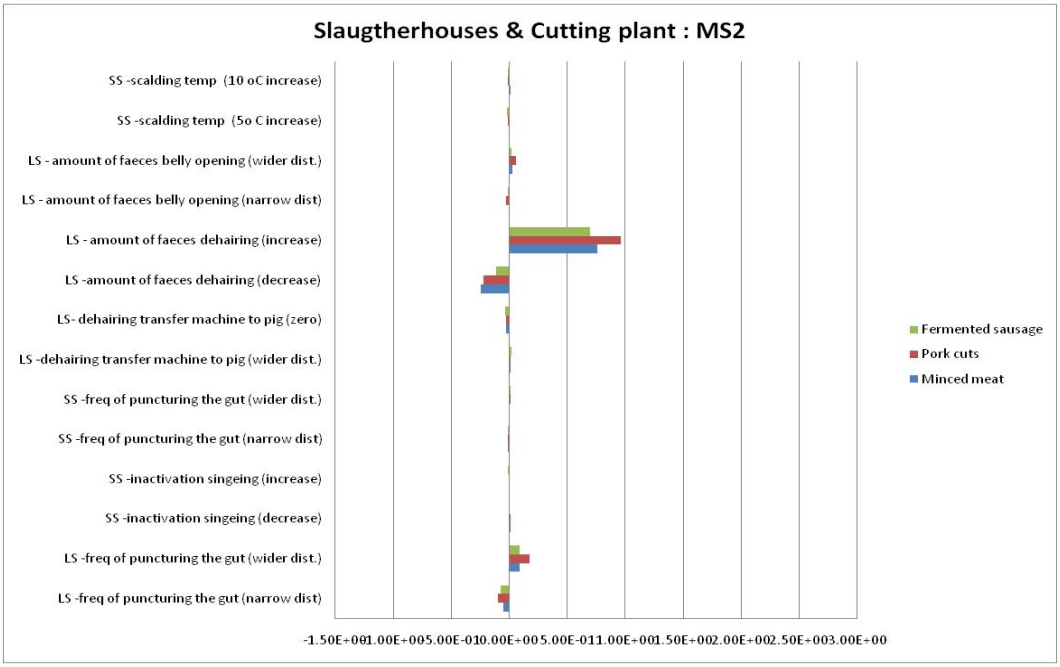

Figure 12.10 Results of the sensitivity analysis considering parameters in the Slaughter & Processing module of the model representing MS2. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the baseline model, i.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk.

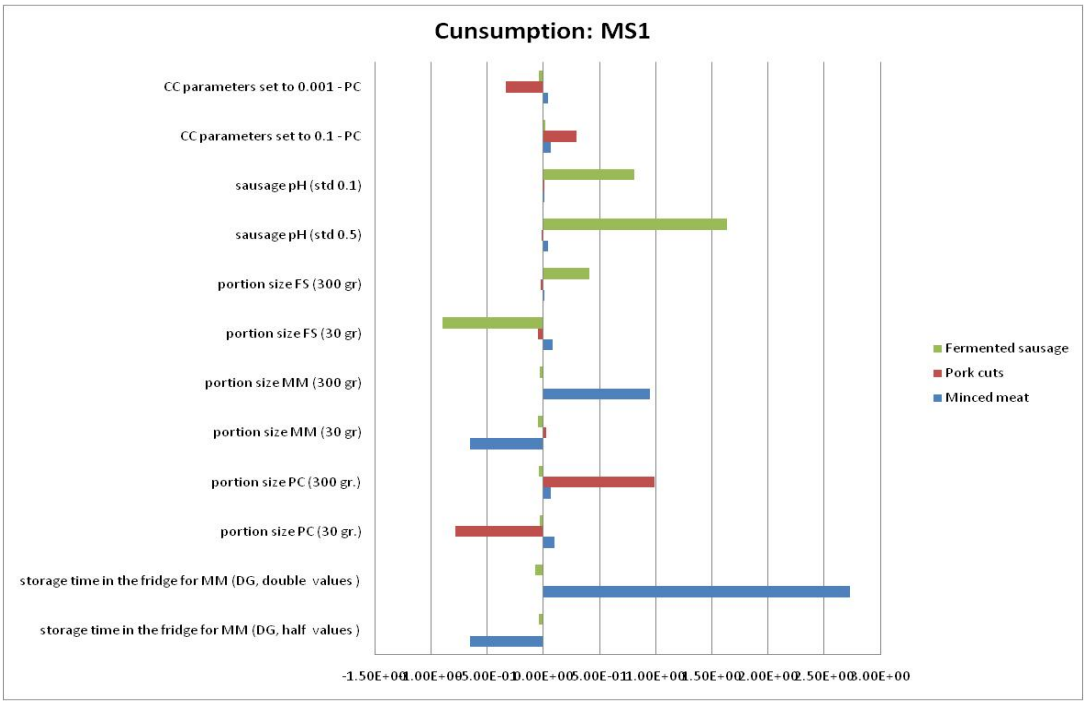

Figure 12.11 Results of the sensitivity analysis considering parameters in the Preparation & Consumption module of the model representing MS1. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the baseline model, i.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk.

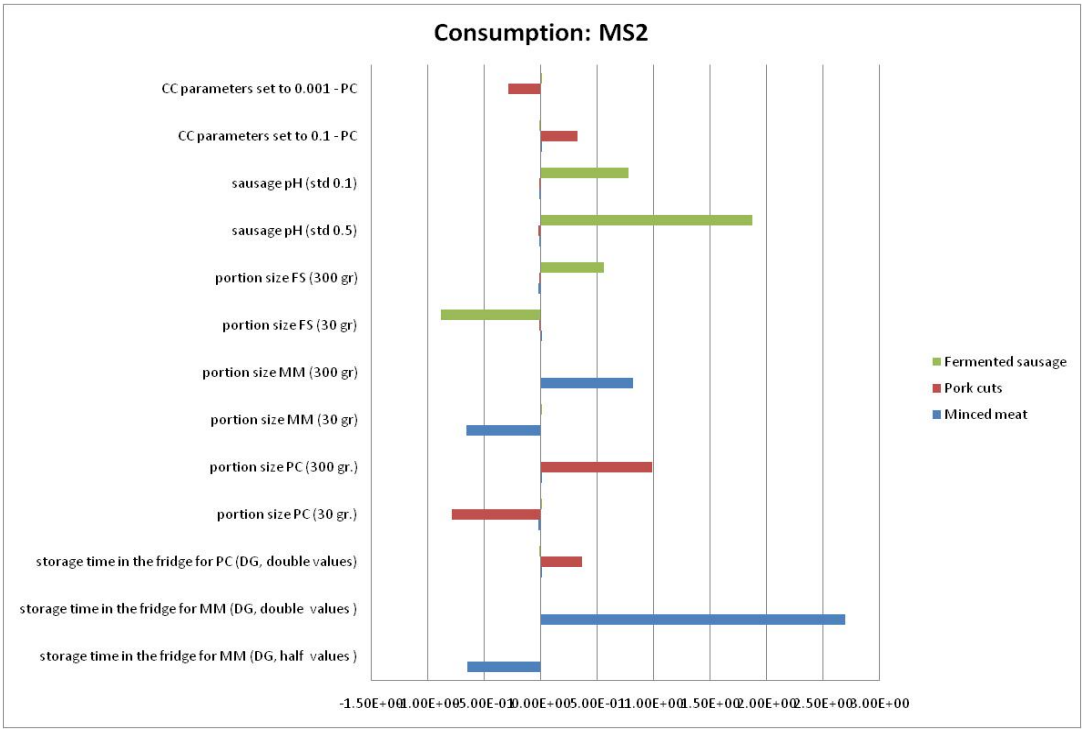

Figure 12.12 Results of the sensitivity analysis considering parameters in the Preparation & Consumption module of the model representing MS2. Bars representing the ratio of risk of illness when the alternative value of the parameter was used in the model compared to the

baseline model, i.e. bars right to zero indicate an increase in risk and bars left to zero indicate a decrease in risk.

Table 12.11: Tabulation of the parameters where the uncertainty of the value has a high impact on the probability of illness.

MS	Farm	Transport & Lairage	Slaughter & Processing	Preparation & Consumption
MS1	- Prevalence of feed contamination; - Prevalence of infection within breeding pig herd	- Probability of pigs being stressed during transport; - α parameter for the dose response;	- Amount of faeces spilled while dehairing;	- Storage time in the fridge (MM); - Portion sizes (all products); - sausage pH
MS2	- Max. mass of faeces ingested per day (finishers); - Prevalence of feed contamination;	- Probability of pigs being stressed during transport;	- Amount of faeces spilled while dehairing;	- Storage time in the fridge (MM); - Portion sizes (all products); - sausage pH

For the Farm module, the influences of changing values of different parameters differ between MS1 and MS2. In MS1 the uncertainty attached to the prevalence on feed contamination has a strong influence on the risk of illness, whereas in MS2 the uncertainty attached to the mass of faeces ingested have a strong influence. In both MS1 and MS2 the uncertainty of prevalence of infection within herd has a strong influence on the result. In the Transport & Lairage module, for both MS1 and MS2, the uncertainty attached to the probability of pigs being stressed during transport has the strongest influence on the probability of illness. Also in the slaughterhouse and cutting plant module, the influence of uncertainty on the probability of illness was similar in MS1 and MS2. In this module, the uncertainties attached to amount of faeces spilled at dehairing and frequency of gut puncturing has the strongest influence on the probability of illness.

12.3.3 Discussion

The results from the uncertainty analysis show that some parameters have a bigger influence in the final output than others. This is a result of a combination of the magnitude of the parameter values and how the actual parameter is manipulated in the model. Strictly speaking the reason why some parameters have a bigger influence then others depends on how the parameter is built into the model and to which value the parameter are changed to.

Discussion of the most important uncertainties

The results of the Farm uncertainty analysis agrees with the work carried out in Section 7 (specifically Figure 7.11), in that feed is relatively important in MS1, but not MS2. Hence, this result also explains the relative importance of the faeces ingested in MS1 and MS2, because pigs in MS1 are probably more likely to pick up infection from feed and the external environment. The probability of feed contamination is a large data gap and this analysis suggests, in concurrence with the recommendation from EFSA, 2008a, that there should be a baseline survey for feed. It was not possible to incorporate the farm management types into the uncertainty analysis (as the way the parameters were estimated did not fit well with the methodology of the uncertainty analysis). However, better data on these parameters is

necessary for contract finishing farms (these did not fall under the EFSA baseline survey for breeding pig herds), as they are crucial in determining the risk of transmission.

For the Transport & Lairage part of the analysis, the probability of pigs being stressed during transport has a relevant impact for both MS1 and MS2. If the probability of stress increases, then more pigs will start excreting higher amounts of *Salmonella* in their faeces (increasing the likelihood of environmental contamination) (Chapter 8). Taking MS1 and fermented sausages as an example, an increase of 2.5 fold on the probability of stress results in an increase of 0.39% on the final probability of illness, and results in an increase of 112 cases of salmonellosis per year, due to consumption of fermented sausages.

From the parameters analysed in the Slaughter & Processing module, for both MS1 and MS2, the uncertainty related to the parameters "amount of faeces spilled at dehairing" and the "frequency of gut puncturing" has a large impact on the probability of illness estimated in the model. This reveals that in this module, the uncertainty in the amount of faeces that contaminated the cutting and dehairing instruments will contribute to the uncertainty in the estimated probability of illness. For instance, for MS1, increasing the amount of faeces by a factor of 5 while dehairing contributed to an increase in the final probability of illness of 75% for minced meat, but since the absolute values for the final $P_{illness}$ are very low, this is translated into a relatively small increase of 35 cases per year.

For the parameters analysed in the Preparation & Consumption module, the effect of uncertainties in the values of the parameters was similar for MS1 and MS2. The parameter where the uncertainties have a high influence on the probability of illness is the storage time for minced meat, followed by the variation in the sausages' pH and portion sizes. The storage time at the consumer's home is an uncertain parameter, and in the model the same general distribution was utilised for MS1 and MS2 (Chapter 10). The high impact of the uncertainties in storage time and portions size on the probability of illness might be the explanation of the high predicted probability of illness in the baseline, in spite of realistic predicted values after farm and slaughtering. By doubling the values in this distribution, an increase of 273% in the final $P_{illness}$ for minced meat was estimated. Doubling the values of the time distribution is not necessarily a real case-scenario, but an educated guess to evaluate the impact of the parameter in the model. On the other hand, specific data on portion sizes should be easier to arrange and verify and this parameters also play an important role on the final output from the model.

12.4 Sensitivity analysis

The results of the sensitivity analysis for each module are shown and discussed in their respective Chapters (Chapters 7-10). As discussed in the methodology section (Chapter 5), it was not realistic to conduct one analysis for the whole model, due to the problems associated with conducting sensitivity analysis across modules where aggregation (e.g. outputs of Farm module used as inputs to Transport & Lairage module) occurs, as mentioned in Frey & Patil (2002). However, the independent analyses highlight the parameters within each module whose variability has a significant effect on the output of that module. It should be remembered that it does not follow that the parameters will have a similar influence of the risk estimate. Table 12.12 below highlights the most sensitive parameters for each module and details the

Table 12.2: Table of most sensitive parameters

Module	Most sensitive parameters	Is this a data gap?	Was there good data for every MS?
Farm	Average amount of *Salmonella* shed by a sow per day	No	No
	Average amount of *Salmonella* shed by piglets	Yes	No
	Average amount of *Salmonella* shed by finishers	No	No
	Average amount of *Salmonella* in feed	Yes	No
Transport	Probability of stress during transport	Yes	No
Lairage	Amount of *Salmonella* in pen before pigs enter	Yes	No
	Dose response relationship	Yes	No
	Number of pigs kept overnight	No	No
	Time in lairage	No	Yes
Slaughterhouse	Length of incision at belly opening	No	Yes
	Time in dehair machine	No	No
	Body Mass at belly opening	No	Yes
	Time in singeing machine	No	No
Cutting Plant	Probability of dangerous cut	Yes	No
	Part of the pig the portion comes from	No	No
P & C: Pork Cuts	Salad consumption	Yes	No
	Knife cleaning	No	Yes
P & C: Minced Meat	Board cleaning	No	Yes
	Salad consumption	Yes	No
	Time in fridge	No	No
	Temperature of fridge	No	No
P & C: Fermented Sausage	None of the variation in the parameter values had a statistically significant effect on the number of *Salmonella* on the fermented sausages at consumption.		

quality of the data used for the parameterisation. This is split up into whether the parameter was a significant data gap (i.e. very little or no data at all) and whether there was good, relevant data for every MS. From the table we can see that the parameters such as average amount of *Salmonella* in feed, stress during transport and salad consumption are of the most concern because as well as being sensitive they are also significant data gaps.

12.5 References

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Appendix 12.1

A12.1.1 Consumption data used for the cluster analysis

For the cluster analysis, it was decided to use data that represented all the major steps in the farm-to-consumption pathway. The data categories chosen were dependent on the data availability. To represent the consumption step, datasets on the amount of pig meat consumed per capita (source *Eurostat* [1]) and on the relative consumption of sausages per capita (source *Eurostat* [1]) were used.

The results from the cluster analysis[35] group the countries in 4 major clusters that are represented in Table 1:

Table 1: Results from the Cluster Analysis. Distribution of the Member States in the four clusters

Cluster	Member States included
1	Austria
2	Belgium, Cyprus, Germany, Denmark, Spain, France, Ireland, Italy, the Netherlands, Portugal, Sweden and UK
3	Bulgaria, Hungary, Lithuania, Poland, Romania, Slovenia
4	Czech Republic, Estonia, Finland, Greece, Luxembourg, Latvia, Malta, Slovakia

For the case studies, one Member State (MS) per cluster was selected. An important consideration in this selection was the need for more detailed data for the chosen MS in order to proceed with the development of the model.

Cluster 1 is represented by MS1, cluster 2 is represented by MS2, cluster 3 is represented by MS3, and cluster 4 is represented by MS4.

A12.1.2 Data requests for the model

To develop the model, the modellers identified the data requirements for all the steps in the farm-to-consumption pathway. Ideally, this data should be specific from each of the representative MS, but in case of unavailability, data from other MS in the same cluster will be used.

To illustrate the diversity in the consumption patterns among the different member states, the data needs for the consumption step include data on portion sizes and frequency of consumption for pork cuts, minced meat and dry-cured sausages. All the parameters required for the consumption step are listed:

- Portion size of pork cuts;
- Portion size of minced meat;

[35] The cluster analysis is described in more detail in the Cluster Analysis – Final Report previously uploaded;

- Portion size of dry cured sausage
- Frequency of consuming minced meat;
- Frequency of consuming pork cuts;
- Frequency of consuming dry-cured sausage;
- Frequency of preparing a non-heated side dish (e.g. salad) accompanying a pork main meal;

A12.1.3 Data search

The data search strategy followed two main approaches:

- Direct request of the data to representatives from each member-state from key institutes in the field (using FOOD-DTU network of contacts);

- Literature/database search;

- EFSA Call for Data

Direct Request

On the 13th of January 2009, an email requesting collaboration was sent to several contacts from the cluster-representative member states and also to contacts from Hungary and Ireland (that could help to fill up eventual data gaps from MS3 and MS2, respectively).

This email provided information about the QMRA project including the purpose of this project, collaborative partners, etc. The cluster analysis procedure and results were also explained.

Attached to the email, a spreadsheet was sent. In this spreadsheet, the data parameters needed were explained and could be easily entered.

Table 2 represents the list of institutes contacted per MS. The email was sent directly to representatives of these institutes that also belonged to DTU-FOOD network of contacts.

Table 2: List of institutes contacted per member state;

Member State	Institute contacted
Austria	- Austrian Agency for Health and Food Safety
Czech Republic	- State Veterinary Administration of the Czech Republic
Hungary	- Hungarian Food Safety Office - Veterinary Medical Research Institute, Hungarian Academy of Sciences - National Disease Control Center
Ireland	- The Food Safety Authority of Ireland - The Irish Agriculture and Food Development Authority - University College Dublin - Department of Agriculture, Fisheries and Food
Poland	- National Institute of Public Health - National Veterinary Research Institute
United Kingdom	- Food Standards Agency; - Health Protection Agency; - Department for Environment, Food and Rural Affairs;

Literature/Database search

The search process started by investigating initiatives already existing on consumption data from experts at FAO [2] or WHO [3].

The following step was to use search engines using search strings including words like pig / pork / meat / consumption/ frequency of consumption / sausages / minced meat, etc

The search engines used were:
- Google scholar [4];
- Digital Article Database Service - DADS [5];
- Pubmed [6];

Through this search it was possible to find some studies on nutrition, household budget surveys or national diet and nutrition surveys to several member states.

In addition, there were a number of projects and initiatives dedicated to consumption and these are listed below

- ILSI Europe study– The micronutrient Landscape of Europe; [7]
- HELENA study – Healthy Lifestyle in Europe by Nutrition in Adolescence [8];
- EPIC studies – European Prospective Investigation into Cancer and Nutrition [9];
- HECTOR project – Healthy Eating Out [10];
- EFCOSUM project– European Food Consumption Survey Method [11];
- WHO MONICA project – Multinational Monitoring of trends and determinants in cardiovascular disease [12];
- EFSA Concise Food Consumption Database [13];
- INFID project– International Food Intake Directory [14];
- DAFNE initiative –Data Food Networking [15]

From these projects, the DAFNE is the one that contains information most relevant to our data needs. The DAFNE initiative shows the results from many European household budget surveys (HSB) in a joint effort of European countries to compare the food habits of their populations and monitor overtime, trends in food availability. The HSB are periodically conducted by the National Statistics Offices of most European countries in country-representative samples of households [15].

The methodology followed is uniform enough to allow comparisons between countries. HSB are not primarily designed to collect nutritional information, but by recording data on the values and quantities of the household food purchases it is possible to depict the dietary patterns prevailing in the representative population samples [15].

The DAFNE databank currently comprises data on 24 European countries (including. MS1, MS2 and MS3) and using a software available at the website, it is possible to extract data on different food categories (eg: pork meat, poultry, seafood) [15].

EFSA Call for data

In the beginning of the QMRA project, a call for data was launched through EFSA. In this call, the crucial data gaps along the farm-to-consumption pathway were identified. The Member States were encouraged to participate via data submission (industry or academic data) or expert opinion [23].

Regarding the consumption data, the countries were requested to fill in forms with data on pork meat products bought and consumed in the country and also data on the most consumed pork-meat dishes.

The data requested for the pork meat products is listed below:
- Description of product type (e.g. pork cuts, ham, roast, minced meat, offal);
- Percentage of persons consuming each product type;
- Amount of product type consumed (gram) per serving;
- Frequency of consumption of the product;

Concerning the data on pork meat dishes:
- Description of the dishes (e.g. meat balls, stew, pork cuts);
- What is the dish usually served with (e.g. potatoes, green salad, raw or heated-treated vegetables, etc);
- How is the pork typically prepared for the dish (boiled, fried, roasted, grilled, smoked, salted, marinated, no preparation);
- How is the pork meat usually consumed (well done, medium, rare or raw);
- How often is the dish prepared and consumed (one a week, twice a month);
- Amount of pork meat consumed (gram) per meal of the dish;
- Percentage of persons ever consuming each dish;

These data requests have the purpose to illustrate the diversity of consumption patterns on the different Member States and to fill in for the data needs in the final model.

A12.1.4 Results

Besides the data available through the DAFNE initiative, more information was found through the literature search. There was also relevant consumption data from the EFSA call [23]. Table 3 shows all the data collected per MS and its different sources.

Table 3: Type of data collected in the literature search, per member state and its sources;

Member State	Type of Data Collected	Source
Austria	- Frequency of consumption-minced meat and sausages - Frequency of consumption-minced meat and sausages	Koenig et al. 1999 [18] DAFNE [15]
Belgium	- Frequency of consumption-pork cuts, minced meat, sausages	DAFNE [15]
Cyprus	- Frequency of consumption-sausages and pork burgers	DAFNE [15]
Czech Republic	- Frequency of consumption - minced meat and sausages - Portion size: minced meat and sausages - Portion size: pork chop, minced meat and sausages;	Anon, 2004 [16] Anon, 2008 [23]
Finland	- Frequency of consumption - minced meat and sausages - Frequency of consumption - pork cuts, minced meat, sausages	Anon, 2007 [17] DAFNE [15]
Germany	- Frequency of consumption-sausages - Frequency of consumption-minced meat, sausages and cold cuts - Percentage of meals where RTE are part of the meal;	Mensink *et al*., 2004 [20] DAFNE [15] Brynestad *et al.* 2008 [24]
Greece	- Frequency of consumption – sausages	Linseisen *et al*. 2002 [19]
Ireland	- Frequency of consumption-sausages - Portion size: pork cuts	DAFNE [15] Anon, 2008 [23]
Latvia	- Frequency of consumption-smoked sausages	DAFNE [15]
Luxembourg	- Frequency of consumption-pork minced meat, sausages - Frequency of consumption pork cuts, minced meat	DAFNE [15] Anon, 2008 [23]

Malta	- Frequency of consumption-pork cutlets, pork minced meat, sausages	DAFNE [15]
Netherlands	- Frequency of consumption minced meat	Anon. 2009 [22]
Poland	- Frequency of consumption-sausages	DAFNE [15]
Portugal	- Frequency of consumption-pork cuts, sausages - Frequency of consumption - pork	DAFNE [15] Anon, 2008 [23
Slovenia	- Frequency of consumption: pork minced meat, sausages	DAFNE [15]
Spain	- Frequency of consumption-sausages	DAFNE [15]
Sweden	- Frequency of consumption-meat dishes with minced meat, pork cuts, sausages - Portion size: minced pork meat, pork sausage	DAFNE [15] Anon, 2009 [21]
MS2	- Frequency of consumption-sausages - Frequency of consumption-pork cuts and pork sausages	Linseisen *et al*. 2002 [19] DAFNE [15]

It was not possible to get any data via the direct request from the contacted institutes.

Values used as input for the model

Table 4 and Table 5 compile the data, from the previous mentioned sources for each MS that is going to be used in the model[36]. The following guidelines were used:

- Data refers to general population (meaning consumers and non consumers);
- In case of the data being split in *men* and *women*, an average was calculated;
- When age information was available, the adult group (approximately 18-40 years old) was chosen;
- When there was no available data from the cluster-representative MS, data from another MS in the same cluster was used. When the latter was also non-available, data from a different cluster was used;

Table 4 describes the values for the frequency of consumption of pork cuts, minced meat and sausages used as input in the model for each cluster. This data comes from the results of the literature search and it is the best available data for the product characteristics: pork cuts, pork minced meat and fermented sausages.

[36] For a more detailed information on the model and how the data is used, please read Arno Swart´s "Modelling of the Slaughterhouse Environment and the Processing of Carcases", also uploaded.

Table 4: Frequency of consumption of pork cuts, minced meat, sausages. The MS are represented between brackets.

Cluster	Pork cuts	Minced meat	Sausages
1	(MS1) – 33 g/day [15]	(MS1) – 2.55 g/day [15]	(MS1) – 10 g/day [15]
2	(MS2) – 3.53 g/day [15]	(BE) – 2.83 g/day[15]	(MS2) – 0.69 g/day [15]
3	(MS3) – 43 g/day[15]	(SI) – 4.34 g/day [15]	(MS3) – 2.25 g/day[15]
4	(LU) – 28.6 g/day[23]	(LU) – 4.48 g/day [15]	(FL) – 8.6 g/day [15]

Table 5 shows the portion (or serving) sizes for pork cuts, minced meat and sausage for each cluster and respective sources.

Table 5: Portion size of pork cuts, minced meat, sausages. The MS are represented between brackets.

Cluster	Pork cuts (g/portion)	Minced meat (g/portion)	Sausages (g/portion)
1	(Ireland) – 146 [23]	(Sweden) – 125 [21]	(Sweden) – 150 [21]
2	(Ireland) – 146 [23]	(Sweden) – 125 [21]	(Sweden) – 150 [21]
3	(Czech Rep) – 200 [16]	(Czech Rep) – 76.7 [16] **(*)**	(Czech Rep) – 110 [16]
4	(Czech Rep) – 200 [16]	(Czech Rep) – 76.7 [16] **(*)**	(Czech Rep) – 110 [16]

(*) – Minced meat in general and not only pork meat;

Bibliography for Appendix

[1] Anonymous. (2006). "Eurostat Metadata in SDDS format: Base Page." from http://epp.eurostat.ec.europa.eu/cache/ITY_SDDS/EN/ef_base.htm.

[2] www.fao.org

[3] www.who.int

[4] http://scholar.google.com

[5] www.dtic.dtu.dk

[6] http://www.ncbi.nlm.nih.gov/pubmed/

[7] http://europe.ilsi.org/

[8] http://www.helenastudy.com/

[9] http://info.cancerresearchuk.org/healthyliving/dietandhealthyeating/theepicstudy/

[10] www.nut.uoa.gr/hector/Home.asp

[11] http://www.public-health.tu-dresden.de/dotnetnuke3/eu/Projects/PastProjects/EFCOSUM/tabid/338/Default.aspx

[12] www.ktl.fi/monica/index.html

[13] www.efsa.europa.eu

[14] INFID

[15] www.nut.uoa.gr/English/index.asp?page=202

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[24] Brynestad *et al*. "Quantitative Microbiological Risk Assessment of campylobacteriosis cases in the German population due to consumption of chicken prepared in homes", Int. J. Risk Assessment and Management, vol 8, No 3, 2008;

Appendix 12.2 Convergence of QMRA model

Figure A12.1 shows convergence of each MS model by around 4,000 iterations. The baseline model is run for 10,000 iterations to ensure complete convergence.

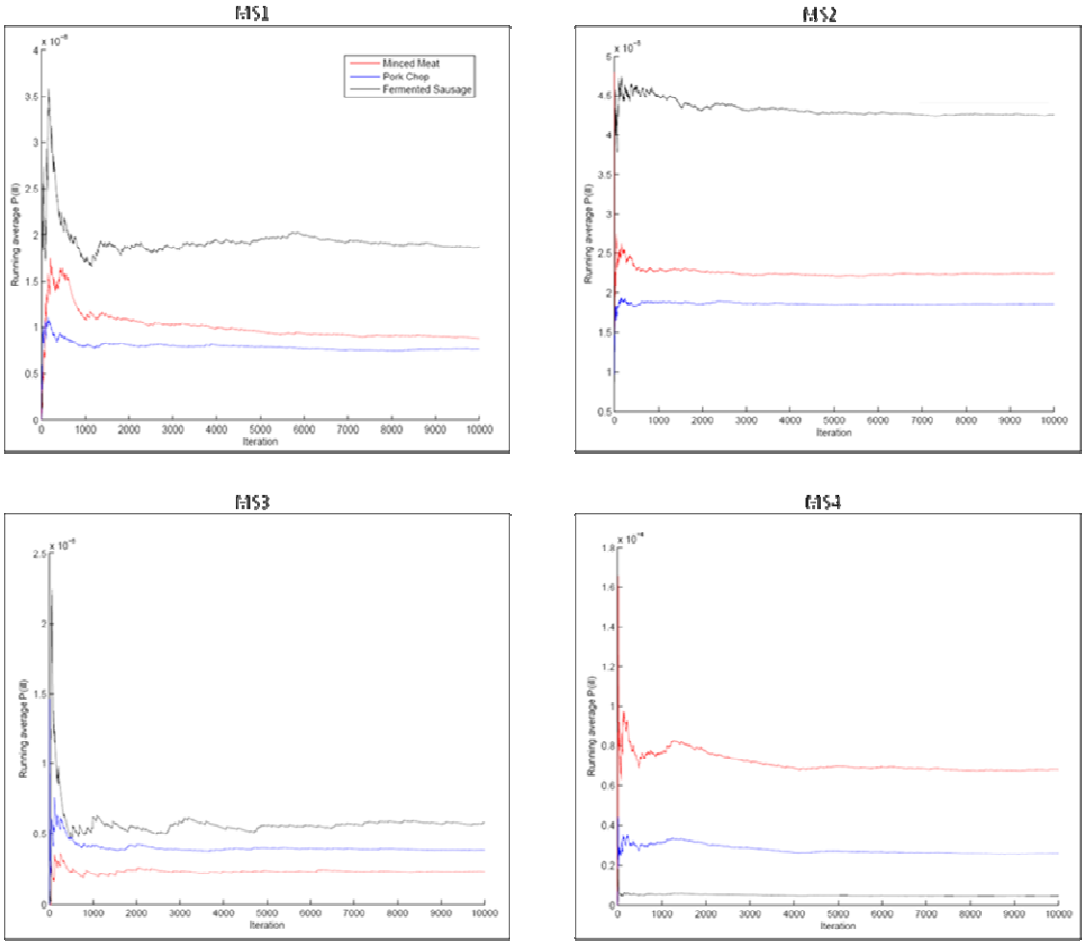

Figure A12.1: Convergence of QMRA models.

13 Intervention Analysis

13.1 Introduction

Within the EFSA ToRs we were contracted to investigate "..the expected reduction of *Salmonella* cases in humans (or pig meat) by the most important control measures at the farm level … [and] during transport at lairage or during the slaughter process". In addition, we were also contracted to assess the:

1. Expected reduction of *Salmonella* cases in humans (or pig meat at retail) by a reduction of *Salmonella* prevalence in slaughter pigs

2. Expected reduction of *Salmonella* cases in humans (or pig meat at retail) by a reduction of *Salmonella* prevalence in piglets from breeder farms

3. Expected reduction of *Salmonella* cases in humans (or pig meat at retail) by the most important potential treatments or control measures at farm level

4. Expected reduction of *Salmonella* cases in humans (or pig meat at retail) by the most important control measures during transport, at lairage or during the slaughter process.

These objectives can be split into two types of intervention: hypothetical and specific.

Regardless of the specific interventions used, it is instructive to assess the effect of points 1 and 2 above. These two objectives hence form the 2 farm hypothetical interventions investigated. Of further interest is a hypothetical reduction of carcass contamination at the slaughterhouse. While not specifically part of the EFSA ToRs, hypothetical reductions in carcass contamination of *x* logs pre-chill are assessed.

In order to assist model development, more information was needed on what specific interventions were desired for the EFSA Working Group to consider as part of their Scientific Opinion. Discussion on the priority of particular interventions, and the feasibility of modelling them, with the Working Group and the EU led to the following specific interventions being included within the scope of the QMRA:

Farm:

1. Reduction of feed contamination
2. Supplier status
3. Improved hygiene/biosecurity
   A. Within farm: increased cleaning, longer downtime
   B. Outside farm: Prevention of external contamination
4. Increased resistance of pigs to *Salmonella* infection by using e.g. wet feed, vaccination or organic acids

Transport:

1. Increased cleaning
2. Logistic transport (i.e. one batch, one vehicle)

Slaughter:

1. Reducing/preventing faecal leakage
2. Logistic slaughter (process high-risk pigs at end of day)
3. Reducing the effect of house flora (i.e. persistent contamination) by extra cleaning efforts

The QMRA model has been developed with these interventions in mind – much of the complexity found within the QMRA model (outlined throughout Chapters 4 – 11) has been included because of these interventions.

Utilising the ability to look at interventions at various point in the farm-to-consumption chain, QMRA focuses on the *relative* effects on the number of human cases. We therefore present, for each intervention, the % reduction in the baseline number of cases for each MS attributable to each product type: pork cuts, minced meat and fermented sausage. This does mean, for example, that a significant 50% reduction in fermented sausage cases might actually represent a smaller absolute number of human cases reduced than a much smaller 5% reduction in the number of pork cuts cases. However, using the relative burden makes it easier to assess the effect of different interventions across MSs.

The effects of many of the specific interventions included in the model have been reviewed comprehensively elsewhere in the literature. We refer to and draw our conclusions from these reviews wherever possible. However, these reviews point towards a very broad trend when investigating specific interventions: that the effects of particular interventions vary markedly between farms and between studies (presumably because the pig environment requiring intervention is in itself complex and variable). It is therefore not possible for any of the interventions to estimate the effect of interventions on the number of human cases to high accuracy. Further research is necessary before we can confidently assess the effect of specific interventions (e.g. vaccination, organic acids) at a MS level.

Nonetheless, a lot of time and research has been put into developing a QMRA that can investigate specific interventions. Therefore, in order to produce usable results for the Working Group, we have investigated how the mechanisms of interventions might reduce human cases, considering biologically plausible ranges of parameter estimates. These intervention results provide insight into the magnitude of reductions that might be achieved by applying control at specified points in the food chain, given particular characteristics of the system (MS-specific or otherwise).

We now describe how the effect of each of these interventions was estimated.

13.2 Methodology

13.2.1 Farm

Hypothetical reductions

We were mandated to look at the effect of reducing slaughter and breeder prevalence (assessed using either bacteriological or serological tests) on the number of human cases.

Breeding pig herd prevalence

From Figures 7.11 and 7.16 (i.e. the Farm model analysis) it is clear that, at least within the model, breeding pig herd prevalence is the dominant factor in determining national slaughter pig prevalence (i.e. low breeding pig herd prevalence ~ low slaughter pig prevalence, and vice versa). There are two major differences between the MS farm models: parameterisation of the breeding pig herd prevalence parameter, p_{herd}, and the allocation of farm types. Further analysis (not shown in this report) has been conducted, where the breeding pig herd prevalence of MS2 has been input to the MS1 MS farm model. The result for slaughter pig prevalence for this MS2/MS1 model was very similar to the original result for the MS2 farm model. This shows that the difference between MS slaughter pig prevalence is largely described (but not completely) by breeding pig herd prevalence. Hence, because of the onerous runtime of the farm model, we have chosen to conduct this analysis of breeding pig herd prevalence for MS4 only. MS4 was chosen as a "middling" MS of the four MSs chosen in terms of farm types and breeding pig herd prevalence. *The trend shown by this analysis of MS4 will apply equally to each of the four case study MSs.*

We therefore adjust the value of p_{herd} to {0.05, 0.15, 0.25, 0.35, 0.45}, and run the model separately for each value. The range of values was chosen to reflect the range of prevalences recorded in the four case study MSs from the slaughter pig baseline survey.

National slaughter pig prevalence

We chose to investigate a 10, 20, 30, 50, 70, 90 and 99% reduction in slaughter pig prevalence, using lymph-node positivity as the sampling test.

For prevalence at slaughter the method of achieving a reduction is important in its effect further down the food chain in reducing human cases of *Salmonella*. For example, reducing the burden of *Salmonella* infection across all batches (i.e. reducing within-batch prevalence) may well produce a very different intervention effect than reducing the proportion of infected batches. We have chosen to represent the reduction of within-batch prevalence, as this would appear a more likely occurrence given the current crop of interventions being suggested at the farm level (e.g. acidified feed, vaccination).

Within-batch slaughter prevalence is reduced in the following way:

1. The farm output matrices (see Section 7.5) are sampled within the transport module.
2. We isolate each batch being sampled from the farm matrices and binomially determine which infected pigs would, because of some intervention, be negative. Hence we revert *s* pigs from positive to negative status, sampling from the following distribution, $s = B(I(j,l,t), p)$ where $I(j,l,t)$ is the number of lymph-node positive pigs within the slaughter batch and *p* the fraction with which to reduce infection by, i.e. the set, respectively. Zero indicates a negative pig, one a positive pig.
3. Having reverted specific pigs to susceptible status, we must also revert shedding status to 0 (negative) as well.

Reduction of feed contamination

There are no national data to suggest how prevalence of feedlot contamination (i.e. the percentage of feed batches that are contaminated with *Salmonella*) might be reduced. We have chosen to reflect hypothetical changes in the prevalence of feedlot contamination, rather than hypothetical changes in the numbers of *Salmonella* present in contaminated feed.

The parameter associated with prevalence of feed contamination is p_{feed}. This is changed to absolute values of {0.01,0.03,0.07,0.1,0.15,0.2} to assess the change in farm prevalence (and human cases) over this range of feed contamination for each case study MS. This range of values is chosen to take into account data that suggests prevalence commonly varies between 1-10% (EFSA 2008b) and expert opinion that suggests prevalence is probably under-estimated using current sampling schemes.

We have already investigated the elimination of feed as a source of infection within Section 7.5.3.

Supplier status

The complexity of the farm model, and a paucity of information on the subject, eventually precluded the explicit inclusion of the supplier status of weaners to a grower-finisher farm.

Investigation of this type of intervention is complex, due to the type of surveillance scheme a MS would use (e.g. serology, bacteriology). For example, the Danish surveillance system classifies all farms to one of three levels; however, while this information on breeding/weaning herds is available, farmers may not utilise this information in deciding where to source their new stock. In addition, MSs may choose their own thresholds for discrimination between low, medium and high prevalence herds.

The Farm model developed and described in this report has been designed, so far as possible, to investigate multiple interventions at the farm level. However, its strength lies more in the consideration of interventions that prevent/reduce within-batch transmission of infection (e.g. cleaning, vaccination). The inclusion of different management structures is a novel development in transmission modelling, which is important when considering the variability between MSs; the management systems considered are, of course, simplified within the model, and at present do not really allow for us to investigate supplier status with confidence.

Improved hygiene/biosecurity

Within the model there are two ways to improve biosecurity or hygiene. First, include downtime between batches of weaning, growing and finishing pigs (in the same way as for farrowing groups). Second, the efficiency of cleaning (between batches) in removing *Salmonella* can be increased.

There are qualitative data that do suggest downtime and cleaning can have an effect on *Salmonella* levels (VLA 2009). However, there are little data to quantitatively estimate the differences in *Salmonella* infection between batches of slaughter pigs produced from farms that have downtime compared to farms that don't, or farms that have good cleaning practices and farms that don't.

Therefore, we again go back to hypothetical changes in the mechanism of that intervention. If we can take a hypothetical but plausible range for a perceived intervention (e.g. if we can assume extra cleaning will have a 1 or 2 log greater reduction in the levels of *Salmonella* present in the pen), then we can suggest a rough estimate for its effectiveness in reducing slaughter pig prevalence and/or number of human cases.

It is assumed that the main mechanism by which downtime achieves a reduction in *Salmonella* prevalence is by the drying out of the pen, which reduces the number of *Salmonella* in the pen environment that are available for carry-over of infection. Assuming this mechanism then we don't explicitly model the emptying of a pen for 1, 2, 7 days etc, but simply calculate the decay of *Salmonella* in the environment over this time period. Given that the flow of pigs is rather regimented within the model, then modelling the inactivation of the *Salmonella*, rather than the emptiness of the pen, is an efficient way to capture this intervention. From published studies we have assumed that *Salmonella* will be inactivated at a rate of 0.4 logs per day, and assume, conservatively, that this rate will also be applicable during downtime. We run the model assuming a 1, 4 and 7 day downtime, or a 0.4, 1.6 and 2.8 log reduction in *Salmonella* numbers per pen between each batch of pigs across weaning, growing and finishing houses.

We currently assume that cleaning of pens after each batch is ineffective in removing all *Salmonella* (most commonly between 20-90% of *Salmonella* being removed during cleaning, assuming that cleaning separates a proportion of the *Salmonella* from the faecal material). Therefore if a pen is highly contaminated a potentially significant level of *Salmonella* may be left behind, where a new batch of pigs entering the pen can be exposed to this residual contamination. There are no sufficiently comprehensive studies to directly estimate the effect of improved cleaning in removing *Salmonella*. However, a study by Small *et al.* 2007 suggests that between 1-2 logs improvement in reducing enterobactiacae numbers is possible through more robust cleaning methods such as pressure washing with sanitiser washing. We therefore assume that an improvement of cleaning will decrease the load within the pen by an additional 1 or 2 logs.

These changes (average 1-log improvement and 2 log improvement) are represented in the model by replacing the baseline estimate for p_{clean} by $\Re(Beta(3,50))$ and $\Re(Beta(3,500))$ respectively (where the modified beta parameterisation was achieved by simply adjusting the distributions until the averages were 1 and 2 orders of magnitude lower than the baseline average). The average reductions achieved by the baseline, 1 log and 2 log reduction models are 0.4, 0.04 and 0.004.

Increased resistance (wet feed, vaccination, organic acids)

As mentioned above, there are no sufficiently extensive intervention studies that provided enough information to confidently quantitatively model these interventions.

A systematic review of vaccination was carried out by Denagamage *et al.* 2007. Their conclusions were that there were few studies that were relevant for assessing the effect of vaccination in reducing *Salmonella* levels in market age pigs. Five clinical trials were reported, none of which achieved a high score for methodology, and (for us) more importantly, the trials were not undertaken to the point of depopulation, therefore reducing the relevance to the QMRA. From these five studies there does appear to be a positive effect of vaccination in reducing *Salmonella* prevalence in pigs; however only one reports the actual prevalence in vaccinated and control groups (Maes *et al.* 2001). Of critical importance for assessing the effects of *Salmonella* vaccination in pigs is the concentration of *Salmonella* in faeces in infected but vaccinated pigs; no study so far has reported this effect.

Introduction of organic acids and wet feed can be considered as manipulating the characteristics of the feed given to pigs in order to alter the gut ecology/microbiology such that *Salmonella* do not survive and multiply as easily within the digestive system (hence

reducing the potential for infection). A recent systematic review of the published literature (O'Connor *et al.* 2008) assessed the evidence for both pH and moisture content of feed as methods that might control *Salmonella* (moisture content obviously relating to wet feed, and pH to both factors). They found little evidence for either pH or moisture content affecting *Salmonella* levels in pigs, however a low-confidence assessment was made that wet feed and acidified feed was effective in reducing *Salmonella* prevalence relative to dry and non-acidified feed respectively. Recent studies on organic acids, not included in the systematic review, are also inconclusive on the effect of organic acids in reducing *Salmonella* in pigs at slaughter (VLA, 2009). Similar conclusions can also be made on non-pelleted feed (Lo Fo Wong & Hald 2000), where evidence does exist for a positive effect, but little data are available to conclusively prove and enumerate such an effect. Again, an important point missing from these studies is the effect on enumeration, rather than prevalence.

The above information for feed does not allow us to assign quantitative values to our intervention analysis. However, as above, we can model the mechanism of intervention over plausible biological ranges. From the evidence available it appears that changing the pH of the feed/gut is fundamental in determining the dose response of the pig: reduce the pH and probability of infection given a particular dose is also reduced, leading to lower numbers of pigs that are *Salmonella*-positive. The underlying biological mechanisms for pH reducing *Salmonella* can be considered well-proven, at least experimentally: therefore, we assume *Salmonella* is inactivated at low pH levels (Hwang *et al.* 2009; Tiganitas *et al.* 2009). The variation in the results of the studies above, in our opinion, is more likely to represent variation in the methodology and the application of the intervention measure than the physical response to pH/moisture content of the *Salmonella* in the pig gut (although of course there will be a varying response from different *Salmonella* spp.). If this assumption is correct, then we can model the 100% correct application of these intervention measures to see their effect (whilst remembering that 100% correct application of acid concentrations etc is extremely unlikely in practical farming conditions). This 100% assumption allows the best-case scenario to be assessed – the true effect will lie between the baseline and the best-case scenarios.

We assess "resistance" interventions – vaccination, feed type or organic acids, via modification of the dose response model for slaughter pigs[37]. The dose response model parameters were adjusted until there was roughly a 1 or 2 log increase in dose needed to cause the same probability of infection (essentially shifting the dose-response curve in Figure 7.9 along the axis by 1 or 2 logs). This can be approximated by re-parameterising β_{DR} to 200,235 and 2,000,235 respectively (using this method the difference in dose needed will vary across the dose range, but the modified dose-response curves, as can be seen from Figure 13.1, are relatively parallel to the baseline). See Figure 13.1 for the effect on the dose-response model.

[37] The mechanisms for increased resistance are obviously different between vaccination and feed: vaccination stimulating the immune response of the pig, feed/organic acids changing the pH/organic acid make-up of the pig's digestive system, making a less favourable environment for *Salmonella* survival/colonisation. However, given we do not know the quantitative effect of each mechanism, we assume the qualitative effect is the same – it takes more *Salmonella*s to cause the same probability of pig infection.

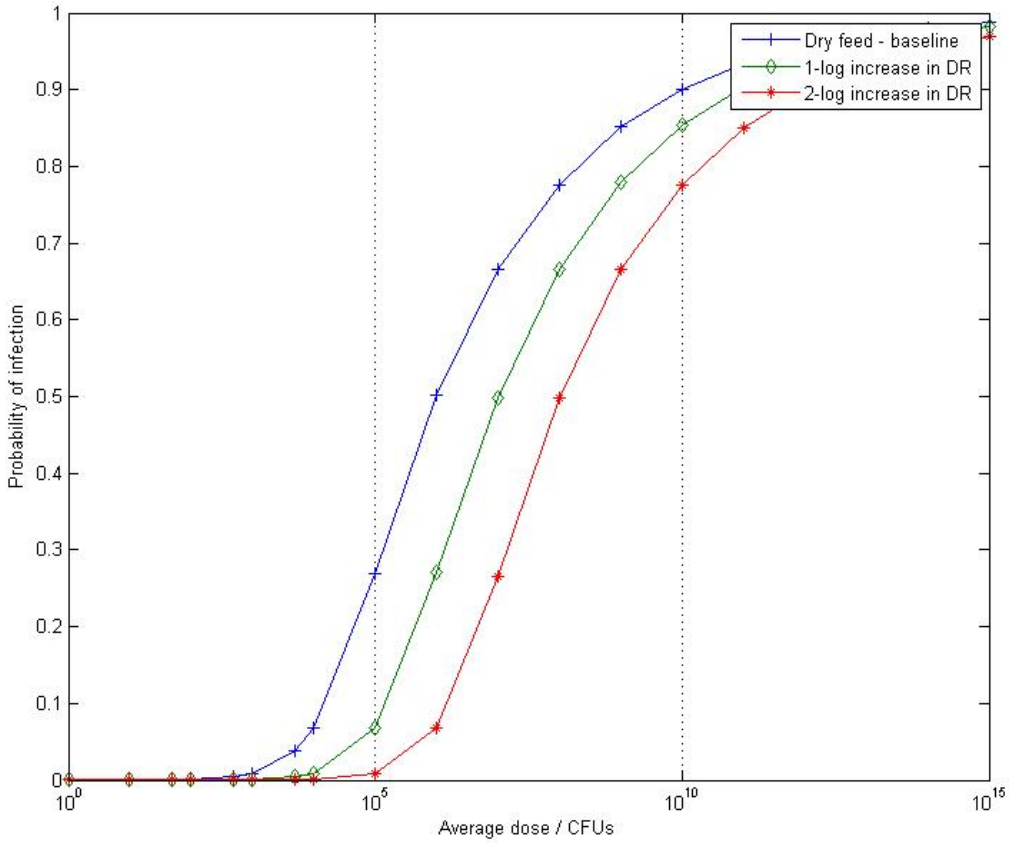

Figure 13.1: Modification of the dose-response model. The beta parameter of the beta-binomial model is adjusted until it takes around, on average, 1 or 2 more logs to cause the same average probability of infection as the baseline model. For clarity, only the average probability of infection is displayed.

The dose-response model has already been modified for wet feed (see Section 7.4).

13.2.2 Transport

Increased cleaning

As per farm pen cleaning, little evidence is available to suggest what the effects of improved cleaning measures are. Small *et al.* (2007) tested the effectiveness of different cleaning measures. The results of these tests for commonly-used cleaning techniques (pressure washing and steam washing) are used in the baseline model. However, the most effective cleaning was pressure washing with sanitiser washing, which had an average 4.5 (+/-0.9) log10 initial reduction and 5.2 (+/-0.5) log10 reduction after one hour. This is a further 2 log reduction on the effect of pressure washing (used as the standard cleaning measure within the baseline model). Following these results we assume a 0.5, 1 and 2 log further reductions in *Salmonella* during cleaning of transport and lairage pens. This is implemented in the model by increasing the estimated reduction in *Salmonella* due to cleaning, χ^{E}_{L}, by a further 0.5, 1 or 2 logs respectively.

Logistic transport (i.e., one batch, one transport vehicle)

Given a paucity of data in the area of transport, and based on available expert opinion, the baseline model assumes that every batch of pigs sent to slaughter is sent on one transport vehicle, and is not mixed with any other pigs on the way. We modified the baseline code so that trucks were filled up to capacity with whatever pigs were next in line (as opposed to restricting to one batch of pigs per truck), thus allowing for the possibility that a truck will contain pigs from multiple farms and thus cross-contamination between clean and infected batches of pigs.

Logistic slaughter (slaughtering highly-infected batches at the end of the day)

Although this is a slaughterhouse intervention it is modelled within the Transport & Lairage module. Logistic slaughter is easy to implement in the current model if we use the actual bacteriological status of the pigs to assess whether a batch is "high-risk" or "low-risk". Instead of a completely random sampling from the farm matrices, we can randomly sample a day's allocation of batches to a slaughterhouse, and then sort the batches by the within-batch prevalence of lymph-node positive pigs, $P_{LN}(b)$, such that for each batch slaughtered that day, x_1 to x_n, then $P_{LN}(x_n) > P_{LN}(x_{n-1}) > P_{LN}(x_{n-2}) \ldots > P_{LN}(x_1)$.

In reality logistic slaughter is carried out via a bacteriological or serological test at the herd level, such that high-risk herds, rather than high-risk batches, are slaughtered at the end of the day. Our representation represents a "perfect" test scenario, and indicates whether the practice of logistic slaughter, as a physical mechanism for preventing significant cross-contamination of carcasses, works or not. The application of a less than 100% sensitive or 100% specific herd test can be modelled if logistic slaughter is assessed to be a significant intervention.

13.2.3 Slaughter

Hypothetical interventions

Decontamination can be performed in several ways, using water or steam, optionally at high temperatures, or using added chemicals. Also, a new technique using ultrasound has been occasionally used. Irradiation is very effective, but prohibited in the EU, as is adding chemicals. Decontamination usually takes place after polishing or before (blast) chilling.

We investigated the effect of a 1, 2 and 3 log decrease in exterior contamination, at an individual carcass level, at the point of pre-chill. Pre-chill was chosen as the final practical point along the slaughter line where intervention can occur.

DG Sanco requested that only hypothetical reductions in carcass contamination were to be investigated, rather than specific interventions that would physically inactivate/remove *Salmonella* from the carcass. Hence the only specific intervention investigated was preventing faecal leakage.

Reducing/preventing faecal leakage

During dehairing and polishing, faecal material may exit via the rectum of the pig. This can also happen after the rectum is loosened, before belly opening. This introduces an extra amount of contamination on the machine and the exterior of the pig. According to Richards & Dodd (2009) it is common practice in Denmark, Norway and Sweden to seal off the rectum of the pig with a plastic bag after loosening. According to Borch *et al.* (1996), after

polishing, the rectum is circumcised, loosened and bagged. This prevents any further leakage. Another option mentioned was the use of a stainless steel plug. The same authors suggest that the protection is near perfect, the single one positive carcass found at the slaughterhouse was probably not faecally contaminated.

This intervention is modelled by simply setting amount of *Salmonella* within an infected pig's gut, *c*, to zero.

13.2.4 Multiple interventions

In reality, the application of just one intervention is unlikely to achieve the elimination, or at least significant reduction, of *Salmonella* from the pig meat food chain, and a more practical approach will be the application of controls at different stages of the food chain. Indeed a comprehensive review of *Salmonella* in pigs (EFSA, 2006), which explored possible interventions across the farm-to-fork pathway, concluded that it was not possible to control *Salmonella* with the adoption of just one measure. In other words, the control of the *Salmonella* can only be achieved by the introduction of multiple interventions across the farm-to-consumption pathway.

We therefore investigated the application of double interventions. The combinations chosen were based on the results of the individual intervention analyses for specific intervention measures.

13.3 Results

13.3.1 Hypothetical interventions

As discussed above, hypothetical interventions describe the effect of reducing some important factor/parameter within the food chain/model, but without defining what intervention might achieve a reduction.

The effect of reducing slaughter pig prevalence, as described above, is shown inFigure 13.2. Reducing slaughter pig prevalence is deemed to be effective in reducing the number of human cases per year for each case study MS. Indeed for MS2, which has a high baseline slaughter pig prevalence, there is a strong linear relationship between reduction in slaughter pig prevalence and reduction in the number of cases. This linear relationship also exists for MS4, but less so for MS3 and MS1. Further discussion on these results are included in Section 3.4.

Breeding pig herd prevalence has already been established as a significant factor within the model via sensitivity analysis – broadly speaking, low breeding pig herd prevalence (low number of positive piglets) equals low slaughter pig prevalence and *vice versa*. The reason for this is that breeding pig herd prevalence has already been shown to dominate the risk of positive pigs at slaughter to a degree that the trend in the change of breeding pig herd prevalence will outweigh all other factors. The result of the breeding pig herd analysis is shown in Figure 13.3. This analysis looks at a broad range of plausible breeding pig herd prevalences (as taken from the EFSA breeding survey), but uses the farm management systems of MS4. The trend observed with this MS4 management model will be much the same as it will for the other three MSs.

It is clear from Figure 13.3 that for MS4 breeding pig herd prevalence is predicted to be strongly correlated with slaughter pig prevalence, and hence is also strongly correlated with the risk of illness in humans. Given the strength of association between breeding pig herd prevalence and slaughter pig prevalence within the model, this same trend will be seen for each case study MS.

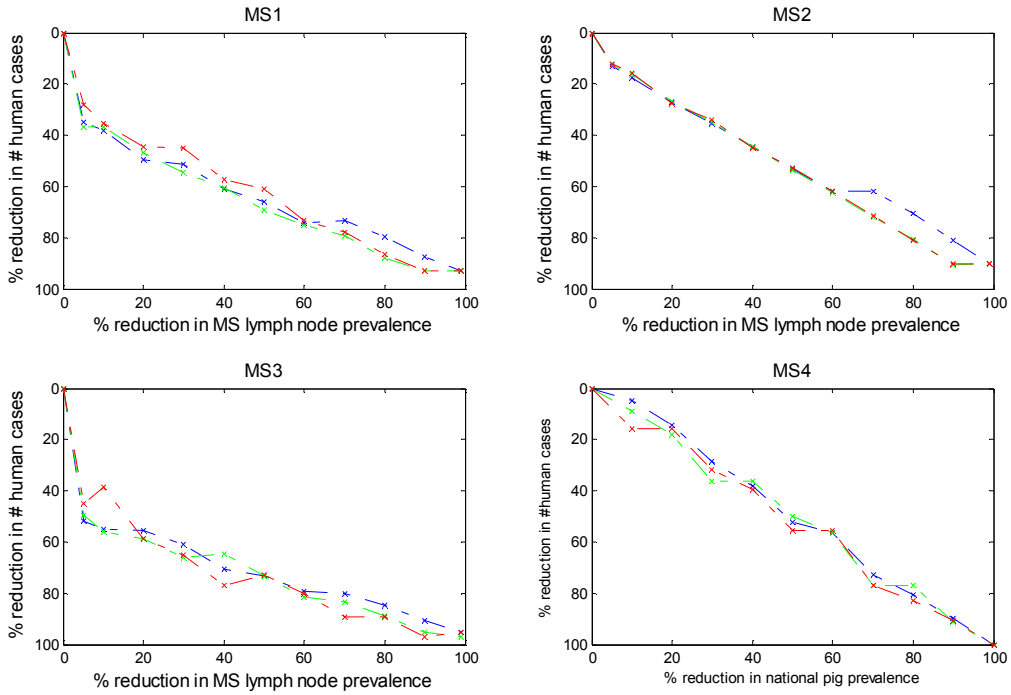

Figure 13.2: Effect of reducing slaughter pig prevalence from 5 to 99% of the baseline national pig prevalence estimated within the baseline model, for each product type and for each case study MS (pork cuts – blue, minced meat – green and fermented sausage – red). y axes are inverted for clarity. Reductions in national pig prevalence are achieved by reducing the number of infected pigs within each batch according to a binomial trial, where the probability of "success" (i.e. subtracting a positive pig), p = {0.05,..0.99}. Hence, the number of infected pigs subtracted from an individual batch varies, but across all batches sent to slaughter the average reduction will converge to p. Small variations in the downward trend can be seen, for MS1 and MS3 in particular; these are due to sampling error within the Monte-Carlo simulations.

We also investigated the effect of a 1,2 or 3 log reduction in contamination of the carcass pre-chill and post-dehair. The results are presented in Figures 13.4 and 13.5. A clear trend is observed in both situations, in that a reduction between 1-2 logs is enough to achieve maximum reductions that could be achieved with decontamination interventions at these stages.

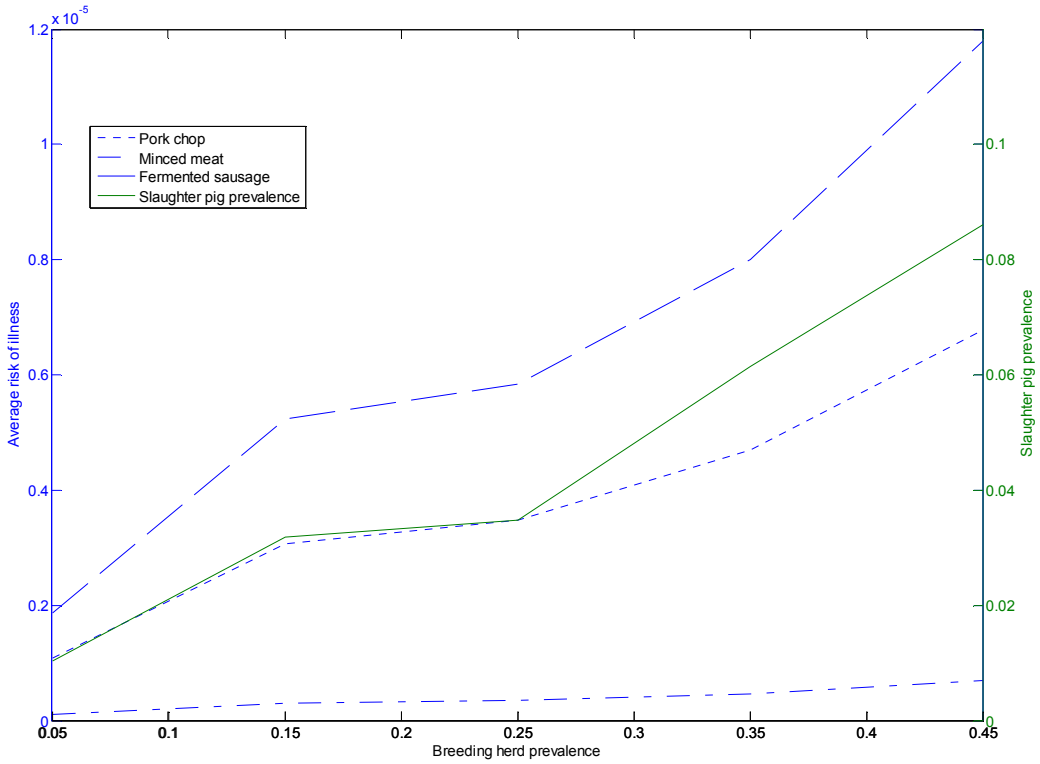

Figure 13.3: The effect of breeding pig herd prevalence on the national slaughter pig prevalence (right-hand axes) and the average risk of illness per serving in humans (left-hand axes).

13.3.2 Specific interventions

As discussed above, no data were available to assess specific interventions quantitatively and confidently, given that any data available are from small studies that could not be used to extrapolate up to a MS level. However, we have investigated the effect of manipulating the mechanisms that we believe influence the interventions.

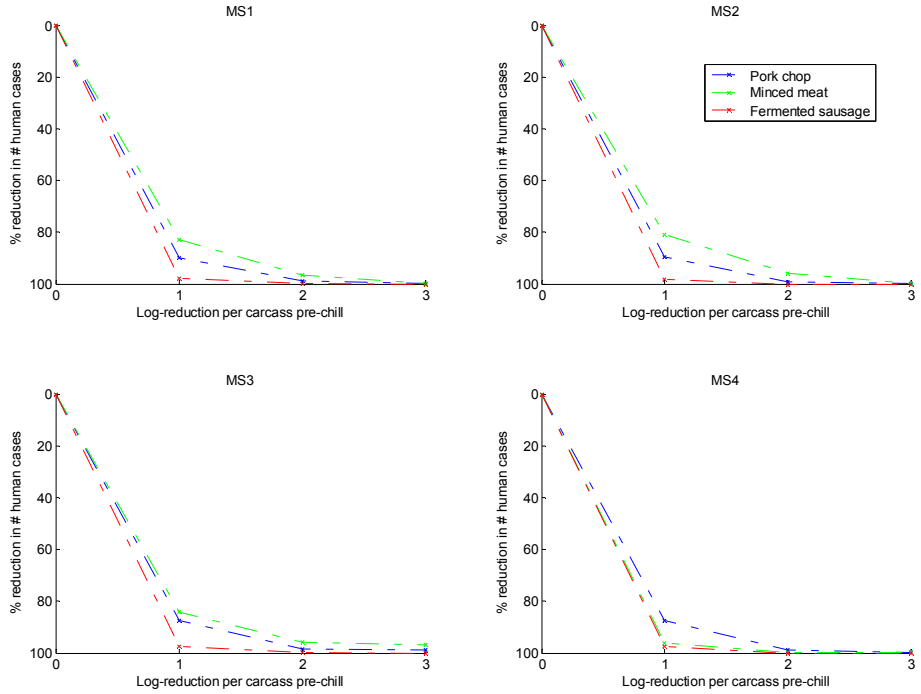

Figure 13.4: Effect of reducing concentrations across all contaminated carcasses in each MS by 1, 2 and 3 logs immediately before chilling of the carcass. For each MS, a log reduction of 2 logs appears to be sufficient to reduce cases by over 90%.

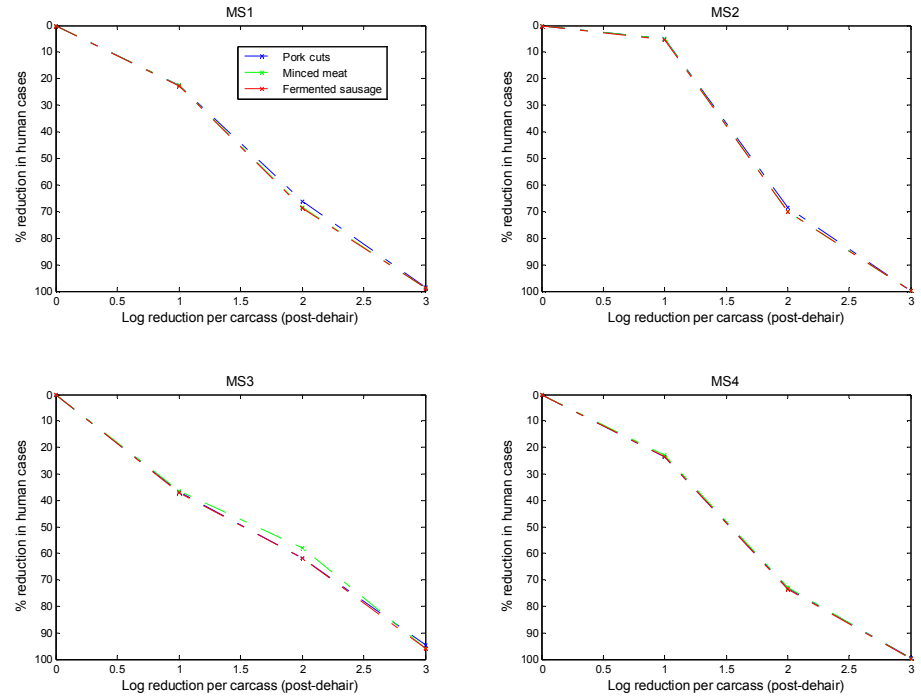

Figure 13.5: Effect of reducing concentrations across all contaminated carcasses in each MS by 1, 2 and 3 logs immediately after dehairing the carcass (e.g. a more efficient singer). For each MS, a log reduction of 2 logs appears to be sufficient to reduce cases due to pork cuts and sausages by over 60%.

Farm

At the farm level we were asked to investigate reducing number of suppliers, increased downtime, increased cleaning efficiency, reduction of feed contamination, acidification of feed/water and vaccination. Increased downtime and increased cleaning have been investigated separately according to the different mechanisms involved. We assume the mechanism for acidification and vaccination has approximately the same effect – i.e. that more *Salmonella*s are required to cause infection (which we can model by modifying the dose response model).

Increased cleaning/downtime

While the mechanisms for removing *Salmonella* are different for downtime and cleaning, the effect is similar – a reduction in the *Salmonella* levels present in a pen at the point where a new batch of pigs enters the pen. However, these reductions in contamination levels between batches do not appear to have a significant effect in reducing slaughter pig prevalence or risk of illness, or at least the reductions are small enough to be outweighed by the stochastic variation inherent in the farm transmission model. A small reduction in pig prevalence is likely because the contamination of the pen at repopulation is not sufficient to cause large numbers of infected pigs, and hence removing the contamination of the pen does not significantly alter the rate of infection.

Increasing resistance of the pig (vaccination, organic acids)

Within the model, the resistance of the pig to infection is governed by the probability of infection given ingestion of a particular dose. Modifying the dose-response relationship for ALL pigs at ALL stages of production across a MS will produce a similar trend in results for each MS, therefore we show only MS4 (see Figure 13.6).

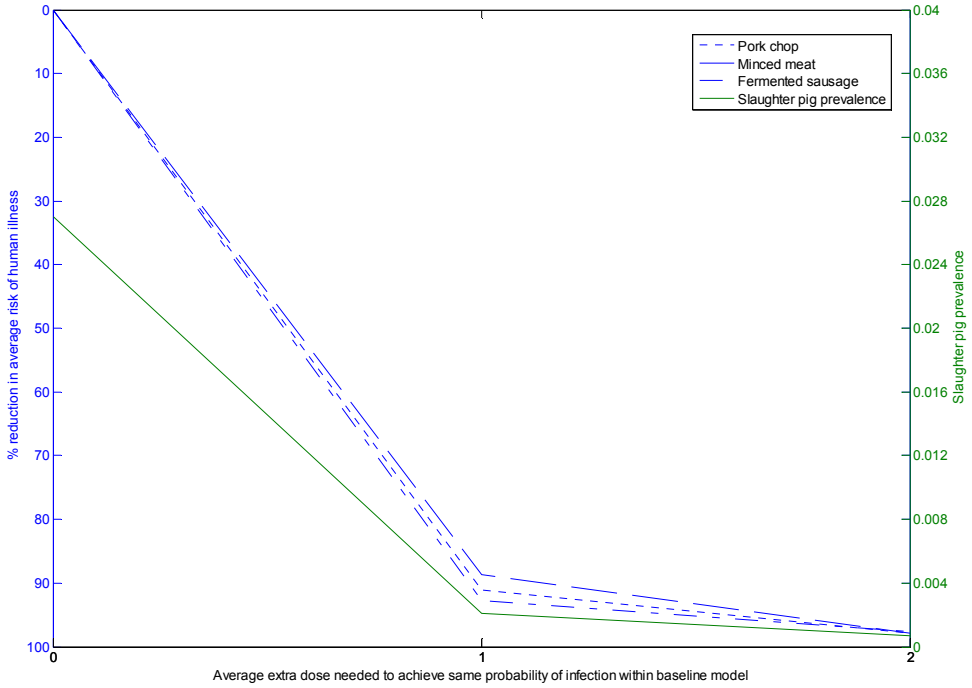

Figure 13.6: Effect on average risk of human illness per serving (left hand y axes) and slaughter pig prevalence (right hand y-axes) by decreasing the average probability of infection of pigs by 1 or 2 logs from baseline model values. MS4 shown.

It is clear that a 1-log increase in the dose needed to cause the average probability of infection will have a significant effect (~ 90% for all product types) in reducing slaughter pig prevalence and subsequently the human risk of illness. It must be remembered that this is the effect of consistently modifying the dose-response relationship for *all* pigs at *all* stages of production – something which has yet to be shown to be practical for such interventions as vaccination or organic acids. There is stronger evidence that feed type might have an effect, but still whether a significant (e.g. 1-log) increase in the dose needed to cause infection can be achieved is debatable.

A further log increase in dose needed to produce the same baseline probability of illness doesn't have the same magnitude of effect, and the published literature suggests this may well be unobtainable with current interventions.

Varying probability of feed contamination

The effects of reducing feed contamination are shown for MS4 and MS1 in Figure. MS1 was also investigated for this analysis due to the identified importance of feed (see Figure 7.11).

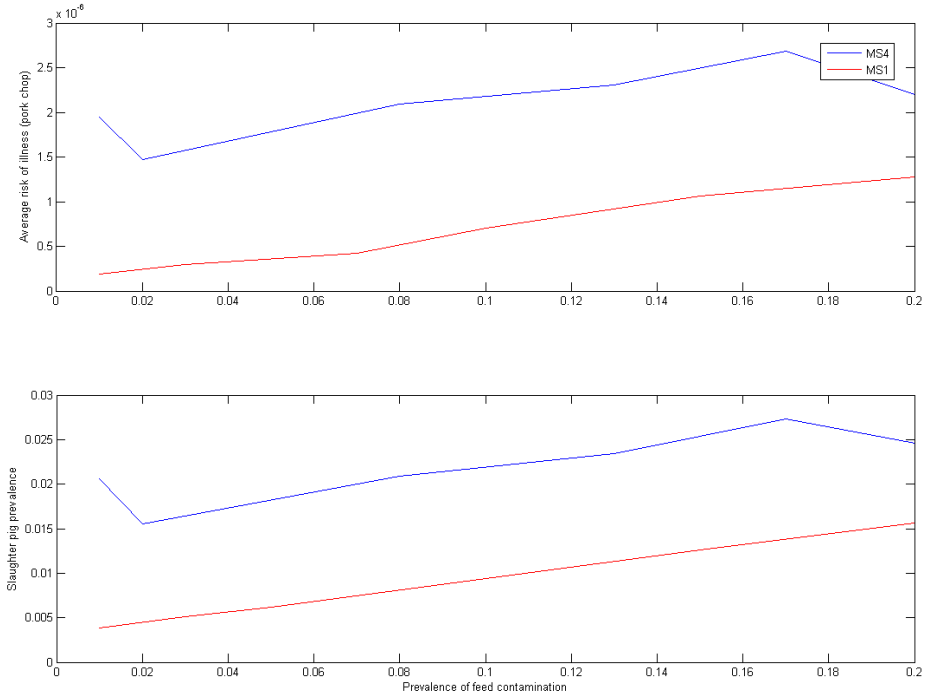

Figure 13.7: Effect of reducing feed contamination for MS1 (red) and MS4 (blue) on the risk of human illness. Shown on log scale to clearly show the greater effect increasing feed contamination has on MS1 compared to MS4.

There is a relatively linear relationship between slaughter prevalence of feed contamination and slaughter pig prevalence (and the human risk of illness) for both MS1 and MS4, although the linear trend is stronger in MS1 because feed is a relatively greater source of infection than in MS4 (where the sow is a greater source of infection).

Transport

Logistic transport

Transporting more than one batch of pigs in one transport vehicle had minimal effect on slaughter pig prevalence, and hence risk of human illness, for any MS.

Logistic slaughter

The effect of slaughtering high-risk batches at the end of the slaughter day was negligible on slaughter pig prevalence, and hence risk of human illness, for any MS. This is because the vast majority of cross-contamination during transport occurs within the same batch, rather than between batches of pigs.

Increased cleaning at transport

Increased cleaning techniques (producing a 0.5, 1 or 2 log reduction in transport contamination before loading of pigs) had minimal effect on slaughter pig prevalence, and hence risk of human illness, for any MS.

Slaughterhouse

Preventing faecal leakage
The majority of contamination on the carcass post-singe originates from faecal leakage, therefore preventing faecal leakage had a similar effect to a 1 log reduction in contamination levels pre-chill, i.e. a reduction in cases of about 90%.

13.3.3 Multiple interventions

Based on the results of the previous section, a number of multiple interventions were investigated, of which we have chosen three to highlight the general trends found:

- Change to wet feed and 1 log decontamination post-dehair
- 1 log modification of dose-response with 1 log decontamination post-dehair
- Change to wet feed with 1 log decontamination pre-chill

The same methodologies for the singular interventions were used, but simply applied in tandem as relevant. The results are shown in Figure 13.8. The combination of interventions can, if applied judiciously, produce reductions greater than the sum of the individual interventions alone. The major reason for this is that both interventions (e.g. changing farms to wet feed and applying a 1-log decontamination step pre-chill) will affect the contamination level of carcasses. Using MS4 wet feed/post-dehair decontamination example, it just so happens that the combination of these two interventions decrease the average concentration on the carcass into the range where significant changes in doses ingested and/or dose-response will occur, leading to a greater increase in the reductions of cases (see Figure 13.8). The application of a decontamination step, regardless of farm intervention, appears a worthwhile intervention, especially if able to decrease contamination levels by 1-2 logs or more.

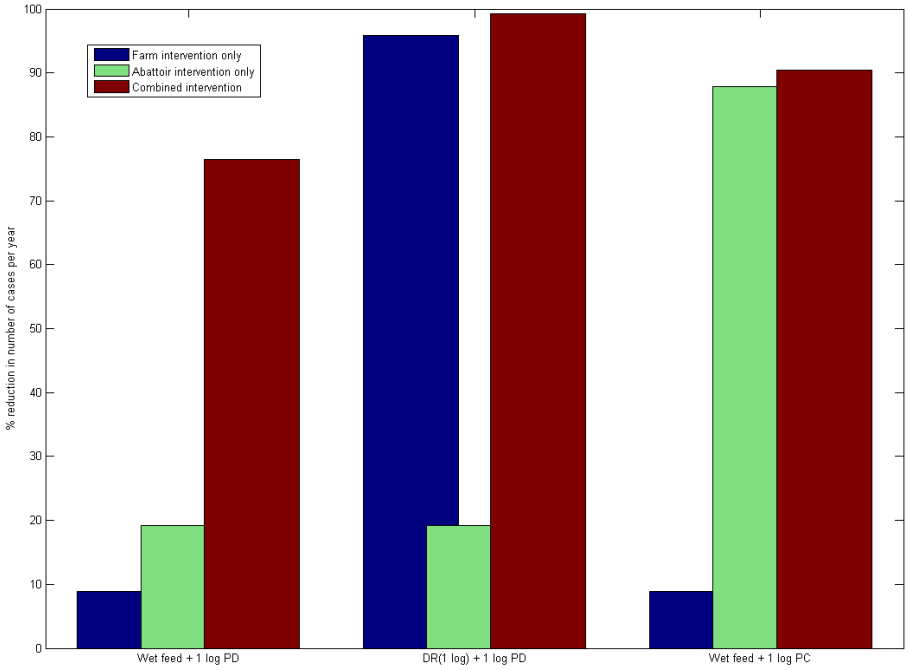

Figure 13.8: Effect of multiple interventions in reducing number of human cases per year, (MS4 and pork cuts only). The effects of multiple interventions for minced meat and fermented sausage were similar. The application of intervention combinations can be much greater than the effect of each intervention applied individually, for example wet feed and decontamination pre-chill (PD – post-dehair, PC – pre-chill).

13.4 Discussion

We have implemented a number of hypothetical interventions in order to investigate the effect of reducing slaughter pig prevalence, breeding pig herd prevalence and finally carcass contamination in reducing the number of human cases per year within the case study MSs attributable to each of the three product types respectively. These hypothetical interventions correspond to the EFSA ToRs for interventions. In addition, we have also investigated the ability of differing specific interventions in achieving the percentage reductions assumed within the hypothetical interventions. We were not able to model the absolute effect of specific interventions, such as vaccination or the feeding of organic acids, due to extreme quantitative data paucity in the area of farm interventions for *Salmonella* in pigs.

In order to implement any of the interventions we have assumed two critical factors: (1) that uptake of each intervention is 100% across all farms/slaughterhouses across MS, and (2) that each intervention would be implemented in such a way to produce the effect desired (e.g. reducing carcass contamination by 1 log, or raising the dose needed to cause a particular probability of infection). Qualitative evidence (VLA, 2009) and expert opinion suggest that uptake and efficient application would be nowhere near 100% in reality. We would strongly advise field studies to assess these two factors.

The hypothetical interventions investigated are illuminating. Interventions that reduce prevalence and/or contamination at each major point in the food chain investigated (breeding pig herds, slaughter pigs and pre-chill) will have a large impact in reducing the number of human illnesses attributable to pig meat consumption - if the reductions are also large. This is a positive result as it does suggest that control will be effective across the whole of the food chain.

Breeding pig herd prevalence is a strong indicator for slaughter pig prevalence (validated in some part by the results of the EU-wide baseline surveys in breeding and slaughter pig surveys), which in turn is a strong indicator of human risk. Hence, by reducing breeding pig herd prevalence major reductions in the number of human cases can be achieved. Greater reductions can be achieved when breeding pig herd prevalence is high, e.g. for MS2. As the sensitivity analysis for the farm suggested (Figure 7.16), the most important factor within the model was the amount of *Salmonella* the pigs (either sows or slaughter pigs) were shedding. Therefore to reduce slaughter pig prevalence the number of infected piglets entering the weaning stage must be reduced. Once the number of infected pigs entering the weaning stage is reduced, then feed and external sources of contamination (e.g. rodents) become more important. This does therefore suggest that as a first step, if breeding pig herd prevalence is high it should be controlled as a first measure – feed and external contamination of finishing pigs can then have a positive effect once breeding pig herd infection is reduced to low levels (perhaps below 5-10%).

Reducing slaughter pig prevalence is also effective in reducing the number of human cases per year for each case study MS. Indeed for MS2, which has a high baseline slaughter pig prevalence, there is a strong linear relationship between reduction in slaughter pig prevalence and reduction in the number of cases. This linear relationship also exists for MS4, but less so for MS3 and MS1. This result is perhaps counter-intuitive, if cross-contamination and growth have a significant role to play at the slaughterhouse and cutting plant/retail. However, the relationship between lymph-node prevalence at slaughter and the prevalence of carcass contamination during the slaughterhouse is yet to be fully proven. A Danish study (Dahl, 2009) suggests that there appears to be a strongly non-linear relationship between the number of sero-positive pigs and the % of positive pooled swabs at pre-chill, but a more representative sample to compare against this model is the % of lymph-node positive pigs and % of contaminated carcasses at evisceration. EFSA (2008a) investigated this as part of their analysis of the slaughter pig baseline survey data, and found what appears to be a modest linear relationship between the two at the slaughterhouse level. Further investigation of the model results shows that there is also a linear relationship between the percentage reduction at slaughter and the percentage reduction in the average prevalence and load of contamination on retail products. Of course, there are factors that may well invalidate this linear relationship past the slaughterhouse (growth at retail, cross-contamination at cutting plant/retail), but there is no information to support/disagree with this at a MS (or large-scale study) level.

From a modelling perspective, the results are logical and intuitive. The main factor determining risk of illness does appear to be the gross contamination (i.e. large numbers of CFUs per carcass) of a carcass at some stage during the slaughterhouse phase, where such gross contamination (usually via faecal leakage from a heavily-infected pig) then cross-contaminates a substantial number of carcasses further down the line (from 10-50). These gross contamination events are infrequent and highly localised (invariably within a batch). At an individual farm/slaughterhouse level, there will be wide variation in the response of the model to the differing percentage reductions investigated. However, averaging over MSs

and time (as for the intervention analysis) then the effect of reducing the number of lymph-node positive pigs entering the slaughterhouse by *x*% simply reduces the number of gross-contamination events due to faecal leakage by a similar percentage. In this case, average retail prevalence and contamination levels will be reduced by a similar percentage. Translating these reductions through the rest of the model, a linear relationship will exist in the percentage reduction in cases, as the gradient of the human dose-response model used within the QMRA over the range of average doses produced from the hypothetical interventions (0.05-0.5 logs) is relatively linear. Therefore, in summary, we believe the current results from the intervention analysis for slaughter pig prevalence are sufficiently representative to at least draw the broad conclusion that intervention at the farm should produce reductions in human illness, and that interestingly reductions in human cases appear to be linear to reductions in slaughter pig prevalence. However, how these large reductions (10-90%) can be achieved is less certain (see later).

Marked reductions can be achieved by applying some decontamination measure, or reducing faecal leakage, at the slaughterhouse. An intervention that could consistently achieve a 1 log decontamination of carcasses pre-chill could reduce the number of cases by up to 90% in all MSs. Further reductions can be achieved by further reducing concentrations on carcasses at pre-chill (e.g. a reduction of 3 logs) with all MSs resulting with a very high reduction (95-100%) in their number of cases. Non-chemical interventions have already been shown to produce reductions in the order of 1-2 logs (Christiansen et al. 2009; James 2009), and hence could be a viable short-term measure for reducing illness in humans if they are shown to be as effective if scaled up to be applied across a MS's slaughterhouses (given interventions at the farm level (e.g. vaccines) are likely to take years before real reductions are achieved).

In contrast, evidence that specific farm and transport interventions work consistently is sparse, if non-existent, presumably due to the more complex environment in which these interventions will have to be applied, and the difficulty in standardising experiments to trial interventions. Hence, while the evidence for consistent effects is lacking, some farm interventions may well be effective. This was the conclusion of Denagamage *et al.* 2007 for vaccination, but no quantitative effect was able to be shown. This lack of evidence for a consistent and/or quantitative effect meant that specific farm interventions could not be modelled. Therefore, in order to provide some assessment of farm interventions, we have only modelled the effect of the varying mechanisms applied to farm interventions (e.g. modifying the dose-response for vaccination, lowering the contamination of pens for cleaning).

The results of these farm interventions suggest that farm interventions could work, although the significant reductions that would be required to achieve the same effect as slaughterhouse interventions would be unlikely for any single farm intervention. Large reduction of slaughter pig prevalence were not seen in the literature for any of the current farm interventions. In addition, transport interventions, even assuming 100% uptake and 100% compliance/effectiveness, would not seem to make a significant difference in the rates of human illness.

On the farm, increased cleaning or downtime did not have in reducing slaughter pig prevalence, and hence human illness. However, it is debatable whether a 1-2 log reduction in contamination could be achieved consistently over all farms (especially in older buildings). Downtime would seem to be a more reliable way to achieve a reduction (as it relies on

simply allowing the drying out of the environment to inactivate *Salmonella*s), but may be prohibitively expensive in terms of lost throughput/production.

Modifying the dose-response model by 1-2 logs, as described above, produces a significant effect in reducing slaughter pig prevalence and human illness. The effect modelled is by a constant modification of the dose-response relationship, and hence current intervention trials where the application of organic acids or vaccination is applied only over limited timeframes are unlikely to achieve similar reductions in slaughter pig prevalence. Therefore, more promising interventions may be changing feed type, as this can be applied over weaning-finishing, and applying organic acids over the whole course of production. Vaccination may be effective in reducing infection in pigs over the entire production timeframe, but only if applied properly.

Reducing feed contamination can have a measurable effect in reducing slaughter pig prevalence, and hence human illness, even where breeding pig herd prevalence is high, as in MS2. A greater relative effect can be seen for MS3 and MS1 where breeding pig herd prevalence is lower. As for all interventions, the magnitude of effect that can be achieved in reality is very uncertain, given we do not really know what the prevalence or contamination levels of feed contamination are across the EU. One option would be to change the method of feed production, as some methods are significantly more associated with *Salmonella* infection (EFSA 2008b).

In summary, the farm and transport interventions are likely to vary in their ability to change slaughter pig prevalence by a sufficient amount to change illness numbers in humans. However, a combination of interventions applied across a large proportion of farms is likely to have a cumulative effect in reducing slaughter pig prevalence. Probably of extreme importance, but not investigated here, is the rate of uptake and correct application of interventions by farmers – if this is not universal across a MS the effect in reducing human illness will be proportionally reduced. The model results lead us to suggest that those MSs with a high breeding pig herd prevalence should focus on these herds in order to reduce the burden of infected new stock entering the weaning/growing/finishing stages, although of course that doesn't mean taking efforts to control *Salmonella* post-weaning won't also be beneficial. However, it may be more efficient in MSs with a low breeding pig herd prevalence to focus their attentions on feed and other sources of infection.

In all likelihood *Salmonella* control in pig production will be implemented at various stages. We have investigated three combinations of interventions, using either wet feed or increasing resistance along with a decontamination measure in the abattoir. A decontamination step achieves a significant reduction for MS4. Certain combinations of interventions are likely to produce even greater reductions than the effects of individually-applied interventions. However, the specific combination of interventions that achieve greater reductions together are dependent on the situation within a particular MS, in particular the contamination levels of carcasses. Investigation of such beneficial combinations can be done with the current QMRA model; the myriad combinations possible prevented us from investigating all of these, but MSs will be able to interrogate potential combinations of interventions if/when the baseline model has been parameterised for their country.

From the current evidence, it would appear that specific slaughterhouse interventions are, at present, more likely to produce greater and more reliable reductions in human illness, at least in a shorter timeframe than can be achieved at the farm. However, the hypothetical

reductions and multiple interventions investigated with the current risk assessment model suggest that MSs can achieve more effective reductions in human cases by targeting both farm and slaughterhouse.

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14 Source Attribution – the public-health impact of pork consumption

14.1 Introduction

Foodborne diseases are recognized as a major public-health problem, and the World Health Organization estimates that up to one third of the population each year suffers from a foodborne infection (WHO, 2005). The economic, social and public-health importance of these diseases has motivated many countries to implement surveillance and intervention strategies to control foodborne illnesses, particularly foodborne zoonoses (Wegener *et al*., 2003; EFSA, 2009). However, a precise evaluation of the effect of such interventions is difficult, partly due to the lack of information of the public-health impact of specific sources on the incidence of foodborne infections.

The ability to attribute cases of human disease to specific reservoirs, food vehicles or other responsible sources is, therefore, recognised as critical for the identification and prioritisation of food safety interventions (Batz *et al*., 2005; Havelaar *et al*., 2007; Pires *et al*., 2009). Efforts to quantify the importance of specific sources for human illness are gathered under the term “source attribution” or “human illness source attribution” and can be defined as the process of determining the proportion of a particular disease that is acquired from a given source (e.g. chicken) and potentially through a given pathway (e.g. food or direct animal contact). Several methods for source attribution have been described, including microbiological approaches, epidemiological approaches, intervention studies and expert elicitations. For a full review of approaches for sources attribution readers are referred to Pires *et al.* (2009) and EFSA (2008a).

Salmonellosis is one of the most common and widely distributed foodborne diseases in Europe. All serovars of *Salmonella* are potentially pathogenic for humans, but the degree of host adaptation varies, which affects the pathogenicity. Non-typhoid and ubiquitous serovars, such as *S.* Typhimurium and *S.* Infantis, affect both humans and a wide range of animals, and are those with principal zoonotic significance. Although these serovars in principle are non-host-adapted, strong associations between certain serovars or phage types within a serovar and a given animal reservoir may occur e.g. *S.* Enteridis in laying hens. In contrast, there exist a group of serovars that a highly adapted to an animal host e.g. *S.* Cholerasuis in pigs, *S.* Dublin in cattle, *S.* Abortus-ovis in sheep, and *S.* Gallinarum in poultry. These serovars only occasionally infect humans, where they may produce no, mild or serious disease (Acha and Szyfres, 1987).

In this chapter, *Salmonella* serovar and phage typing data collected as part of the EU-wide Baseline Surveys (BS) conducted in the period from 2005-2008 as well as data reported by the EU Member States in 2005-2008 published in the Community Summary Reports (CSRs), were analysed to make inferences about the most important sources of human salmonellosis in EU, as well as to highlight regional differences. No Member State specific data on the distribution of serovars in humans was available, meaning that it was not possible to develop a source attribution model estimating the quantitative contribution from each animal-food source based on the subtyping data. As an alternative the relative contribution of different sources to human disease was quantified using an analysis of data from outbreaks reported in the EU in 2005 and 2006.

14.2 Materials and Methods

14.2.1 Analysis of serovar and phage typing data

Animal data obtained from the EU BS on the prevalence of *Salmonella* in broiler flocks (2005-2006), slaughter pigs (2006-2007), laying hens (2004-2005) and turkeys (2006-2007) were used for the herd/flock level of the production chain. For slaughter pigs, lymph node samples and carcass swabs were considered as herd and carcass level, respectively. The 2008 BS on the prevalence of *Salmonella* and *Campylobacter* in broiler carcasses provided data for the carcass level in broilers. The study design, sampling schemes and data collection methods can be found in the respective BS reports (EFSA, 2007a; EFSA, 2007b; EFSA, 2007c; EFSA, 2008b; EFSA, 2008c; EFSA 2008d; EFSA, 2008e). No Baseline survey has been conducted in cattle and beef, consequently, we obtained data on this reservoir from the Community Summary Report in 2007 (CSR) (EFSA, 2009a).

Information on phage types was provided for a limited number of isolates of *S*. Enteritidis and *S*. Typhimurium, and not by all countries. The relative frequencies were calculated for all available data in each reservoir and in humans, but in the Netherlands a different phage typing scheme for *S*. Typhimurium was used, consequently showing a different set of phage types when compared with other countries.

The serovar distribution of reported human cases and *S*. Enteritidis and *S*. Typhimurium phage types was collected from the CSR from 2005 - 2008 (EFSA, 2006; EFSA, 2007d, EFSA, 2009a; EFSA, 2010). Published data corresponds to the top 10 serovars causing human disease each year and the most frequent *S.* Enteritidis and *S*. Typhimurium phage types in all reporting countries. Data were aggregated at the EU level, meaning that no country-specific data were available.

All data were analysed and compared by simple frequency distributions and, when possible, utilised for making inferences about the most important sources of human salmonellosis. To estimate relative frequencies, the numerator was the number of units (herds, flocks or samples) positive for a specific serovar and the denominator was the number of positive units. In the relative frequency graphs, “Not typeable” and “*Salmonella* spp, unspecified” were excluded in order to show the distribution among known serovars. Thus, values shown on top of the bars are the total number of units given an unambiguously named serovar.

Data were stored and analyzed in SAS Enterprise Guide, SAS Institute., SAS/STAT® User’s Guide, Version 8, Cary, NC: SAS Institute Inc., 1999.

14.2.2 Spatial analysis

Prevalence data from the BS in laying hens, broilers, slaughter pigs and turkeys in the EU were utilised for the spatial analysis (EFSA, 2007a; EFSA, 2007b; EFSA, 2008b; EFSA 2008c). The geographical analysis of the serovar distributions was limited to the country level, as the location (coordinates) of the individual holdings participating in the studies was not available. Also, the lymph node samples representing slaughter pigs were collected at the slaughterhouse. ArcGIS 9.3 was used to create maps showing the distribution of the most frequently identified *Salmonella* serovars among MS and non-MS participating in the baseline studies. In order to standardize animal sources at herd/flock level, we used data from laying hen flocks, broiler flocks, fattening turkey flocks, and slaughter pigs (lymph node samples).

Statistical analysis of the spatial distribution of *Salmonella* positive holdings was performed by applying both a global clustering test (Moran's I) and a local cluster detection test (spatial scan statistics (SaTScan™ developed by Kulldorff (1997; 2009). The Moran's I test measures the spatial global correlation, indicating whether the evaluated *Salmonella* prevalences were globally clustered or dispersed. In order to explore the specific location of potential spatial clusters, the local cluster detection test was used. The presence and location of local clusters were investigated for all evaluated serovars, since even in the absence of significant global spatial autocorrelation, clusters at the local level may still occur.

The local cluster test estimated the probability that the frequency of events per trial at each vertex surpasses the expected frequency by chance. SaTScan uses ellipses and a non-parametric test statistic. It takes into account the observed number of cases inside and outside the ellipse when calculating the highest likelihood for each ellipse. SaTScan tests the null hypothesis against the alternative hypothesis that there is an elevated rate of cases within the windows as compared to the outside. The method uses the likelihood ratio λ as the test statistic. The significance of the test statistic λ is determined by a large number of replications of the data set generated under the null hypothesis in a Monte Carlo simulation. The likelihood ratio λ for each replica is computed, and the result is significant at the 0.05 level if the λ value of the real data set is among the top 5% of all the values, including the replicas.

The Poisson model was chosen, which requires information about the estimated number of *Salmonella* positive holdings or samples in each country and animal population data. The estimated number of positive cases of each evaluated *Salmonella* serovar was calculated from the estimated prevalence. All estimated *Salmonella* positive holdings or samples were geocoded to the centroid of its respective country. The maximum window size was defined here as 50% of the cases and 999 replications were performed. The cluster analyses were performed separately for the results obtained by each baseline survey (laying hens, broilers, fattening turkeys and slaughter pigs). Only the most likely cluster is displayed in this analysis. The SaTScan output was imported into Arc GIS 9.3 to create maps of the identified clusters.

14.2.3 Source attribution using outbreak data

Human foodborne illnesses may be attributed to the responsible sources by analysing of data collected through outbreak investigations (Grieg et al., 2009). Foodborne outbreak data are usually freely available to the public and may provide detailed information over several years (EFSA, 2006; EFSA, 2007d; EFSA 2009b). In addition, these data are observed at the public health point which gives important information to the authorities for immediate control of individual events (EFSA 2008a). A simple analysis of foods implicated in outbreaks could be sufficient to estimate the proportion of cases that can be attributable to different food types (Adak et al, 2005; EFSA 2008f). However, often the food implicated in the outbreak is a complex food containing several food items, which leads to the necessity of using methods that analyse the relative contribution of each food category to the burden of human disease (Painter *et al.*, 2006; Pires, 2009).

A source attribution analysis using data from outbreak investigations was conducted to estimate the relative contribution of food sources to human salmonellosis in EU. Data on

Salmonella outbreaks from 2005 and 2006 were supplied by EFSA, which in collaboration with the European Centre for Disease Prevention and Control (ECDC) is responsible for the analysis of national data on foodborne outbreaks from all the Member States (EFSA, 2006; EFSA, 2007d).

14.3 Outbreaks of human salmonellosis in European Member States, Norway and Switzerland

Twenty two European Union MS and one non-MS in 2005 and 22 MS and two non-MS in 2006 reported outbreaks of human salmonellosis; in total, data from outbreak investigations from 26 countries were used in the analysis. *Salmonella* was the most common zoonotic agent in foodborne outbreaks reported in the EU, being responsible for 64% and 54% of all reported outbreaks in 2005 and 2006. These outbreaks affected more than 22,700 people in each year, of which 14% were admitted to hospital (Table 14.1) (EFSA, 2006; EFSA, 2007d). For cases for which specific information of the location of exposure was available, restaurant outbreaks affected around 80% more people than outbreaks in family homes in 2005, whereas in 2006 household outbreaks represented 47% of the total *Salmonella* outbreaks.

All *Salmonella* outbreaks reported by MS and non-MS were used as input data in the analysis, including confirmed and suspected outbreaks as well as outbreaks where evidence for an implicated source was not provided (source unknown). Implicated foods were reported based on epidemiological and/or laboratory evidence. Analytical epidemiological evidence corresponds to evidence of a statistically significant association between a food item (foodstuff) and the human cases in the food-borne outbreak, demonstrated by either a cohort study or a case-control study. Descriptive epidemiological evidence corresponds to information linking two or more persons with clinical symptoms consistent with the same disease, with a possible food vehicle in common. Laboratory evidence implies that the causative agent was detected by laboratory methods in the food source (e.g. in leftovers or ingredients) or in the food production and preparation environment (EFSA, 2007e).

Serotype and phage type information was provided for a part of the *Salmonella* related outbreaks reported by the MS. In the study period, 54% of the causative agents were serotyped and 17% were phage typed. When no specific serotype was reported, the pathogen was classified as *Salmonella* spp. *S.* Enteritidis was the predominant *Salmonella* serovar associated with outbreaks and accounted for 56% of all reported outbreaks where serotyping was performed.

Table 14.1 Outbreaks caused by *Salmonella* in Europe*, 2005 and 2006

	Outbreaks		**Human cases**		
	N	**% of total**	**N**	**No. admitted to hospital**	**No. of deaths**
2005	3,406	63.9	25,760	3,554	16
2006	3,131	53.9	22,705	3,185	23

*22 EU MS and 2 non-MS (Norway and Switzerland)

14.3.1 Location

Outbreaks were classified as general or household outbreaks, accordingly to the setting of the outbreak (Figure 14.1). The location describes where the food was consumed or exposure occurred (e.g. cafe/restaurant, institution e.g. school and nursing homes, household), or where the food was prepared (e.g. catering establishment). The main category *general outbreaks* included all outbreaks that took place outside a private home (*household outbreaks*).

Reported outbreaks associated with travelling abroad were analyzed separately and were not attributed to any of the specific sources. Travelling abroad is not considered to constitute a source/route of exposure by itself, as the main sources described will also apply for travellers. However, because information on the implicated foods was lacking, attribution of these cases to a specific source was not possible. In addition, information on the destination was not reported making it impossible to assign illnesses to be acquired within and outside the EU. Outbreak cases with travel history were thus attributed to a separate category *travel*.

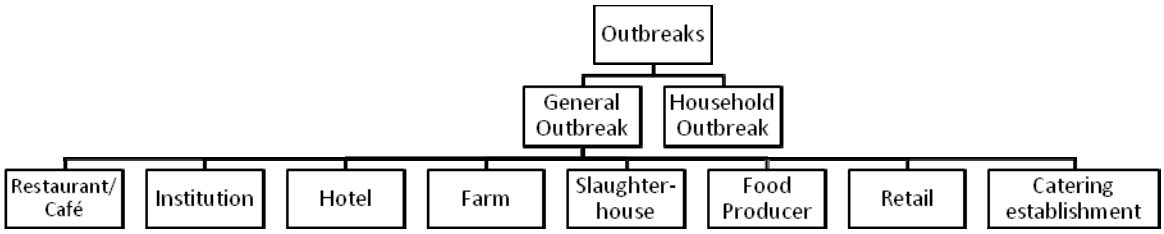

Figure 14.1 Hierarchical scheme for categorising the location of the outbreak.

14.3.2 Food categorisation

Food items were categorized using the hierarchical scheme presented in Figure 14.2. Foods that contained only one category (e.g. steak contains beef; fruit salad contains fruit, even though it contains multiple fruits) were considered “simple foods”, while foods containing ingredients belonging to different categories (e.g. meatloaf contains beef, egg, bread, and spices) were considered “complex foods”. Each implicated food was assigned to one or more mutually exclusive food categories, according to its ingredients. For outbreaks caused by complex foods for which ingredients were unavailable, an ingredient list was obtained by a review of recipes on the World Wide Web, as described by Painter *et al.* (2006): the top three recipes from a Google search were selected; when recipes were conflicting, the ingredients listed in at least two of the three recipes were included. Non-reported sources of infection were classified as *unknown*. Categories belonging to the main group “land animals” were in some cases grouped together in *meat and poultry*, depending on the level of detail of information available.

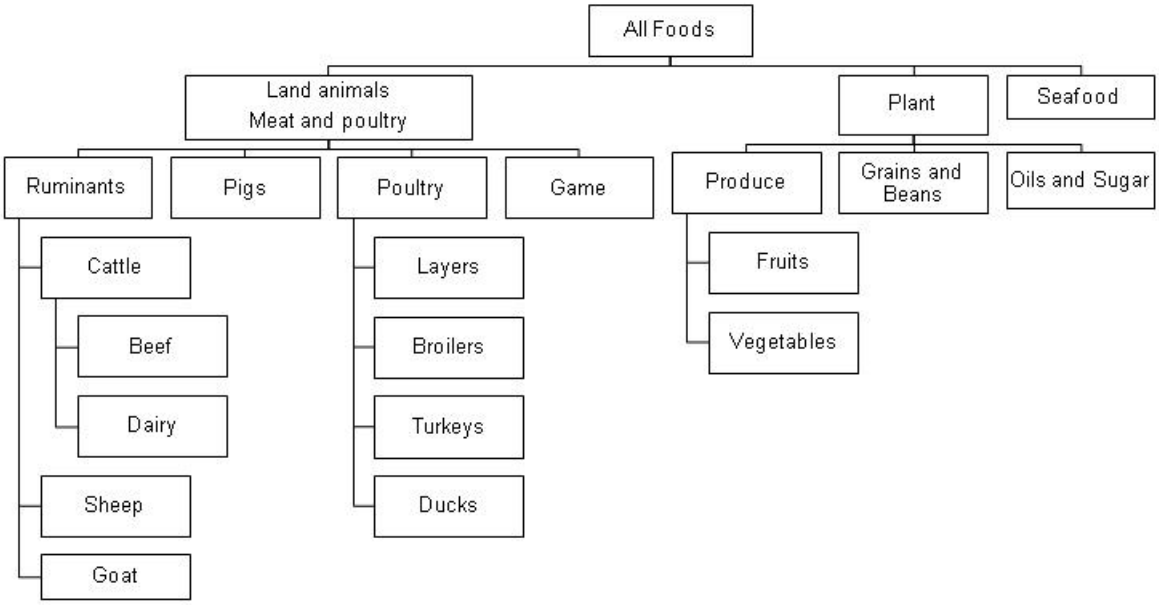

Figure 14.2. Hierarchical scheme for categorising food items into commodities within the main animal reservoirs.

14.3.3 Attribution of outbreak-related cases to specific sources

For the majority of the dataset, the data were organized so that one observation corresponded to one outbreak. For each observation, information on the year of occurrence, country, number of ill people, hospitalisations and fatalities associated with the outbreak, travel information, location of the outbreak, and implicated source was included. When any of the fields was incomplete, the parameter was included as *missing* or *unknown*. Analyses of the data were complicated by the fact that some countries report aggregated outbreak data. In the analyzed period, 5.2% of the *Salmonella* outbreaks were reported in an aggregated form. To include these aggregated data in the dataset, an additional variable was introduced: “number of outbreaks”.

For simple-food outbreaks, all illnesses were attributed to a single food category. For complex-food outbreaks, illnesses were partitioned to each implicated category relative to the proportion of illnesses attributed to each of the categories in outbreaks caused by simple foods. As a result, illnesses in an outbreak due to a complex food were only attributed to categories that had been implicated in at least one outbreak due to a simple food. As an example, outbreak-associated illnesses caused by *lasagne* would be attributed to the categories dairy, beef, vegetables, grains and beans, and oils and sugar. If any of these categories was not implicated in any outbreak caused by simple foods, the category would be excluded from the analysis of the attribution of illnesses to the separate ingredients composing the complex food. For categories implicated also in simple food outbreaks, the proportion of illnesses in complex food outbreaks was estimated based on the number of illnesses caused by the categories involved in simple foods-outbreaks and the sum of illnesses caused by all the commodities that composed the food (see example in Table 14.2). The total number of illnesses caused by each category in simple and complex food outbreaks was then summed, and the proportion of illnesses attributed to each source was estimated on the basis of the total number of illnesses analysed.

The proportion of reported human illnesses attributable to specific sources was estimated both on the basis of the number of reported outbreaks of salmonellosis and on the number of ill people reported in the outbreaks. The first analysis was performed in an attempt to explore for potential overestimations of the proportion of disease attributed to sources that caused large outbreaks, e.g. egg-associated outbreaks. The proportion of hospitalisations and fatalities linked to *Salmonella* outbreaks attributed to specific sources was also estimated.

The attribution estimates (in %) based on the number of outbreaks was multiplied with the total number of sporadic cases reported in EU to estimate the number of sporadic cases by source. The number of reported outbreak-related cases was then added to the output of this analysis, either to the specific sources implicated in the outbreaks or to "outbreaks with unknown source". The underlying assumption of this final step was that each outbreak contributes with one case to the total number of sporadic cases.

To illustrate potential regional differences within Europe, separate analyses for the 4 United Nations regions were performed (as available in http://www.un.org/depts/dhl/maplib/worldregions.htm). Table 14.3 shows the regions and countries belonging to each.

Table 14.2 Example illustrating how to attribute the number of illnesses associated with a *Salmonella* outbreak caused by a complex food (lasagna) to specific commodities.

Implicated food	**Dairy**	**Beef**	**Vegetables**	**Grains and beans**	**Oils and sugar**	**Total**
Simple Foods	80	0	2	4	3	89
Complex foods						
Lasagne	2*(80/89) = 1.79	Excluded	2*(2/89) = 0.045	2*(4/89) =0.09	2*(3/89) =0.101	2

Table 14.3 European macro regions and components, as defined by the United Nations

United Nation Region	***EU Member States and non-MS considered***
Eastern Europe	Bulgaria, Czech Republic, Hungary Poland, Romania, Slovakia
Northern Europe	Denmark, Estonia, Finland, Ireland, Latvia, Lithuania, Norway, Sweden, United Kingdom
Southern Europe	Greece, Italy, Malta, Portugal, Spain, Slovenia
Western Europe	Austria, Belgium, France, Germany, Netherlands, Switzerland, Cyprus, Luxemborg

14.3.4 Uncertainty

Confidence limits of the proportion of cases and outbreaks attributed to specific sources was obtained using bootstrap re-sampling of the original data, in order to generate the bootstrap distribution of the parameter of interest. Using this approach we assumed that the data were a random sample of outbreaks within the population of interest. For each source attribution analysis, we used a set of 10,000 replications obtained from the original data. The source attribution estimations were performed using each replication separately (totally 10,000 analyses), and the 10,000 estimates of each parameter were used to obtain the bootstrap distribution of the parameter of interest. The 95% confidence intervals for the parameters

were then given by the two values that encompass the central 95% of the distribution (the percentile method to obtain confidence intervals).

Data were stored and analyzed in SAS Enterprise Guide, SAS Insitute., SAS/STAT® User's Guide, Version 8, Cary, NC: SAS Institute Inc., 1999

14.3.5 Source attribution modeling using microbial subtyping data

The principle of the subtyping method is to compare the subtypes of isolates from different sources (e.g., animals, food) with the same subtypes isolated from humans. The microbial subtyping approach is enabled by the identification of strong associations between some of the dominant subtypes and a specific reservoir or source, providing a heterogeneous distribution of subtypes among the sources. The approach utilizes a collection of temporally and spatially related isolates from various sources, and thus it is facilitated by an integrated foodborne disease surveillance programme that is focused on the collection of isolates from the major food animal reservoirs of foodborne diseases (Pires *et al.*, 2009). This method typically focuses on sporadic cases and attributes infections to the reservoir level, meaning that the original infectious source is identified, whereas the route from reservoir (primary production) to consumer is not described. The results have provided information for the implementation and evaluation of control strategies in the major reservoirs (Wegener *et al.*, 2003; EFSA 2008b).

Several developed countries including EU MS have implemented laboratory-based surveillance and monitoring programmes for *Salmonella* infections in humans and in the main food-reservoir animals (Wegener *et al*., 2003; Hopp, 1999; Korsgaard *et al*. 2009; EFSA, 2009a). However, the extracted data is often not comparable between countries due to differences in sampling schemes and analytical methods. This highlights the importance of initiatives like the EU-wide Baseline Surveys for *Salmonella* organised by the EU Commission in collaboration with the Member States and EFSA.

For this report, we originally intended to develop a hierarchical source attribution model based on the principles described by Pires & Hald (2009) using MS-specific animal and food data from the EU baseline surveys and human data as reported by the MS to The European Surveillance System (TESSy). However, this idea was abandoned, since MS-specific data on the distribution of serovar and phage types in humans was not available. As an alternative, we made some descriptive comparisons of animal, food and human data as described above. The results were supplemented with results from the spatial and outbreak data analyses, and all results were discussed in an attempt to make inferences and rank the most important sources of human salmonellosis in EU.

14.4 Results

14.4.1 Analysis of serovar and phage typing data

Slaughter pigs and carcasses

Data from the BS in slaughter pigs were collected between 2006 and 2007. As mentioned before, it was assumed that lymph node samples represent the occurrence of *Salmonella* at herd level (although estimation of herd prevalences were not possible), and carcass swabs represent the slaughterhouse level. Twenty-five MS and Norway participated in this study. The overall EU lymph node-prevalence was estimated to 10.3% (95% CI: 9.2-11.5%) varying from 0% to 29% among MSs. Data on carcass swabs were submitted by 13 MS, resulting in an overall EU prevalence of 8.3% (95% CI: 6.3-11%) varying from 0% to 20% among MSs.

The most frequent serovar isolated from lymph nodes and carcass swabs was *S.* Typhimurium (40% and 49% of positive samples, respectively) followed by *S.* Derby (14% and 24% of positive samples, respectively). These serovars were widely distributed in EU occurring in most MSs, where *Salmonella* was isolated from pigs or pork. *S.* Enteritidis was on the top-ten serovar list among both lymph node samples and carcass swabs, and although the absolute prevalence in most MSs was low, *S.* Enteritidis appeared to be more prevalent in Eastern European countries corresponding to the MS clusters represented by MS3 and the MS4 in the QMRA (see Chapter 6). The frequencies of the most important serovars in humans and each of the investigated animal sources are presented in Figures 14.3-14.4, where the latter presents serovars other than *S.* Enteritidis and *S.* Typhimurium to facilitate visualization of the distribution of less frequent serovars.

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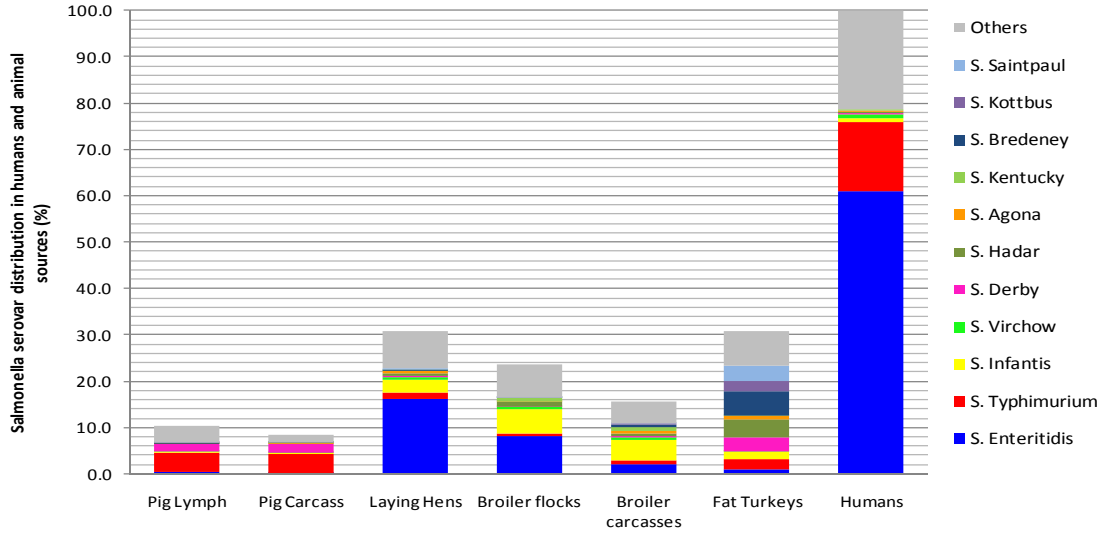

Figure 14.3. *Salmonella* prevalence and serovar distribution in animal and food sources as reported in EU-wide baseline surveys, and the serovar distribution in humans as reported by TESSy in the community summary reports (2005-2008).

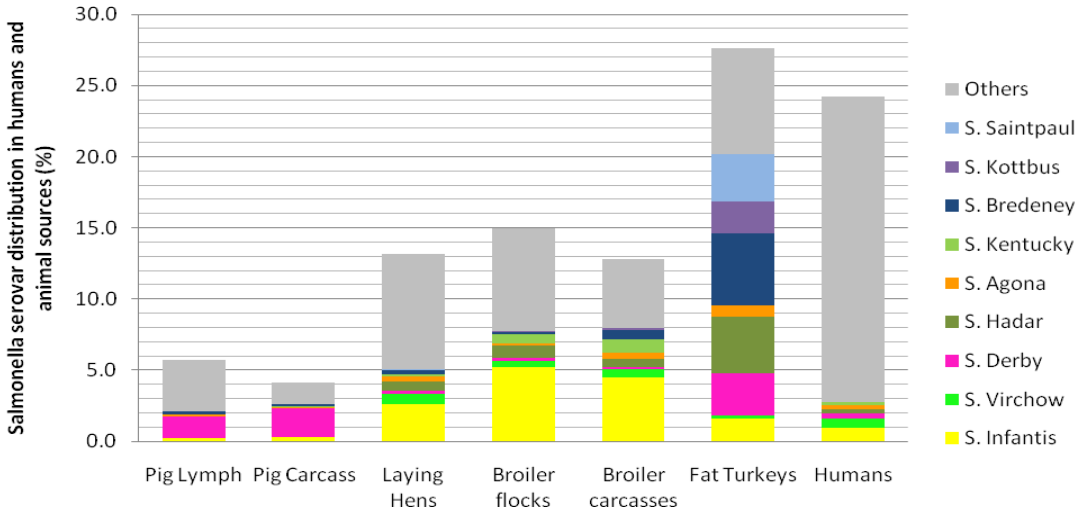

Figure 14.4. *Salmonella* prevalence of other serovars than *S.* Enteritidis and *S.* Typhimurium and the serovar distribution in animal and food sources as reported in EU-wide baseline surveys, and the serovar distribution in humans as reported by TESSy in the community summary reports (2005-2008).

Laying-hen flocks (holdings)

Twenty-three MS and Norway took part of the BS in laying hens conducted between 2004 and 2005. The overall EU holding prevalence was estimated to 30.8% (95% CI: 29.8-31.8%) varying between 0% and 79.5% between MSs. A particularity for this study was that several types of samples were used, including pooled-faeces from dropping belts, followed by boot swabs, and dust or faeces from different locations in the production system. A holding was assumed positive if one or more samples were found positive (EFSA, 2007a).

The most prevalent and most widely distributed serovar in laying hens was *S*. Enteritidis, which occurred in 18.3% of holdings (59.9% of positive holdings) and was found in 18 MSs. *S*. Typhimurium had an overall EU holding prevalence of 2.6% (8.3% of positive holding) and was found in 15 MSs. *S*. Infantis was the second most frequently occurring serovar. It was isolated from 11.5% of the positive holdings from 13 MSs. The frequencies of the most important serovars in humans and each of the investigated animal sources are presented in Figure 14.3-14.4.

Broiler flocks and carcasses

The BS in broiler flocks was conducted between 2005 and 2006, with 23 Members States and Norway participating. The overall EU flock prevalence was estimated to 23.7% (95% CI: 23-24.5%) varying from 0% to 68% among MSs. The BS on the prevalence of *Salmonella* in broiler carcasses was conducted in 2008 with 26 participating Member States. The overall EU prevalence of contaminated broiler carcasses was 15.7% (95% CI: 13.7-18%) ranging from 0.0% to 85.6% between MSs.

S. Enteritidis and *S.* Infantis were clearly the most frequently reported serovars in broiler flocks in the EU, being reported in 37% and 20% of positive flocks and from 17 and 14 MSs, respectively. The next most frequently observed serovars were *S.* Mbandaka (in 7.9% of flocks in 12 MSs), *S.* Typhimurium (in 4.6% of flocks in 15 MSs), and *S.* Hadar (4.1% and 8

MSs). Although *S.* Virchow was found in only 2.1% of all positive broiler flocks, it was reported by 11 MSs indicating that it is among the more widely spread serovars throughout the EU.

In broiler carcasses, *S*. Enteritidis, *S*. Infantis, *S*. Typhimurium, *S*. Mbandaka and *S*. Agona were the most widely spread serovars occurring in 10-15 MSs. Overall, the serovar distribution mirrored the distribution in broiler flocks, although some serovars tended to be more clustered – or not as wide spread as compared to the broiler flocks. The latter means that the overall serovar distribution in some instances was hugely driven by the dominant occurrence of a specific serovar in one or a few countries. For example, *S*. Infantis was isolated from 358 positive broiler carcasses, but 269 of these isolates was from a single MS. Likewise, *S*. Kentucky was isolated from 76 carcasses, where 68 isolates were from three MSs and 39 of these were from only one MS. The frequencies of the most important serovars in humans and each of the investigated animal sources are presented in Figure 14.3-14.4.

Turkey flocks

The BS in fattening turkeys was conducted between 2006 and 2007. Twenty-two MS and Norway participated in the survey and *Salmonella* was reported from turkey fattening flocks in 19 countries. The overall EU flock prevalence was estimated to 30.7% (95% CI: 28.2-33.2%) varying from 0% to 78.5% between MSs.

S. Bredeney was the most frequently reported serovar from the fattening turkey flocks in EU, representing 17.2% of the *Salmonella* positive flocks. The three next most frequent serovars were
S. Hadar, *S.* Derby and *S.* Saintpaul (14%, 11.3% and 10.4% of the positive flocks, respectively). *S.* Saintpaul and *S.* Typhimurium were the serovars most widely distributed found in 12 MSs. Generally, the serovar distribution in turkey flocks was characterised by the predominace of serovars that are infrequently found as the cause of human infections. The frequencies of the most important serovars in humans and each of the investigated animal sources are presented in Figure 14.3-14.4.

Cattle and beef

As no EU-wide baseline survey has been conducted for cattle, all data presented was retrieved from Community Summary Report 2007. Data from herd level was very sparse and not representative, and was therefore excluded. Most data were reported from the slaughterhouse level, where a total of 30,134 samples of fresh beef from 9 MSs was reported. The prevalence varied from 0% to 0.7% between MSs, with the exception of one MS having 6.7% positive samples. Results from sampling fresh beef during processing plants and at retail were also reported, but only by a few MSs. The results are, therefore, not described in detail here.

Data on the serovar distribution was very scarce and not very informative for the purpose of this report. In brief, the most frequent serovar reported in fresh beef sampled at the slaughterhouse was *S*. Typhimurium (19% of positive samples from 5 MSs), followed by *S*. Dublin (13% from 1 MS) and *S*. Enteritidis (4% from 2 MSs). Serovar information on milk and dairy products was also scarce, since only three out of 47,596 tested units were positive for *Salmonella*.

Humans

Human cases caused by the most frequent serovars in all reporting countries were collected from the CSR from 2005 to 2008 (EFSA, 2006; EFSA, 2007d; EFSA, 2009a; EFSA, 2010). Data were reported through The European Surveillance System (TESSy) and represents uploaded case-based and aggregated data that has been approved by each MS. In the CSR reports, the top-ten serovars are reported, but since the ranking of serovars differs between years, more than 10 different serovars are presented in Table 14.4. The aggregation also mean that serovars reported individually in one year may be reported in the group of "other" in other years (for example *S.* Bovismorbificans, which was reported individually in 2005 and 2008, was most likely included in the group of other in 2006 and 2007).

Overall, the incidence of human salmonellosis decreased from 2005 to 2008. *S*. Enteritidis and *S*. Typhimurium are by far the most frequent serovars reported in humans. Together these serovars constituted between 63% and 85.7% during the four year period. However, while the reported incidence as well as the relative frequency of *S*. Enteritidis has been decreasing from 2006 to 2008, the opposite trend has been observed for *S*. Typhimurium. Other important serovars reported on the top-ten in humans during all four years included *S*. Infantis, *S*. Virchow and *S*. Derby. The frequencies of the most important serovars in humans and each of the investigated animal sources are presented in Figure 14.3-14.4.

Table 14.4 *Salmonella* serovars reported in humans in the EU, CSR 2005-2008.

Serovar	Year							
	2005 (N=23 MS + 2)		2006 (N=24 MS + 4)		2007 (N=26 MS + 3)		2008 (N=26 MS + 3)	
	N	%	N	%	N	%	N	%
S. Enteritidis	86,536	53.7	90,362	71.0	81,472	64.5	70,091	58.0
S. Typhimurium	15,058	9.3	18,685	14.7	20,781	16.5	26,423	21.9
S. Infantis	1,354	0.8	1,246	1.0	1,310	1.0	1,317	1.1
S. Bovismorbificans	621	0.4	-	-	-	-	501	0.4
S. Hadar	577	0.4	713	0.6	479	0.4	-	-
S. Virchow	535	0.3	1,056	0.8	1,068	0.8	860	0.7
S. Derby	259	0.2	477	0.4	469	0.4	624	0.5
S. Newport	245	0.2	730	0.6	733	0.6	787	0.7
S. Stanley	-	-	522	0.4	589	0.5	529	0.4
S. Agona	-	-	367	0.3	387	0.3	636	0.5
S. Anatum	179	0.1	-	-	-	-	-	-
S. Goldcoast	173	0.1	-	-	-	-	-	-
S. Kentucky	-	-	357	0.3	431	0.3	497	0.4
Other	55,619	34.5	12,790	10.0	18,562	14.7	18,495	15.3
Total	*161,156*		*127,305*		*126,281*		*120,760*	
Unknown	56,619		17,359		9,814		6,636	

Phage type distributions in humans and animal sources

Similar to the serovar information in humans, data on phage types was only available at an aggregated level and only a minority of MSs routinely perform phage typing. Phage typing data available from the baseline surveys was in general also sparse. Since phage typing was not mandatory, only a proportion of the countries in each study reported these data and the results should, therefore, be interpreted with care.

The most frequent *S.* Enteritidis phage type in humans reported from 2005 to 2006 was PT4, causing between 23% and 30% of all *S*. Enteritidis infections, followed by PT1, PT8 and PT 21. *S*. Enteritidis observed phage types varied among food sources, but PT8 and PT4 were present at high relative percentages for almost all sources. In laying hens, these two types corresponded to around 60% of all typed samples. The exception was turkeys, where the most frequent phage types were PT13 and PT14b. Depending on the animal host, other types also showed some expression, such as PT2 and PT21 in broilers.

S. Typhimurium infections in humans were mainly caused by phage types DT104, DT120 and DT193, which constitutes more than 50% of all typed isolates reported from 2005 to 2008, but with the relative importance of each fluctuating over the years. Although S. Typhimurium phage types varied widely among the different animal sources, DT104 was present in all animal species and it was the main observed phage type among broilers and turkeys. Source-specific important phage types included U288, DT193, DT120, DT208 and U302 for pigs, DT208 and DT85 for broilers, and DT104b, DT135 and U302 for turkeys.

14.4.2 Spatial analysis

To investigate the spatial distribution of the most prevalent *Salmonella* serovars from the baseline studies, both a global and a local spatial cluster tests were performed. No spatial analysis was performed for cattle or beef because of insufficient data availability.

The Moran's I test for global cluster detected global spatial autocorrelation for the distribution of *S.* Typhimurium in pigs and in turkeys. *S.* Derby in pigs and in turkeys was also globally clustered. The Moran I tests indicated less than 5% likelihood that these clustered patterns could be the result of random chance. However, for the remaining prevalent serovars reported by the baseline studies, no spatial autocorrelation was statistically significant, meaning that the presented patterns were neither globally clustered nor dispersed. Still, since even in the absence of significant global spatial autocorrelation, clusters at the local level may occur, subsequent local spatial cluster analyses were performed for the overall most prevalent *Salmonella* serovars. Table 14.5 shows the most likely spatial local clusters of the most prevalent *Salmonella* serovars, with their respective relative risk (RR) and level of significance (p-value), for laying hens, broilers, pigs and turkeys. Figures 14.5-14.8 show the location of the significant spatial cluster of *Salmonella* serovars for laying hens, broilers, pigs and turkeys in EU.

Table 14.5 Spatial global and local clusters of the most prevalent *Salmonella* serovars, with their respective relative risk (RR) and level of significance (p-value), for laying hens, broilers, pigs and turkeys.

Serovar	Production type	Local cluster - Area included	Relative Risk (RR)	*p*-value
S. Typhimurium	Laying hens	GR	2.5	<0.01
	Broilers	SK, HU, PL	9.6	<0.01
	Pigs	PT, ES, IE, FR, UK, LU, BE	2.5	<0.01
	Turkeys	IT	2.8	<0.01
S. Enteritidis	Laying hens	PL, CZ	2.1	<0.01
	Broilers	PT, ES	6.2	<0.01
	Pigs	HU, SK, SI, CZ, PL	5.1	<0.01
S. Derby	Pigs	PT, ES, IE, FR, UK, LU, BE, NL, IT	3.9	<0.01
	Turkeys	ES	7.6	<0.01
S. Hadar	Broilers	PL	5.7	<0.01
	Turkeys	ES	21.5	<0.01
S. Infantis	Broilers	SK, HU, PL	20.5	<0.01
	Pigs	DK, DE	3.6	<0.01
S. Rissen	Pigs	PT, ES	201.4	<0.01
S. Mbandaka	Broilers	IE	48.3	<0.01
S. Saintpaul	Turkeys	CZ, AT, SI, SK, PL, HU	12.3	<0.01
S. Bredney	Turkeys	HU, CY, IT	68.4	<0.01
S. Kottbus	Turkeys	UK, IE, BE	10.8	<0.01

Figure 14.5. Spatial clusters of *S. Typhimurium* for laying hens, broilers, pigs and turkeys in EU.

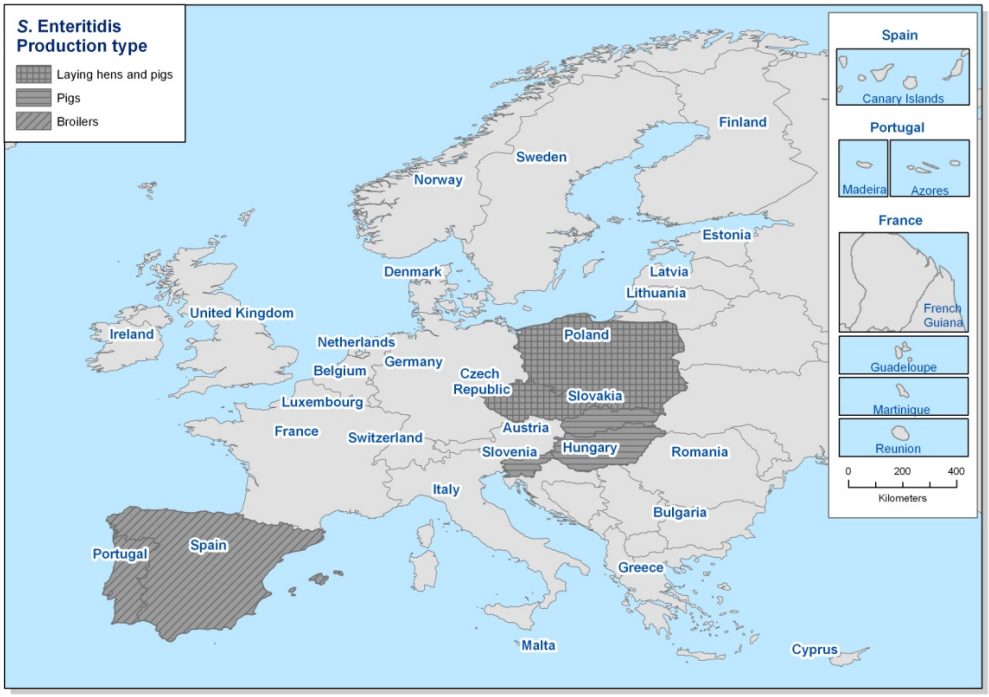

Figure 14.6. Spatial clusters of *S. Enteritidis* for laying hens, broilers and pigs in EU.

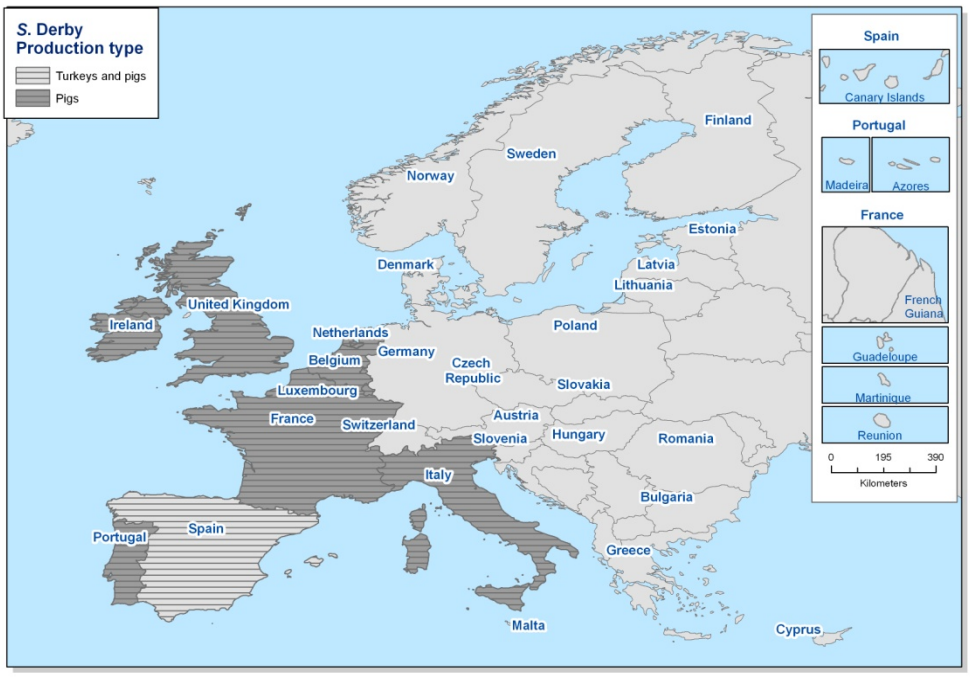

Figure 14.7. Spatial clusters of *S. Derby* for pigs and turkeys in EU.

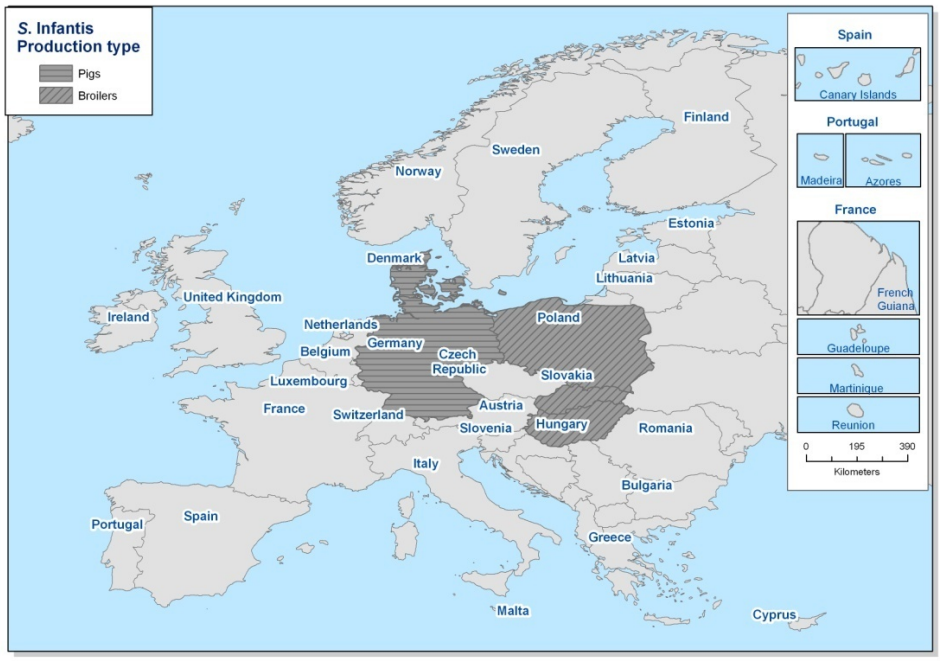

Figure 14.8. Spatial clusters of *S. Infantis* for broilers and pigs in EU.

14.4.3 Source attribution using outbreak data

The proportions of foodborne outbreak-associated salmonellosis attributed to specific sources, to travelling abroad and to an unknown source are presented in Table 14.6. Presented results are from an overall analysis performed by the total number of ill people and by the number of outbreaks, which included all countries in the study, cases caused by all *Salmonella* serotypes, and outbreaks that took place both outside and in the household.

Table 14.6 Attribution estimates showing the proportion of outbreak-associated salmonellosis cases attributed to specific sources in Europe, 2005 and 2006 (median, %)

	Proportion of number of ill	**95% CI**	**Proportion of number of outbreaks**	**95% CI**
Eggs	32.45	[20.89, 47.00]	25.72	[15.95, 40.33]
Meat and poultry	11.10	[4.12, 22.07]	9.47	[4.08, 20.83]
- Chicken	1.83	[1.05, 3.10]	1.60	[0.95, 2.67]
- Pork	0.72	[0.19, 1.59]	0.29	[0.13, 0.56]
- Poultry	0.44	[0.04, 1.62]	0.25	[0.05, 0.79]
- Beef	0.20	[0.03, 0.54]	0.12	[0.03, 0.28]
- Lamb	0.13	[0.00, 0.42]	0.04	[0.00, 0.12]
- Turkey	0.04	[0.01, 0.10]	0.21	[0.06, 0.50]
- Game	0.00	[0.00, 0.00]	0.00	[0.00, 0.00]
Dairy	2.21	[0.89, 4.46]	1.78	[1.09, 2.89]
Fruits and Nuts	0.04	[0.00, 0.15]	0.02	[0.00, 0.08]
Vegetables	1.39	[0.48, 2.85]	0.49	[0.24, 0.92]
Grains and Beans	0.04	[0.00, 0.10]	0.13	[0.00, 0.31]
Oils and Sugar	0.52	[0.24, 1.05]	0.71	[0.36, 1.32]
Seafood	0.97	[0.35, 2.17]	0.92	[0.54, 1.56]
Travel	3.89	[0.21,12.65]	2.16	[0.54, 6.45]
Unknown	42.02	[24.42, 59.33]	54,92	[31.86, 71.42]

In the analysis by the number of ill, it was estimated that 32.4% (95% CI: 20.9 - 47.0%) of the outbreak-associated salmonellosis cases were attributable to the consumption of eggs, making it the most important source of illness. For many outbreaks the source reported was meat i.e. it was not specified from which animal species the meat originated. This of course limited the ability of the results to point at specific sources. The general category *meat and poultry*, which include pork, was estimated to be responsible for 11% (95% CI: 4.1 – 22.1%) of the cases, and dairy products (2.21%, 95% CI: 0.9 – 4.5%) and chicken (1.8%, 95% CI 1.1 – 3.1%) followed in the contribution for human salmonellosis. When summarizing the proportion of cases caused by all meat and poultry-meat categories (see categorization scheme in Figure 14.2), it was estimated that 14.5% of the outbreak-associated cases of salmonellosis could be attributed to this main food category. 4% of the cases were attributed to international travel, and 42% could not be attributed to any source. No outbreaks and therefore no cases were attributed to game meat. The analysis by the number of outbreaks attributed, in general, a lower proportion of salmonellosis to each of the sources and a higher proportion to an unknown source, but the results were not substantially different.

A comparison between the proportion of outbreak-associated salmonellosis attributed to specific sources in general outbreaks and household outbreaks suggested that eggs were the major contributor to human illness in both types of outbreaks (data not shown); chicken (4.0% versus 1.6%), dairy products (4.0% versus 2.5%) and vegetables (2.9% versus 0.5%) were estimated to cause a higher proportion of salmonellosis in general outbreaks, whereas the general category *meat and poultry* (3.4% versus 5.7%) was a more important source of illness in private homes. When all the subcategories within meat and poultry-meat were summed up, no significant difference between the two types of outbreaks was apparent. In contrast, meat products within the poultry category appeared to cause a higher proportion of cases in outbreaks outside the household.

A comparison between the proportions of illnesses attributed to the various sources in the different categories of general outbreaks showed no significant differences in the order of importance of the sources to human illness. Our results suggest that eggs caused a higher proportion of the cases associated with outbreaks in institutions when compared to restaurants/cafés and catering establishments, whereas the proportion attributed to chicken was higher in catering establishments' outbreaks than in the other locations (data not shown).

The analysis of human cases caused by different serotypes showed that *eggs* was the most important source of outbreak-associated cases caused by *S*. Enteritidis and *S*. Typhimurium, and that foods within the category meat, particularly pork (7.5%, 95% CI 2.2 – 16.4) and beef (3.3%, 95% CI 0 – 11.4), were important sources of *S*. Typhimurium infections. Overall, around 18% of the *S*. Typhimurium cases were attributed to the category *meat and poultry.* For *S*. Typhimurium, 13.5% (95% CI 0 - 31.8%) of the cases caused by this serotype were attributed to vegetables, whereas only 1.5% (95% CI 0.3 - 4.8%) of the *S*. Enteritidis outbreak infections could be associated with this source.

Source attribution estimates differed when the analysis was performed stratifying by European regions. Figure 45 shows the differences in the proportion of cases attributed to specific sources in each region. Results suggest that eggs were the most important source of outbreak-associated salmonellosis in Eastern (61%, 95% CI 50 – 71%) and Western Europe (27%, 95% CI 14 - 47%), that in Northern Europe chicken was the most important source (24%, 95% CI 6 – 52%) and eggs contributed for a high proportion of cases, and that the majority of the salmonellosis cases were attributable to meat in the south of Europe (39%, 95% CI 0 – 76%). No outbreak-related illnesses were associated with international travel in Southern and Eastern Europe. The proportion of cases that could not be attributed to any source in Western Europe was substantially higher than in other countries.

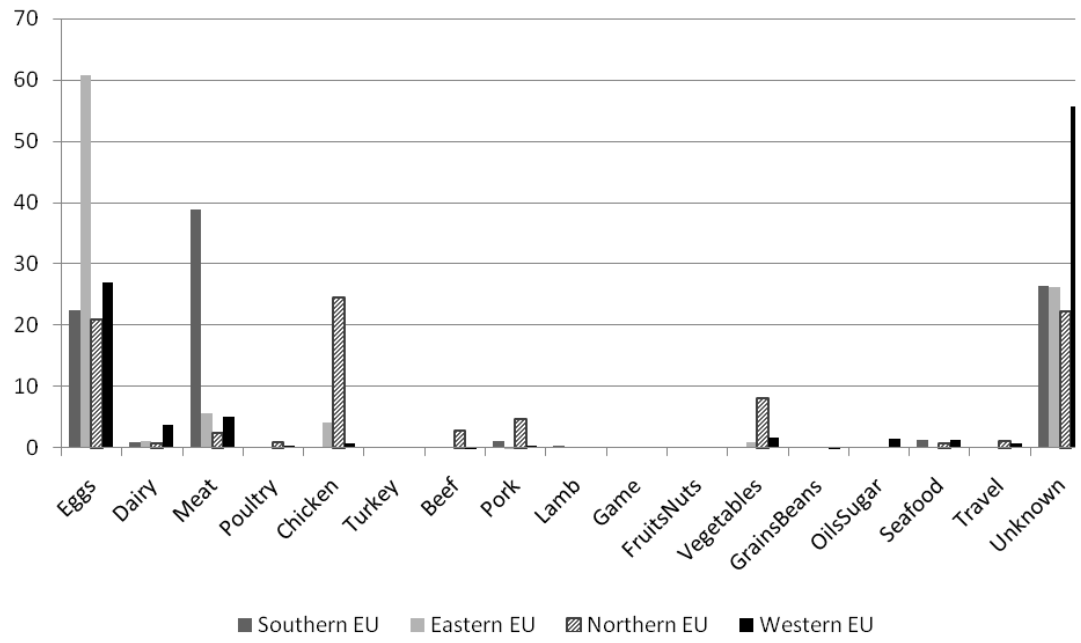

Figure 14.9 Proportion of salmonellosis outbreak-associated cases attributed to specific sources, travel and unknown in different European regions.

The vast majority of the salmonellosis outbreak cases that resulted in death were attributed to eggs (67.2%, 95% CI 47.3 – 82.1%). All the remaining sources were estimated to have a minor contribution to reported fatalities, and attribution estimates varied between 0 and 3.8%. Around 16% of the outbreak-associated deaths were attributed to an unknown source. Of the *Salmonella* infections that required hospitalization, 32.5% were attributed to eggs (95% CI 21.5 – 42.8%), and around 40% of these cases could not be attributed to any source.

A total of 173,379 human laboratory-confirmed cases of salmonellosis were reported in 2005, and 165,023 in 2006. On the basis of the proportion of disease attributable to each source estimated in the analysis by the number of outbreaks, a total of 82,539 cases of salmonellosis were attributed to the consumption of eggs in the overall population and study period. The general category meat and poultry-meat was estimated to be responsible for 38,772 cases, and 8,124 cases were associated with international travel. 177,135 cases could not be attributed to any source.

14.5 Discussion and interpretation of the findings

Application of microbial subyping techniques for source attribution has gained a lot interest in recent years, particular for *Salmonella*, where several approaches have been described (e.g. Van Pelt *et al*., 1999; Sarwari *et al*., 2001; Hald et al., 2004; Hald et al., 2007; Pires & Hald, 2009). The microbial subtyping method involves characterization of pathogen isolates by phenotypic and/or genotypic subtyping methods. Currently, serotyping and phage typing appear as the more relevant subtyping methods for *Salmonella*, as these are more generally applied for surveillance than genotypic methods (EFSA 2008a). However, the latter are expected to take over in the future, and approaches for attributing human campylobacterioses using MLST typing have recently been described (Mullner *et al*., 2009).

The contribution of each animal-food source for human cases depends on the prevalence of the *Salmonella* subtypes causing disease in that specific source, on the consumption of the food source in the population, on the ability of the subtype to cause infection (which depends on the survivability of the subtype in the food chain and on the pathogenicity), and on particularities of processing and preparation of the food source. Both consumption patterns and processing and preparation practices may vary between countries, reflecting, among others, cultural differences.

The *Salmonella* serovar and phage type distribution in animals, foods and humans in European countries was analysed on the basis of data from two different data sources, the CSRs, which publish data reported from individual countries, and the EU-wide baseline surveys (BS) conducted at the major animal reservoirs. The BS data were assessed to be more appropriate for source attribution, since they are uniform, representative and provide information on several animal sources at the reservoir level. They have, however, the downside of being cross-sectional studies providing only a snapshot of the situation. Furthermore, they were conducted in different years, which limited the comparison of the serovar and phage type distribution between sources and humans as these may change over time.

Data from the CSR was the only source of data available for the cattle reservoir and for human *Salmonella* infections. Human *Salmonella* data were aggregated for all European countries, and the unavailability of MS-specific information limited the comparison of *Salmonella* subtypes distributions from animal-food sources and humans. Consequently, quantitative estimates for the relative importance of each source for human disease could not be provided. Additionally, only a minor proportion of isolates from both animal-food sources (including from the BS) and humans was phage typed, and only from a limited number of countries. The phage typing distribution observed does, therefore, not represent all European regions.

The main observed serovars varied between animal reservoirs, but the frequent occurrence and wide distribution of *S*. Enteritidis followed by *S*. Typhimurium and *S*. Infantis was clear throughout the analyses. Other serovars, however, also appeared as important for specific animal sources, such as *S*. Derby in pigs, *S*. Dublin in cattle, *S*. Hadar in broilers and *S*. Saintpaul, *S*. Kottbus and *S*. Bredeney in turkeys.

The most important serovars in humans were *S*. Enteritidis, *S*. Typhimurium and *S*. Infantis. Together these three serovars accounted for up to 81% of the human *Salmonella* cases in

the period 2005 to 2008, with *S*. Enteritidis alone being responsible for between 54% and 64% of cases. When comparing between animals/food sources, table eggs (i.e. layer flocks) showed a higher proportion of *S*. Enteritidis, which is in line with the results of the source attribution analyses based on outbreak data, where it was estimated that eggs were the most important source of human salmonellosis in EU countries, and that the majority of *S*. Enteritidis cases was attributed to egg consumption.

S. Enteritidis in pigs appeared to be more prevalent in Eastern Europe including MSs of both MS cluster 3 and 4 used in the QMRA (Chapter 6). The overlapping of *S*. Enteritidis clusters in laying hens and pigs (Figure 14.6) further suggest the possibility of a common source and/or transmission of infection between these two species due for instance to a more extensive pig production for instance characterized by a relatively large proportion of smaller holdings. The predominance of PT4 and PT8 among most animal reservoirs was not surprising, given that those, along with PT1, were the main phage types involved in human cases. In laying hens, these two types corresponded to around 60% of all typed samples, emphasizing the role of eggs in human infections. Still, *S.* Enteritis was also the most frequently isolated serovar in the BS in broilers, where also PT8 and PT4 dominated. Broiler meat is, therefore, likely to be an important source in countries with a high *S.* Enteritidis prevalence in broiler flocks.

Along with those results, *S.* Typhimurium showed different clusters for turkeys, pigs, broilers and laying hens, where the cluster for pigs was located to Western Europe corresponding to MS cluster 2 represented by MS2 in the QMRA (Chapter 6). This concurs with the current knowledge about the widespread distribution of *S.* Typhimurium both in area and in types of sources. Very broadly speaking, these clustering patterns of *S*. Typhimurium suggest that pigs and pork are a main source of human *S*. Typhimurium infections infections in Western Europe, whereas the disease burden is more evenly shared between broilers and pigs in Eastern Europe (MS cluster 3 and 4 in the QMRA).

Although *S*. Typhimurium phage types varied widely among the different animal sources, DT104 was present in all animal species, which was also expected, given its wide distribution and its multi-resistant characteristics. The phage type distribution observed in humans with DT104, DT120 and DT193 as the main types, concurs well with the animal data and to some extent supports, that pigs and to a lesser extent poultry are important sources of human *S*. Typhimurium infections.

Interpretation of the sources of human *S*. Infantis infections tended to be more complex, given its widespread occurrence including in animal feed. However, it is notable that it clustered in countries with intensive pig production (North-east of cluster 2 in the QMRA), and also in broilers in Eastern Europe (particularly MS cluster 3 in the QMRA) indicating that pork and broiler meat may be important sources of these infections. However, *S*. Infantis was also commonly observed in laying hens and turkeys, so a proportion of infections originating from these sources cannot be ruled out.

S. Derby was, as expected, mostly found to be pig-associated, and the cluster analysis provided a cluster for pigs that was very similar to that found for *S*. Typhimurium in pigs i.e. MSs belonging to MS cluster 2. *S*. Derby was, however, also present in poultry, mainly turkeys. Compared to the other animal species, turkeys were in general not assessed to be a major source of human infections, even though many of the important serovars seen in humans were also found in turkeys. This is supported by the finding that *S*. Saintpaul and *S*. Kottbus, which play an important part in turkeys, cause only a few human cases, suggesting

that turkey meat does not have as high an impact on human salmonellosis. This may be explained by the lower consumption and maybe more varied consumption patterns across countries.

S. Hadar was associated with poultry with high prevalences observed in turkeys in a few MSs. Still, broilers are considered, quantitatively, to be a more important source due to a higher consumption of broiler meat as compared to turkey meat. The same pattern was observed for *S*. Virchow, which was very widespread in broilers and was also found in turkeys, but less spread and in lower prevalences. The importance of *S*. Virchow among human cases has increased in the last years, which may be explained by an increase in broiler consumption in European countries (Magdelaine et al., 2008).

As human data were not available at the country-specific level, it was not possible to compare the spatial serovar distribution between humans and animal sources, However, it is clear that *S*. Enteritidis, *S*. Typhimurium and *S*. Infantis are also the most frequently observed and widely distributed serovars in the animal source population. In contrast, *S*. Saintpaul and *S*. Kottbus, which play an important role in fattening turkey flocks, are not appearing on the human top-ten for any of the years included (2005-2008). However, given that these serovars show some very area-specific clustering, we would expect them to represent a higher proportion of human cases in these areas.

For some animal sources, the serovar proportions were driven by the findings in only one or a few countries. Such observations reinforces the importance of the spatial analysis as a strong tool to help drawing conclusions based on data provenient from a large area with different realities, such as the EU. In general, the clustering of specific *Salmonella* serovars in specific geographic areas may mirror common sources or reservoirs of infection such as specific raw feed ingredients, infected breeding pig herds, or endemic wildlife species. Spatial clustering is also consistent with the potential for the clonal spreading of a particular *Salmonella* serovar among farms following the introduction into a region, e.g. through the movement of infected animals, or through feed or animal transport vehicles, as suggested by Emborg et al. (2007). Finally, clustering may reflect a selection pressure for a specific serovar or phage types in a region for example due to the use of specific antimicrobials, which is consistent with the observations of Emborg et al. (2008).

Overall, the results of analysing the serovar and phage type distributions tended to confirm the current knowledge on sources (Hald et al., 2007; Pires et al, 2008; EFSA. 2008f; Pires, 2009). The observed data also concurred with the results of the source attribution study based on outbreak data, which renders credibility to both approaches. We acknowledge, however, that we could have done a more robust analysis if more detailed information on the human data as well as data over time had been available. Still, all included BS were conducted within a five-year period, and we would not expect this to be a major issue.

Results from an analysis of data from outbreak investigations suggested that eggs were the most important source of human illness, followed by meat (including pork) and poultry-meat and dairy products. The analysis of human cases caused by different serovars showed that eggs were the most important source of human disease caused by *S*. Enteritidis, and that meat products, particularly pork and beef, were important sources of *S*. Typhimurium infections. Source attribution estimates revealed regional differences in the relative importance of sources of salmonellosis. These differences are in line with differences in the epidemiology of *Salmonella* in different countries, such as the identified clusters of *S.* Enteritidis in MS3 and Slovakia and high prevalence of *Salmonella* in broilers and pigs in

Southern countries. Additionally, regional differences in the source attribution estimates can reflect differences in the effectiveness of the surveillance systems and completeness of the available data. Other source attribution studies have shown differences between countries in the relative importance of sources for human salmonellosis (Pires et al., 2008). Specifically, table-eggs were estimated to be the most important source of *Salmonella* infections in the Netherlands and Denmark followed by pork.

The results of the analysis of outbreak data were found useful to investigate the relative importance of food sources for human salmonellosis and to a wide extent support other results. It is, however, acknowledged that extrapolation from outbreak data to the population level involves making certain assumptions that may bias the results and that the unharmonised outbreak reporting in EU in 2005 and 2006 added to the uncertainty of the results. A more elaborate discussion on the limitations of the method can be found in Pires (2009).

It should be emphasised that the Consortium originally intended to develop a hierarchical source attribution model based on microbial subtyping (Hald et al., 2004; Pires & Hald, 2009) using MS-specific animal and food data from the EU baseline surveys and human data as reported by the MS to The European Surveillance System (TESSy). It was, however, necessary to abandon this approach, since MS-specific data on the distribution of serovar and phage types in humans was not available. As an alternative, the Consortium made some descriptive comparisons of animal, food and human data, which were supplemented with results from a spatial analysis and an outbreak data analyses. The conclusion below should, therefore, be considered as a guesstimate and is based on very simple deductions:

Human *S. Typhimurium* infections represented between ca. 10-20% of all cases, and this proportion seems in fact to be increasing (relatively and absolutely). Based on the comparison of phage types occurring in humans and animals sources, it is assessed that the majority of human S. Typhimurium cases are caused by pig-related phage types leading to the conclusion that the majority of human S. Typhimurium infections overall is coming from the pig reservoir. Certainly broilers and beef also contribute to these infections, but the contribution is in general assessed to be low due to low prevalences and/or lower impact through the food production chain. The latter is derived from the fact that some of the dominant S. Typhimurium phage types in broilers only occur in low frequencies in humans. Still, as illustrated by the spatial analysis there are geographical variations, where S. Typhimurium appears to be more prevalent in pigs in Western Europe and in broilers in Eastern Europe suggesting that broilers contribute relatively more in the latter region.

S. Enteritidis is recognised to be associated primarily with the poultry reservoir and particular laying hens and table eggs. Still, in Eastern Europe a small proportion of these infections may also come from the pig reservoir, as the prevalence of S. Enteritidis in pigs in this region generally is higher. This is also supported by the spatial analysis indicating a common cluster for S. Enteritidis in pigs and laying hens in the eastern part of Europe, This may add a few percentages to the overall pig-associated burden.

S. Derby is another very important serovar in pigs and most human infections of this type is assessed to originate from the pig reservoir. Although, it is also occurring in turkeys, the much lower consumption and production of turkey meat point at pork. In addition, it can be seen that some of the turkey-specific serovars (e.g. Saintpaul, Bredeney and Kottbus) have hardly any impact in humans. Of course this may be due to for instance lower infectivity of

these serovars as compared to S. Tm., but without the detailed human data, it was not possible to estimate these differences.

Finally, the interpretation of the sources of human S. Infantis infections tended to be more complex, given its widespread occurrence including in animal feed. However, a certain proportion of S. Infantis infections and minor proportions of other serovars will most likely also be associated with pigs.

In conclusion, it is "guessed" that 10-20% of human infections in EU is attributed to the pig reservoir.This is to some extend supported by the outbreak data analysis that indicated that meat products, particularly pork and beef, were important sources of S. Typhimurium infections. This is furthermore in concordance with a recent attribution study done by Pires et al. (2008) and Pires (2009), where the proportion of pork-associated cases acquired domestically was estimated for four EU countries: Denmark (3.6-9.7), The Netherlands (7.6-15.2%), Sweden (0.1-0.3%) and UK (3.4-3.7%).

14.6 Conclusions

- The relative importance of different sources varies between EU regions according to differences in prevalences, consumption patterns and preferences, and animal and food production systems.

- The overall EU incidence of human salmonellosis has been decreasing from 2005 to 2008, which is mainly explained by a decrease in the number of *S*. Enteritidis infections presumably as a result of an improved surveillance and control of *S*. Enteritidis in laying hens in many MSs (EFSA 2010; Korsgaard *et al*. 2009). In contrast, the incidence of *S*. Typhimurium infections has increased from 2006 to 2008 indicating that one or more sources of these infections are increasing in importance.

- Besides the decreasing trend of *S*. Enteritidis cases, eggs from laying hens are still considered the most important source of *S*. Enteritidis infections and consequently the most important single source of human salmonellosis in EU. This is supported by the source attribution analysis based on outbreak data, where table eggs were found as the most important source. A certain proportion of human *S*. Enteritidis infections are also assessed to be attributable to broilers, particularly in countries with a high *S*. Enteritidis prevalence in broiler flocks.

- *S*. Typhimurium showed different clusters for pigs and broilers suggesting that pigs is a main source of these infections in Western Europe (MS cluster 2), whereas in Eastern Europe (MS cluster 3 and 4), the disease burden may be more evenly shared between broilers and pigs.

- *S*. Infantis tended to cluster in countries with intensive pig production in the North-east corner of MS cluster 2, whereas in Eastern Europe (MS cluster 3 and 4) the focus was on broilers, but the sources' relative contribution to human infections was difficult to assess.

- Although *S*. Derby was mainly associated with pigs in Western Europe (MS cluster 2), it was also present in poultry, particularly turkeys. However, compared to the other animal

species, turkeys are in general not assessed to be a major source of human infections, presumably due to its lower consumption and maybe more varied consumption patterns across Europe.

- *S*. Hadar was associated to poultry meat consumption, with particularly high prevalences among turkeys in a few countries. Also *S*. Virchow had a very widespread distribution in broilers in Europe, and its increasing importance among human cases since 2005 is likely to be due to increasing broiler meat consumption.

- Based on the above discussion of the relative importance of different animal sources, a cautious assessment would be that around 10-20% of human infections in EU may be attributable to pigs and pork. However, this "guesstimate" is believed to vary considerably between MSs depending on for instance *Salmonella* prevalence in pigs and pork, consumption patterns and preferences, and the relative importance of other sources.

- In order to obtain more reliable and quantitative estimates for the importance of different source to human salmonellosis in EU, it is recommended to develop a model for the attribution of human salmonellosis based on the microbial subtyping approach. This will require MS-specific data on the distribution of *Salmonella* subtypes in the most important sources and in humans. Particular, the latter data has been very difficult to obtain, which is considered most unfortunate as these data are essential for understanding the trends and sources of human salmonellosis.

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15 Discussion

15.1 Modelling the EU

The real challenge of this QMRA has been the requirement of developing a model for the whole of EU. The variability between MSs in all areas of pig production, slaughter, processing, preparation and consumer preferences is vast and hence a single model for the EU was deemed not to be feasible at an early stage of the QMRA. We have tried to overcome this by producing a generic EU model, where the processes are fixed but the parameterisation is MS specific. In terms of processes the QMRA includes models for large farms; small farms; large slaughterhouses; small slaughterhouses and 3 product types (pork cuts, minced meat and fermented sausage). Indeed within the Farm modules further variability is accounted for by the consideration of key management factors, e.g. all-in-all-out vs. continuous production; wet feed vs. dry feed etc. These sub-models are linked together with MS information on ratios of large-to-small farms and large-to-small slaughterhouses. Unfortunately, due to lack of data, it was necessary to assume that all pigs from large farms go to large slaughterhouses and all pigs from small farms go to small slaughterhouses. However, if such data become available the model can be easily the amended to take this into account. Of course, compared to reality, the categorisation of the pig production into small and large farms/slaughterhouses is coarse and within these there will be significant variation, both within and between MSs. However, within the resources available, we believe that a large proportion of the variability between production types has been captured; risk assessment is an iterative process and can always be further improved upon – when better or new data becomes available. The advantage of producing a generic model that can be parameterised for any MS is that, it is hoped, each MS will have the opportunity to use the model to assist with the development of its own national control plan.

The generic EU QMRA is a fully stochastic farm-to-consumption Monte-Carlo simulation model, and includes several novel developments for *Salmonella* in pigs QMRAs and EU QMRA methodology. Specifically, this includes detailed modelling of the pig and pig slaughterhouse environments (farm management, faecal shedding and cross-contamination at the slaughterhouse). In order to demonstrate the QMRA and, in particular, to identify key differences between MSs which might be important for intervention at a national level, four case study MSs were selected. Rather than simply choose a MS from each geographical region within the EU or MSs with the best data, a cluster analysis was undertaken. The aim of the cluster analysis was to objectively identify case study MSs that would be likely to have differing impacts for different interventions. Although, many attributes were originally suggested for the clustering criteria, due to data gaps/deficiencies the EU MSs were clustered according to production practices (ratio of large-to-small farms; ratio of large-to-small slaughterhouses) and consumption practices (amount of pork consumed; relative amount of fermented sausage consumed). *Salmonella* prevalence was not included as a factor within the analysis as this would be an outcome of the QMRA. Therefore the clusters do not reflect similarities in *Salmonella* prevalence but similarities in production and consumption practices. The MSs within each cluster were then selected based on data availability. The aim of the QMRA is for it to be applicable to any MS and therefore discussions are currently underway between EFSA and the QMRA team on how best to provide other MSs access to the model.

15.2 Validation of the Results: Further Discussion

The baseline results indicate that, as expected, the probability of illness and number of cases varies between products and between MSs. In particular, the probability of illness (per serving) is highest for fermented sausage for all MSs. However, when taking into account consumption practices, the products with the highest number of attributable cases are predicted to be pork cuts (MS1, MS3, MS4) and minced meat (MS2). Overall, it is predicted that, from the 3 product types modelled there will be 949 cases of *Salmonella* in MS1; 25248 cases in MS2; 1509 in MS3 and 2686 in MS4.

The QMRA model has been validated at three important stages of the food chain: prevalence of infection in slaughter pigs; prevalence and concentration of contamination at retail, and the number of human cases per year attributable to pig meat consumption. Validation of any QMRA is always challenging particularly since there are so many uncertainties present within the model; consequently QMRAs will often over-estimate the overall risk (e.g. Hartnett, 2001; Nauta *et al.,* 2001, 2005; Havelaar *et al.,* 2008). In addition, the observed data to which the outputs of the model are compared are not accurate. For example, as discussed in Chapter 12, sampling at the slaughterhouse or at retail may not be totally randomised (or may even be risk-based), the microbiological test may not be 100% sensitive and the reported number of human cases may be significantly under-reported (which will vary between MSs). However, we have concluded that the results of the QMRA look fairly reasonable at the points of post-lairage (compared to the EFSA Baseline Survey (EFSA, 2008a)) and at retail (compared to data from EFSA 2009, Little *et al.,* 2008). At the point of post-lairage, the model appears to give relatively accurate predictions for the prevalence of lymph-node infection in three of the four case study MSs, which suggests that a large proportion of the variation between MSs in farm practices/*Salmonella* prevalence is captured. However, the model underestimates the prevalence of infection for MS3 as the model predicts that 0.7% of pigs will be lymph-node positive and the EFSA baseline study states that an average of 5.1% [3.7 – 6.9]. As suggested in Chapter 8, it is likely that this discrepancy is due to the model not capturing a specific farm management aspect of MS3; this is likely within the small farm as this is highly uncertain and MS3 has a much larger proportion of small farms than the other three MSs.

Although, at the point of retail, it is only possible to compare the MS1 and MS2 results against MS-specific data, the results for MS3 and MS4 are not unreasonable when compared to the other MSs that reported the results of retail sampling to EFSA. Certainly, the model appears to predict values for prevalence and concentrations that are similar to those observed in studies from across the EU.

Validating the number of cases estimated by the model is complex and must take into account a number of uncertainties associated with both the model predictions and also the reported number of cases, especially due to under-reporting of human cases which is discussed in detail within Chapter 3 (Section 3.3.2). For all four MSs the model does seem to be over-estimating the number of cases, particularly as the number of reported cases for each MS will be attributable to all sources of *Salmonella*, not just those related to pig/pork-meat or, indeed, not just the 3 product types considered here. The reasons for this are hard to determine given the complexity of the system being modelled, and the lack of data that would enable us to determine the effect of immunity and age- and food- related dose-response.

As mentioned above, the number of reported cases for each MS will be attributable to all sources of *Salmonella*, not just those related to pig/pork-meat. Therefore, as part of this project (Chapter 14), we investigated the main animal-food sources of human salmonellosis in EU and assessed these to be table eggs, pork and broiler meat. However the relative importance of different sources varies between EU regions according to differences in prevalences, consumption patterns and preferences, and animal and food production systems. Overall the results tended to confirm the current knowledge on sources (Hald *et al.,* 2007; Pires *et al*, 2008; EFSA, 2008b; Pires, 2009), and it also concurred with the results of the source attribution study based on outbreak data, which renders credibility to both approaches. We could have done a more robust analysis if more detailed information on the human data as well as data over time had been available. In relation to pork, the source attribution work suggested that overall 10-20% of all *Salmonella* infections within the EU may be attributable to pork; however this estimate is highly uncertain and will vary between countries depending e.g. on prevalences and consumption patterns. Unfortunately, quantitative estimates for the relative importance of each source for human disease could not be provided, because human data was only available at an aggregated level and only for the top-ten serovars found each year. However, for MS2, it was recently estimated that 3.4-3.7% of cases were attributable to pork (Pires *et al.,* 2008) and in Denmark between 5-11% of cases have been estimated to be related to the consumption of pork during the past years (Pires & Hald, 2009). Therefore, no matter how well controls for *Salmonella* in pig meat work in reducing pig-meat attributable *Salmonella* cases, there is only likely to be a small effect in reducing the *total* burden of *Salmonella* illness in the EU.

Within the mandate, EFSA were asked "to consider all serovars in pigs that are of human health significance". EFSA, 2006 concluded that "all *Salmonella* serovars in pork are to be regarded as a hazard for public health" and recognised that there will be variability between strains in their behaviours across the food chain. It was therefore deemed acceptable by EFSA (as stated in the call for proposals) for the QMRA to consider all types similarly and hence that a QMRA for *Salmonella* Spp. would be appropriate. However, this assumption will lead to an over-estimation of the risk, which is now discussed. The *Salmonella* serovar and phage type distribution in animals, foods and humans in European countries was analysed within Chapter 14 on the basis of data from two different data sources, the Community Summary Reports, which publish data reported from individual countries, and the EU-wide baseline surveys conducted for the major animal reservoirs. Within the EU, the most important serovars in humans were *S*. Enteritidis, *S*. Typhimurium and *S*. Infantis representing up to 81% of all infections. The main observed serovars varied between the animal reservoirs, but the frequent occurrence and wide geographic distribution of *S*. Enteritidis, *S*. Typhimurium and *S*. Infantis was clear throughout the analyses. The most frequent serovar isolated from slaughter pigs (lymph-nodes and carcass swabs) was *S*. Typhimurium and *S*. Derby. These serovars were widely distributed in EU occurring in most MSs. The prevalence of S. Enteritidis in most MSs was low, although S. Enteritidis appeared to be more prevalent in Eastern European countries, whereas S. Infantis more prevalent in Northern Europe. Other serovars, however, also appeared as important for specific animal sources, such as *S*. Dublin in cattle, *S*. Hadar in broilers and *S*. Saintpaul, *S*. Kottbus and *S*. Bredeney in turkeys. This therefore highlights the difficulties of producing *Salmonella*-specific QMRAs. To develop serovar specific QMRAs would be highly challenging due to the number of anticipated data gaps (there are still data gaps when considering *all Salmonella*) and time available. It is therefore assumed within the QMRA that all *Salmonella* are equal in terms of, for example, their survival in the environment and infectiousness to

pigs and humans. However it is known that this assumption is not really valid as, f*or* example, many serovars are commonly seen in pigs but rarely observed in human infections (e.g. *S.* Derb*y*) whilst others are commonly detected in both pig populations and in human cases of salmonellosis (e.g. *S.* Typhimurium). This could be attributable to many factors, including differences in their survival during the slaughter and processing environment or the dose-response relationship.

15.3 Uncertainty Analysis: Identification of Data Gaps and Assumptions

The model parameters, for which there is a high degree of uncertainty, were investigated in the uncertainty analysis (Section 12.3). Many were assessed to have an important impact on the model results; hence it is recommended that future research in the area of *Salmonella* in pigs focuses on these parameters if the aim is to reduce the amount of uncertainty associated with the output of the QMRA. In addition to uncertainty, the probability of illness will also be highly influenced by the variability described within the model. The variability is incorporated into the model using probability distributions and includes variability both at the individual animal/product level and variability between farms/slaughterhouses. The consideration of variability is important because human cases of illness will most likely occur when in the tails of the distribution, i.e. the relatively rare occasions when the number of *Salmonella* in a pork product is high and so the probability of illness is also high (from the dose-response model). Although not possible to perform a full sensitivity analysis (i.e. assessing the impact of the distribution of each variable parameter on the final probability of illness), it was carried out at the end-point of each module. In turn, each of the exposure assessment modules (Farm, Transport & Lairage, Slaughter & Processing, Preparation & Consumption) and the hazard characterisation are now discussed with particular focus on the uncertainties present within each module.

15.3.1 Farm

The farm model is a stochastic SIR model, modified to specifically incorporate faecal-oral, feed and external routes of *Salmonella* transmission. These modifications allow us to differentiate between sources of infection, but also allow the description of varying farm types between EU MSs. The results from the baseline model appear to capture the variability in the dynamics of infection, and much of the variability between MSs has been captured, given that three of the four MSs were well-validated at the point of national prevalence of lymph-node positive pigs at slaughter. We estimate that MS breeding pig herd prevalence is a strong predictor of national slaughter pig prevalence, which is validated to some degree by the comparison of the two baseline survey results.

The farm model is necessarily complex in order to capture the wide variation in transmission and management practices across the EU. However, despite this complexity a number of simplifying assumptions have been made. Of importance is the homogenous mixing of faeces and *Salmonella*, but also the generic dose-response model used. Perhaps of greatest importance is the simplification of farm categorisation used within the model. Despite incorporating 56 different farm types into the model, this categorisation is still an over-simplification of reality, especially for the small farm.

The above assumptions were primarily made to reduce model complexity, but also because of data gaps in transmission dynamics and management practices. A crucial data gap identified from the farm analyses is the probability of feed contamination. In addition, while not explicitly included within the uncertainty analyses, more detail is required on management practices and dose-response.

Given these inherent model and data uncertainties, care must be taken when interpreting the farm model results, especially when assessing the effect of interventions (see below), but we consider the current model as a large first step towards capturing previously unaddressed differences in farm type, and a useful tool for assessing the effectiveness of hypothetical and specific interventions at a national level (although the validity of such a model to make recommendations to an individual farmer has not been tested).

15.3.2 Transport & Lairage

The Transport & Lairage module was developed to incorporate factors that are thought to influence the prevalence of *Salmonella* in slaughter-age pigs, including stress during transport, contamination of the environment and cleaning of the environment. These factors were included with the aim of assessing the effect of various interventions implemented at the transport and lairage stage.

The results from this stage show that the prevalence does increase, both during transport and lairage. The average batch prevalence for each of the four member states compares favourably with the findings of the EFSA slaughter pig baseline survey (EFSA, 2008a), albeit with a few deviations (particularly the lower prevalence for MS3 predicted by the model) suggesting, as would be expected, that the (Farm and Transport & Lairage) models do not capture all the factors associated with *Salmonella* transmission and prevalence. Part of the reason for this may be data gaps associated with some of the parameters. Sometimes this is a lack of adequate quantitative data across all member states (such as estimating the skin contamination at the start of the slaughter line and the effect of stress). In other cases we have good data for some member states and not others (e.g. the effect of cleaning of lairage, proportion of pigs kept overnight in lairage) so it was necessary to estimate the value based on data from another member state. However, it has been shown that the within batch prevalence before transport (i.e. the farm model output) is more influential than any of the parameter distributions within the Transport & Lairage module.

There is little quantitative data on stress so expert opinion had to be used to estimate the proportion of pigs during transport that become stressed. The uncertainty analysis showed that the probability of pigs being stressed during transport has a significant impact on the probability of illness for pork cuts, minced meat and fermented sausages. It was concluded that this is the most important data gap in the Transport & Lairage module. This parameter was also identified in the sensitivity analysis as the variation associated with this parameter having an important impact on the variation associated with the lymph-node prevalence at the end of transport.

15.3.3 Slaughter & Processing

The slaughterhouse module is described in Chapter 9. It is clear that significant data gaps remain. Firstly, there are quite some uncertain parameter values, for which additional

measurements or laboratory experiments would be needed in order to obtain more accurate numerical values. Some parameter estimates would actually be very simple to measure, for example time and temperature data from large slaughterhouses, often recorded by automated systems. However, unfortunately, such data is not usually available to researchers. Secondly, there is a lack of validation data. By this we mean, data to which we can compare the outputs of the model, e.g. *Salmonella* counts on carcasses before and after a process step, or *Salmonella* numbers on machinery throughout the day. Thirdly, the production process itself is sometimes not known explicitly. A case in point is the small slaughterhouse, where the model was based on the slaughter process as observed in a single Dutch slaughterhouse. Slaughter practices in other MSs (and other slaughterhouses) remain unknown to us; this is called model uncertainty and although not possible to be included within the uncertainty analysis *per se* needs to be highlighted as a data gap.

Another form of model uncertainty is the inclusion of certain stages in the slaughterhouse, which is often based on arguments presented in previous QMRAs. This is potentially dangerous, since it may lead to certain stages being overlooked. Consider for example the possibility of contamination of the lungs of the pigs with scalding water, which may be hazardous at the pluck removal stage leading to carcass contamination (Hald *et al.,* 2004). Or, another example could be cross-contamination when using gas stunning of batches of pigs, which is not usually modelled. This may be the result of a lack of data, or perhaps it is simply ignored because it is hardly ever considered at all in previous studies (perhaps due to lack of data!).

With respect to the baseline slaughterhouse results, we find that the results do not look unreasonable and, in particular, when comparing the profile of microbial loads over the different slaughter stages (see Section 9.4). Currently, our model suggests that house flora has very little effect on the final risks of illness. However, it does impact the prevalences to a large degree, due to the additional contamination (in low amounts) on many carcasses.

After the slaughterhouse module follows the cutting plant module. Here, the half-carcass is processed and partitioned into consumer cuts. The process of cutting a half carcass into consumer portions at the cutting plant is not standardised at all. Furthermore there is considerable confusion of terminology, to the point that encyclopedia and dictionary lookup of meat products would yield incompatible results. Our model describes a possible implementation of the cutting plant process, but by no means exhaustive with regards to variation over MS. Although the model takes into account cross-contamination via the cutting equipment, it does not take into account any contamination between carcasses (via e.g. the table surface or improperly cleaned knives). This is an opportunity for further research. Unfortunately, no suitable data were found for validation of the cutting plant model.

The uncertainty analysis identified that the amount of faeces released during dehairing in the large slaughterhouse (A_k) to be an important parameter. This suggests that the prevention of faecal leakage, for example by means of bunging, would be an effective intervention. This was further considered in section 13.2.3.

15.3.4 Preparation & Consumption

The consumer model consists of three parallel pathways: pork cuts, minced meat and fermented sausage. These products are chosen as a proxy for a wide range of products.

e.g. the category 'pork cuts' represents all meat that is cut by the consumer, and the fermented sausage would cover every conceivable dry cured sausage in production. The three pork products were selected (in a sense) to cover the spectrum of risks. Specifically: pork cuts represent all cut pork associated with the risk of cross-contamination, minced meat represents hamburgers, meatballs and other pork patties, with the associated risk of undercooking (and cross-contamination), while the fermented sausages collectively represent the risk of a ready-to-eat product.

Also in the consumer model we face data gaps. Specifically, data on transfer coefficients are severely lacking, for example between pork product and chopping board. Further laboratory experiments on bacterial transfer would be extremely beneficial for further QMRAs, perhaps even allowing for the use of distributions instead of point values. Consumer behaviour in the domestic kitchen is reasonably well known from surveys.

The most useful data on time / temperature combinations during various transport and storage phases was taken from one French study (Derens *et al.* 2006). In this study a small time / temperature recorder was embedded in packs of retail pork, yielding large quantities of realistic data. Replication of such an experiment in additional MS would be of great value for future QMRA work, especially because the storage time of minced meat is influential on the probability of illness in both MSs considered in the uncertainty analysis and also for pork cuts in MS2.

An unanticipated result of the fermented sausage model is that outbreaks do not seem that relevant as compared to sporadic cases. Our model, based on the model of Hwang *et al.* 2008, predicts more sporadic cases from successful[38] fermentation than reported cases from known outbreaks, which we assume to be the result of failed fermentation.

The uncertainty analysis identified that portion sizes (of all products) had an important impact on the probability of illness. EFSA are currently carrying out further research in this area of consumption data. Should these data, when published, be more relevant then they can be used to better parameterise a MS's QMRA model.

Finally, we would like to point out that it is hard to intervene at the consumer stage (although one can think of government information campaigns, but the efficiency is debatable). Nonetheless, a detailed model is certainly useful for gaining insight into the hazards at the consumer phase.

[38] Here, successful means that there was no obvious shortcoming in the process. A high load of *Salmonella* is purely by chance. In contrast, when we speak of 'failed' fermentation, there was a clear defect during the fermentation process.

15.3.5 Hazard characterisation (dose-response)

The QMRA uses a dose-response model to predict the outcome of exposure to *Salmonella* on pork products. For any dose-response model there is always a high degree of uncertainty due to the availability of data. In general, two types of data are available for the construction of a dose-response model: feeding trial data and outbreak data. Feeding trial datasets (e.g. McCullough & Eisele 1952; Bemrah *et al.* 2003) have the benefit of recording accurately the dose each participant in the trial would have received; however on the downside they often use high doses to initiate infection, consider only one combination of food matrix and organism and, lastly, typically enrol healthy, young male volunteers and are therefore not representative of the overall population. Dose-response models have also been developed using data from outbreaks. These datasets have the advantage of being a more realistic reflection of reality, for example the exposed population will differ in their susceptibility; however in outbreak situations it is difficult to assess who has actually been exposed and, if so, what dose they received. The QMRA applies the FAO/WHO *Salmonella* dose-response model (FAO/WHO, 2002). This is based on a total of 21 *Salmonella* outbreaks of which 13 were *S*. Enteritidis; 3 were *S*. Typhimurium and there was 1 outbreak each for *S.* Heidelberg*, S.* Cubana*, S.* Infantis*, S.* Newport and *S.* Oranienburg (Table 11.1). It also covers a large range of food stuffs, some meat (e.g. chicken, hamburger) and some non-meat (e.g. water, peanut sauce). None of the outbreaks were related to pork/pork products. Bollaerts *et al.* (2008) comment that the FAO/WHO model, which is modelled using a Beta-Poisson, does not take into account heterogeneity due to the fact that the Beta-Poisson reflects the biological process of infection; not illness. The authors therefore developed a dose-illness model using generalised linear mixed models and fractional polynomials of dose which allows for heterogeniety due to differences in host susceptibility and the serovar and food matrix (in combination). In this QMRA, the heterogenoety between host, pathogen and food is not considered and we therefore apply the Beta-Binomial to the outbreak data summarised in Table 11.1. The Beta-Binomial incorporates the variability in the pathogen-host given a certain, variable dose, rather than the average dose considered in the standard Beta-Poisson model (FAO/WHO, 2002). Considering the above discussion, it can be summarised that there is a high degree of uncertainty associated with the data underlying the dose-response model which cannot be quantified. In addition there is also model uncertainty, i.e. which model is the most appropriate for describing the dose-illness relationship. Considering this, for any QMRA it is important to place more emphasis on the *relative* risks (e.g. the intervention analysis) than the *absolute* risk (Havelaar *et al.,* 2007).

15.4 Intervention Analysis

We have implemented a number of hypothetical and specific interventions in order to investigate the effect of reducing slaughter pig prevalence, breeding pig herd prevalence and finally carcass contamination on the number of human cases per year within the case study MSs attributable to each of the three product types.

In order to implement any of the interventions we have assumed two critical factors: that uptake of each intervention is 100% across all farms/slaughterhouses across the MS, and that each intervention would be implemented in such a way to produce the effect desired

(e.g. reducing carcass contamination by 1 log, or raising the dose needed to cause a particular probability of infection). Qualitative evidence (VLA, 2009) and expert opinion suggest that uptake and efficient application would be nowhere near 100% in reality. We would therefore strongly advise that field studies are carried out to assess these two factors.

The hypothetical interventions investigated are illuminating. Interventions that reduce prevalence and/or contamination at each major point in the food chain investigated (breeding pig herds, slaughter pigs and pre-chill) will have a large impact in reducing the number of human illnesses attributable to pig meat consumption - if the reductions are also large. This is a positive result as it does suggest that control will be effective across the whole of the food chain.

We were not able to confidently model specific farm or transport interventions because of a lack of evidence showing consistent quantitatively-estimated reductions. We have therefore investigated hypothetical changes in the mechanisms of these interventions (e.g. vaccination, cleaning). The results show that large changes in these mechanisms are necessary before significant reductions can be made (e.g. the dose-response of each pig would have to be raised by 1 log). Whether these large changes can be achieved consistently across a whole MS is debatable, but certainly there is little current evidence to suggest that these farm interventions can achieve marked reductions on their own. The broad conclusion must be that a sustained program of farm interventions would be needed to be effective on the wide range of farm types in the EU.

Results from the intervention analysis highlighted that interventions modelled during the Transport & Lairage phase had little effect on the risk of illness, e.g. logistic slaughter and cleaning of lairage. While it is clear that the conditions in lairage can have a significant effect on the prevalence of skin contamination at the start of the slaughter line, the intervention analysis suggests that this is of lesser importance than the effect that the various farm or slaughter processes have.

Marked reductions can be achieved by applying some decontamination measure, or reducing faecal leakage, at the slaughterhouse. An intervention that could consistently achieve a 1 log decontamination of carcasses pre-chill could reduce human illness by up to 90%. Non-chemical interventions have already been shown to produce reductions in the order of 1-2 logs, and hence could be a viable short-term measure for reducing illness in humans if they are shown to be as effective if scaled up to be applied across a MS's slaughterhouses (given intervention at farm level are likely to take years before real reductions are achieved).

In summary, the farm and transport interventions are likely to vary in their ability to reduce slaughter pig prevalence by a sufficient amount to reduce the number of salmonellosis cases in humans. However, a combination of interventions applied across a large proportion of farms, probably combining changing feed type, is likely to have a cumulative effect in reducing slaughter pig prevalence. Probably of extreme importance, but not investigated here, is the rate of uptake and correct application of interventions by farmers – if this is not universal across a MS the effect in reducing human illness will be proportionally reduced. The model results lead us to suggest those MSs with a high breeding pig herd prevalence should focus on these herds in order to reduce the burden of infected new stock entering the weaning/growing/finishing stages, although of course that doesn't mean taking efforts to control *Salmonella* post-weaning won't also be beneficial. However, it may be

more efficient in MSs with a low breeding pig herd prevalence to focus their attentions on feed and other sources of infection.

In all likelihood *Salmonella* control in pig production will be implemented at various stages. We have investigated three combinations of interventions, using either wet feed, increasing resistance or downtime along with a decontamination measure at pre-chill. Certain combinations of interventions are likely to produce even greater reductions than the effects of individually-applied interventions. However, the specific combination of interventions that achieve greater reductions together are dependent on the situation within a particular MS, in particular the contamination levels of carcasses. Investigation of such beneficial combinations can be done with the current QMRA model; the myriad combinations possible prevented us from investigating all of these, but MSs will be able to interrogate potential combinations of interventions if/when the baseline model has been parameterised for their country.

From the current evidence, it would appear that specific slaughterhouse interventions are currently best placed to produce consistently large reductions in the number of human cases. For high breeding prevalence MSs, reducing infection in breeders would seem to be an important control measure as has been successfully implemented by the poultry industry. However, multiple intervention investigations suggest that MSs can achieve more effective reductions in human cases by targeting both farm and slaughterhouse. Such information will be valuable to the EU Cost-Benefit Analysis project currently underway, where with the results of the intervention analyses they will be able to assess the cost-effectiveness of different intervention options.

15.5 Comparisons to other Member State QMRAs

It is worthwhile comparing the QMRA described here to other work in the area of *Salmonella* in pig QMRA. In particular, we compare the results of this model to a qualitative risk assessment (De Sadeleer *et al.,* 2009) and QMRAs developed by Delhalle *et al.* 2009 (Belgium); Titus 2007 (New Zealand); VLA 2009 (MS2) and Barron *et al.* 2009 (Ireland).

A qualitative Belgian risk assessment (De Sadeleer *et al.,* 2009) concluded that the risk of *Salmonella* infection in humans in Belgium was low, but that it could be reduced further by implementing additional measures in the slaughterhouse and the domestic kitchen. The current model also highlights the effectiveness of slaughterhouse control measures. Interventions in the domestic kitchen were out of the remit of the current project, so were not considered.

In addition to the above, a modular risk model to assess the risk of salmonellosis through the consumption of mixed pork meat has been developed in Belgium (Delhalle *et al.* 2009). Within this model the exposure assessment starts at the end of the lairage and finishes at the point of human consumption. Similar to the current model (post-lairage) this model comprised modules for the slaughterhouse, Post-Harvest, Distribution & Storage and Preparation & Consumption, with each module generating an output that was the input to the next module. Some results from the Belgian risk assessment are shown in Figure 15.1 and Figure 15.2. At the end of the slaughterhouse (i.e. at chilling) the Belgian model estimated the prevalence of

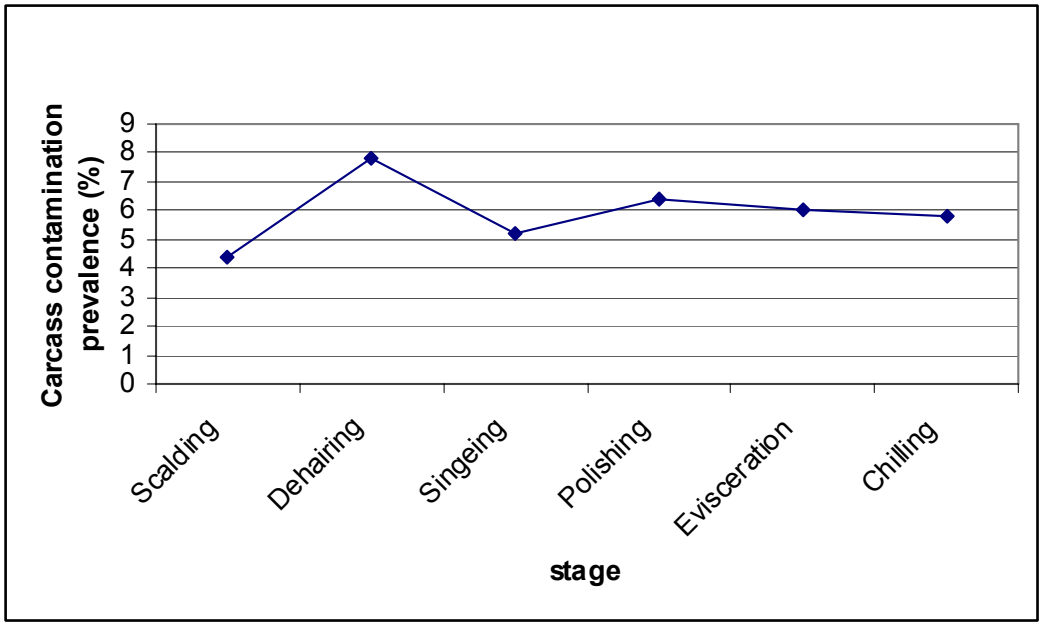

Figure 15.1: Average prevalence of carcass contamination at different stages of the Slaughterhouse from a Belgian risk assessment (Delhalle *et al.,* 2009)

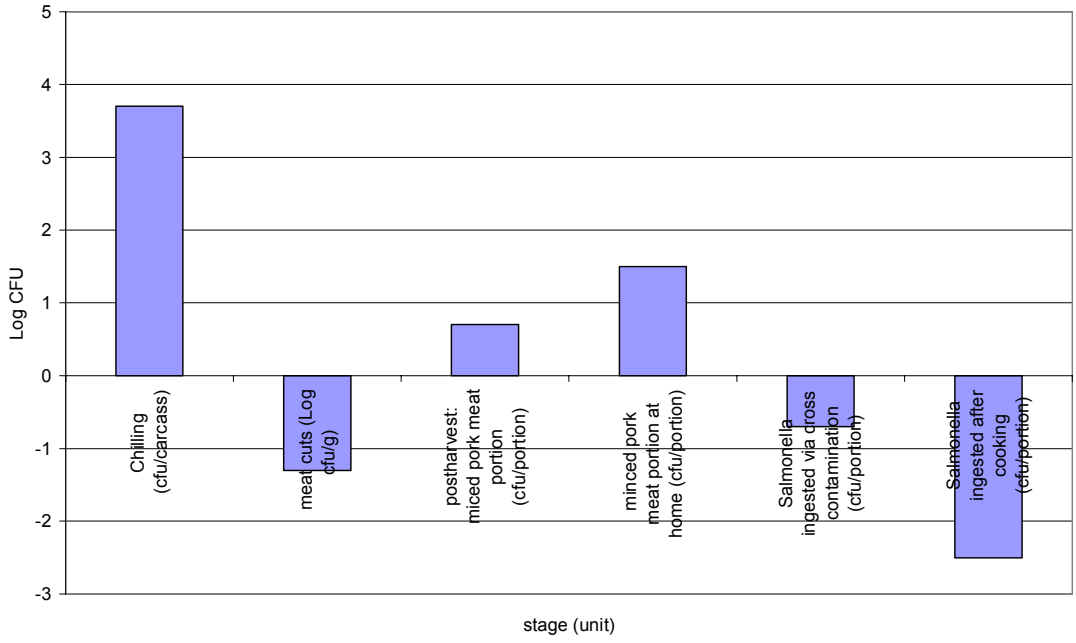

Figure 15.2: Average concentration of *Salmonella* (Log CFU/unit) at different stages of a Belgian risk assessment (Delhalle *et al.,* 2009)

contaminated carcasses to be 5.8% with an average of 3.7 log cfu/carcass. The number of *Salmonella* decreases to 0.7 cfu/portion of minced meat after processing rising to 1.5 log

cfu/portion at the home, comparable to the current model estimates of 0.5 log cfu/portion after processing rising to 1.05 log cfu/portion at home.

Similar to the results of the intervention analysis provided in Chapter 13, the Belgian QMRA also found that the risk of illness could be significantly reduced all along the pork meat production chain (by the implementation of hypothetical reductions such as reducing the prevalence of *Salmonella* in pigs at lairage by 25%, 50% and 75%, which is similar to the farm intervention in the current model where we reduce the within batch prevalence from 10-99%.). They also found the risk could be reduced by consumers however, as also mentioned above, consumer interventions were not in the remit of the current project.

We can also discuss the similarities and differences of our QMRA compared to a model described by Titus 2007. The Titus QMRA is also based on the MPRM paradigm, taking into account numbers of *Salmonella* on individual pigs. The main focus of the QMRA is the pig slaughterhouse where it was concluded that many carcasses are contaminated with low numbers of *Salmonella* and only a small number are heavily contaminated; this is consistent with our findings. Looking more precisely at the various stages (Figure 3.4 in Titus 2007) we find a significant decrease at scalding (about 3 logs) and a modest increase at dehairing. This is also observed in our model. In all other phases, the contamination slowly decreases (but note that polishing and blast chilling were not modelled). Again this is compatible with our findings, except at evisceration where we predict an increase and Titus shows a reduction. The intervention 'prevention of faecal leakage' was studied and was found to result in a 10% - 44% reduction in prevalence. This is less than estimated by the EFSA QMRA, where a 90% reduction in the risk of illness/number of cases per year for all MSs. The author concludes with some statements that we also subscribe to: the need for proper inclusion of cross-contamination, the need for mechanistic modelling, and the need for more quantitative data to fill the data gaps.

We can compare the MS2 results of the current model with that of a MS2 *Salmonella* in pigs farm-to-consumption risk assessment developed by the Veterinary Laboratories Agency (VLA, 2009). The VLA model follows a similar model framework to the current model incorporating: Farm, Slaughterhouse, Further Processing, Distribution and Consumption modules. The current model is, in many ways, a more complex model than the VLA model, building on the methods of the VLA model and, due to the European-wide scale of the project, being able to acquire more data for parameterisation of processes that could not be considered in the VLA QMRA (such as cross contamination in the slaughterhouse). The model explicitly considers variability and, similar to here, sensitivity and uncertainty analyses were carried out. It was found that the model was sensitive to the parameters: cooking temperature, duration of infection of pigs on the farm, degree of clustering of *Salmonella* on the product, whether the product is frozen and use of a chopping board. Uncertainty analysis suggested that the human dose-response, the transfer of *Salmonella* from pig meat to hands and the duration of infection of pigs on the farm were important.

Comparison between the two models should be done with care (and certainly only for MS2), particularly as the VLA model looks at the risk associated with pork chops, bacon and sausages (typical MS2 sausages meant for heat treatment before consumption as opposed to the fermented ready-to-eat sausages considered here). Comparing the two farm modules, there are methodological differences between them, for example the VLA model makes the distinction between ‘excretor’ and ‘carrier’ pigs, with excretors always shedding *Salmonella* in their faeces and carriers never (although a pig may switch between the states over time) while the current model does not make this distinction (infected pigs are assumed

to shed intermittently, which is analogous to the excretor and carrier state combined). However, the average prevalence of 'infected' (i.e. excretor + carrier pigs in the VLA model) slaughter-age pigs is similar; the VLA model predicts an average prevalence of 25%, and the EFSA QMRA predicts 18%.

Results from the VLA QMRA at the end of the consumption stage are shown in Table 15.1. Comparing the EFSA QMRA results to the VLA results, we can see that there is a difference in the average risk and number of illnesses predicted per year for MS2, with the VLA risk assessment predicting an average of 557 cases per year while the current model predicts 13,802. However, it should be remembered that this was for different product types (pork chops are only a fraction of all types of pork cuts and likewise sausages could be considered only a fraction of all minced meat).

The VLA risk assessment also looked at the effect of interventions at both the farm and slaughterhouse. It found, similar to the current study, that prevention of faecal leakage at the slaughterhouse was effective at reducing the number of human cases. As part of the project the results of the QMRA intervention analysis were used as part of a cost-effectiveness analysis.

An Irish model (Barron et al. 2009) looked at the transmission of *Salmonella* at the slaughterhouse (modelling process from stunning to jointing). They modelled the different stages using different approaches including; stochastic regression analysis and meta analysis. The model estimated a mean prevalence of *Salmonella* on pork joints at Irish boning halls of 4%, with a 95% confidence interval of (0.3%-12%). This compares favourably with our predicted MS2 retail prevalence of 4% for pork cuts. As MS2 and Ireland are in the same EU cluster and have similar slaughter pig *Salmonella* lymph-node prevalence (16.1% for Ireland compared to 21.2% for MS2 (EFSA, 2009)) the comparison of the Irish results to MS2 is appropriate.

Table 15.1: Average risk of infection from chops, bacon and sausages, associated confidence intervals and percentage of contaminated products (VLA, 2009)

Product	Average risk and 95% confidence interval	% portions contaminated (A_H)	% of contaminated portions resulting in illness	No. of servings consumed per person per year	Average number of illness per year
Pork Chop	3.56×10^{-07} (3.46×10^{-07}, 3.67×10^{-07})	4.19×10^{-06}	8.5	13	220.4
Bacon	1.69×10^{-09} (1.37×10^{-09}, 2.00×10^{-09})	1.05×10^{-07}	1.6	26	2.1
Sausage	2.71×10^{-07} (2.68×10^{-07}, 2.74×10^{-07})	1.25×10^{-05}	2.17	26	334.9
Total					557.4

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16 Conclusions

The QMRA consortium has developed a fully mechanistic farm-to-consumption QMRA to estimate the probability of *Salmonella* illness (and resulting number of cases) from three product types (pork cuts, minced meat and fermented sausage), and to estimate the effect of interventions at the farm and/or slaughterhouse level. The three products were chosen to represent a range of different production practices, and also different consumption patterns within the EU. The model has been designed to be a generic model for the EU; therefore having a clearly defined set of parameters that may vary between countries, the values of which can be easily input for any specific EU MS. The model is stochastic, using Monte Carlo sampling as a means for dealing with variability in the parameters. To demonstrate the QMRA four case-study MSs were selected: MS1, MS2, MS3 & MS4.

As requested by the Terms of Reference, the model covers the whole food chain. In particular it includes the role of piglets as a source of *Salmonella*, cross-infection between batches during transport and lairage, due to carry-over of *Salmonella* within the environment and cross-contamination within the abattoir and during the preparation phase. Finally, a dose-response model allows the outcome of exposure to be assessed, providing an estimate of the probability of illness.

For all four MSs the average probability of illness, across all product types, is between 1 in 100,000 and 1 in 10 million servings. Across all products, MS2 is predicted to have a higher probability of illness than MS1, MS3 and MS4. For all of the MSs, the product with the highest probability of illness per serving is fermented sausage. The lowest risk per serving is associated with pork cuts (MS1, MS2); minced meat (MS3, MS4). The total number of cases attributable to the three product types are 949 (MS1); 25248 (MS2); 1509 (MS3) and 2686 (MS4). In MS1, MS3 and MS4, the highest number of cases was attributable to pork cuts and in MS2 to minced meat products.

Although difficult to validate the outputs from any QMRA, due to the uncertainty associated with the observed data, it is probable that the QMRA is overestimating the number of cases. Reasons for this may be that the observed data is uncertain due to the under-reporting of *Salmonella* in different MSs and also that the proportion of cases attributable to pork is unknown. Investigations undertaken as part of this project concluded that around 10-20% of human infections in EU may be attributable to pigs and pork, but this is a cautious assessment. However, this “guesstimate” is believed to vary considerably between MSs depending on, for instance, *Salmonella* prevalence in pigs and pork, consumption patterns and preferences, pig production systems and the relative importance of other sources. In terms of the QMRA, the over-estimation could be attributable to a number of factors, including the consideration of all *S*. spp within the QMRA, with no account taken for differences between *Salmonella* serovars in their ability to grow/survive in the environment or to infect humans (virulence). Other factors include uncertainty associated with the consumption data, the dose-response model and many other parameters used within the exposure assessment, particularly MS specific parameter estimates (where sometimes, due to lack of data, the parameters for one MS had to be estimated using data from another MS).

Considering this likely over-estimation, it is necessary to address the impact this may have on drawing conclusions about the effect of interventions. Given current uncertainties listed above, and the fact that many QMRAs currently over-estimate human illness attributable to a particular pathogen/host pair, then it is important to place more emphasis on the *relative*

risks than the *absolute* risk. We must also consider how far out from reality the model predictions are. Subjectively, we could say we are perhaps an order of magnitude out (if MS2 results are representative of our case study MSs): in this case it is not unreasonable to think we are at least close enough such that the results of the intervention analysis stand.

The QMRA was also validated at earlier points within the farm-to-consumption pathway and, in particular, post-lairage and at retail. However, similar to the epidemiological data, it cannot be assumed that the observed data are perfect as, for example, tests used to detect *Salmonella* at abattoir or retail will not be 100% sensitive. At post-lairage the output of the QMRA (average proportion of *Salmonella* positive lymph nodes) was compared to the EFSA slaughter pig baseline survey results. From this, it was concluded that the QMRA was producing realistic estimates for MS1, MS2 and MS4 at the start of the Slaughter & Processing module. It is uncertain why the model may be underestimating the prevalence in MS3, but it is likely to be attributable to the model not capturing a specific aspect of MS3 at the farm and particularly within the small farm model as MS3 has a much larger proportion of small farms than the other MSs.

At the point of retail validation data were only available for MS1 and MS2 and these compared reasonably well to the QMRA predictions. Although it was not possible to get data for all product types in each case study MS EFSA data provided ranges of *Salmonella* prevalence across different EU MSs. For pork cuts the prevalence ranged from 0%-6.1%, for minced meat 1.3% - 5.9% and for ready-to-eat minced meat/minced meat products (which includes fermented sausages) of 0%-3.3%. The model predictions are in the same order of magnitude, with the results from all case-studies falling within or slightly below these observed intervals. Across a number of EU MSs, studies show that contamination on retail cuts is comparatively low (scaling up to the unit of a serving commonly less than 10 CFU/portion). The average number of *Salmonella* contaminating the three product types was predicted by the QMRA to range from 1-11CFU/portion for all MS/product-type combinations. It was therefore concluded that the QMRA is producing realistic enough results at the point of retail to differentiate between MSs and provide a baseline from which to conduct an intervention analysis.

A key part of the QMRA was the investigation of interventions. In this respect, EFSA provided a number of scenarios that the QMRA needed to address. Each of these is considered below:

The expected reduction of Salmonella cases in humans (or pig meat at retail) by a reduction (e.g. 5- or 10-fold) of Salmonella prevalence in slaughter pigs (based on bacteriology or serology at slaughter).

Marked reductions in cases can be achieved by reducing slaughter pig prevalence, and indeed for MS2 and MS4 there is a strong linear relationship between slaughter pig lymph-node prevalence and the number of human cases. The major effect of reducing slaughter pig prevalence was to reduce the number of infected pigs with high infection/contamination loads entering the slaughterhouse, hence eventually reducing the number of highly-contaminated servings consumed by consumers.

The linear relationship shows that factors that would be expected to introduce a non-linear relationship into the model, such as cross-contamination at the slaughterhouse, growth during retail storage and dose-response, although accounted for in the model, seem to have

limited importance for the assessed relationship between pig prevalence and human incidence. Data from the EFSA baseline survey support a modest linear relationship at a MS level, at least for infection and carcass contamination at evisceration.

The sources of infection for slaughter pigs at farm level.

We have investigated the relative importance of source of infection by simply turning off each source of infection within each MS model. The results show that for MSs with a higher breeding pig herd prevalence (MS2, MS4) switching breeding pig herd prevalence to zero, hence assuming that the breeding pig herd cannot be re-infected from the finishing herd, removes the vast majority of infections at depopulation of the fattening herds. Conversely, removing feed or external contamination from the model does little to change the national slaughter pig prevalence in MS2 and MS4. The reverse trend is true in MSs with low breeding pig herd prevalence (MS1, MS3) as feed contamination seems to be the most important factor for the national slaughter pig prevalence in these MSs. This strongly indicates that breeding pig herd prevalence is a strong indicator of national slaughter pig prevalence – i.e. if a relatively low number of breeding pig herds are positive, national slaughter pig prevalence will be relatively lower than in MSs with more infected breeding pig herds. Finally, external sources of contamination appear to have a general low impact on the slaughter pig prevalence.

The reduction of the prevalence in slaughter pigs by the most important potential treatments or control measures at farm level

Evidence that specific farm and transport interventions consistently work is sparse. This is presumably due to the more complex environment in which these interventions will have to be applied and the difficulty in standardising experiments to trial interventions. Hence, while the evidence for consistent effects is sparse, some farm interventions may well be effective. This lack of evidence for a consistent and/or quantitative effect meant that specific farm interventions could not be modelled. Therefore, in order to provide some assessment of farm interventions, we have modelled the effect of the varying mechanisms applied to farm interventions (e.g. modifying the dose-response for vaccination, lowering the contamination of pens due to cleaning).

Modifying the pig dose-response relationship to *Salmonella* exposure, perhaps by changing feed type, adding organic acids to feed/water, or vaccination, could have a significant effect in reducing slaughter pig prevalence within a MS, which would subsequently reduce number of cases. However, a large increase in this dose-response relationship – broadly speaking increasing the resistance of ALL of a MS' pigs such that an extra half-log to a log dose is needed to cause the same previous probability of infection – would be needed to see significant change in the MS slaughter pig prevalence. This type of effect has rarely been seen in the literature and it is debatable whether such an effect could be achieved consistently at a national herd level. Cleaning and disinfection appeared to have a minimal effect in reducing slaughter pig prevalence or human illness.

Reducing feed contamination appears to be an effective measure in reducing slaughter pig prevalence and human cases and for large scale producers would translate into a widespread decrease in pig exposure to *Salmonella* from feed. The effect was greater in

MSs with a low prevalence (MS1) of positive breeding pig herds than in MSs with relatively high breeding pig herd prevalence (MS4).

The results of these farm interventions suggest that farm interventions could work, although the significant reductions that would be required to achieve the same effect as slaughterhouse interventions would probably be unlikely for any single farm intervention. Large reductions in slaughter pig prevalence were not seen in the literature for any of the current farm interventions.

The impact of transport, lairage and slaughter processes on contamination of carcasses

Due to the unavailability of data on the contamination of hides, it was not possible to model the cross-contamination of hides during .Transport & Lairage. Therefore the contamination on the skin was estimated at the point of slaughter and used as an input to the Slaughter & Processing module.

Within the Slaughter & Processing module, cross-contamination has been extensively modelled. The QMRA results predict that, for all four MSs, the evisceration step in a large slaughterhouse model greatly increases both the microbial load and also the prevalence of carcass contamination. This increase is due to the possibility of the gut being punctured during evisceration, therefore allowing the carcass (and subsequent carcasses on the line) to become highly contaminated. The increase in prevalence is also attributable to house flora, although the microbial load transferred from this source to the carcass is assessed to be low. In addition, the load and prevalence is increased during the dehairing phase (primarily due to faecal leakage) in MS2 and MS4, which had the higher infection prevalence at the point of slaughter. In the small slaughterhouse, the microbial load decreased over each phase but there was a small increase in the prevalence of contamination during the combined step of trimming/singeing.

The expected reduction of Salmonella cases in humans (or pig meat) by the most important control measures during transport, at lairage or during the slaughter process.

Transport interventions (logistic transport, increased cleaning), even assuming 100% uptake and 100% compliance/effectiveness, were assessed to have an insignificant effect in reducing the probability of human illness.

Marked reductions can be achieved by applying some decontamination measure, or reducing faecal leakage, at the slaughterhouse. An intervention that could consistently achieve a 1-2 log decontamination of carcasses pre-chill could reduce the number of cases by over 90% in all case study MSs. Further reductions can be achieved by further reducing concentrations on carcasses at pre-chill (e.g. a reduction of 3 logs) with all case study MSs predicted to achieve a very high reduction (95-100%) in their number of cases Practical non-chemical interventions have been shown to produce reductions in the order of 1-2 logs. If such interventions are shown to be as effective when scaled up and applied across a MS's slaughterhouses, it is concluded that a control measure that reduces *Salmonella* concentrations on carcasses pre-chill would be a viable option for reducing the number of human salmonellosis cases.

The consideration of multiple interventions

Reviews of *Salmonella* in have concluded that it was not possible to control *Salmonella* with the adoption of just one measure. In other words, the control of the *Salmonella* can only be achieved by the introduction of multiple interventions across the farm-to-consumption pathway. In order to investigate the impact of multiple interventions we considered three combinations of interventions:

- Change to wet feed and 1 log decontamination post-dehair
- 1 log modification of dose-response with 1 log decontamination post-dehair
- Change to wet feed with 1 log decontamination pre-chill

The analysis was carried out for MS4 only and it is concluded that a combination of interventions can, if applied judiciously, produce reductions greater than the sum of the individual interventions alone. The major reason for this is that both interventions will affect the contamination level of carcasses. We also predict similar results for MS1, MS2 and MS3 although, of course, the impact of the combination of interventions that achieve the greatest reductions will be dependent on the situation within a particular MS, in particular the contamination levels of carcasses.

Summary of the intervention analysis

In summary, the farm and transport interventions are likely to vary in their ability to change slaughter pig prevalence by a sufficient amount to change numbers of salmonellosis cases. However, a combination of farm interventions applied across a large proportion of farms is likely to have a cumulative effect in reducing slaughter pig prevalence. Probably of extreme importance, but not investigated here, is the rate of uptake and correct application of interventions by farmers – if this is not universal across a MS the effect in reducing human illness will be reduced. The model results lead us to suggest those MSs with a high breeding pig herd prevalence should focus on these herds in order to reduce the burden of infected new stock entering the weaning/growing/finishing stages However, from the results of the intervention analysis we predict that it may be more effective for MSs with a low breeding pig herd prevalence to focus their attentions on feed and other sources of infection.

From the current evidence, it would appear that specific slaughterhouse interventions are currently best placed to produce consistently large reductions in the number of human cases. For high breeding prevalence MSs, reducing infection in breeders would seem to be an important control measure as has been successfully implemented by the poultry industry. However, the hypothetical reductions and multiple interventions investigated here suggest that MSs can achieve larger reductions by targeting farm and slaughterhouse together. Reducing the prevalence at farm level is also considered important for preventing the transmission of *Salmonella* from pigs to other livestock species such as laying hens and broilers, where the prevention and control efforts are focused on the farm.

Comparison of the current QMRA model against similar national QMRAs for *Salmonella* in pigs highlights similar conclusions across all model results: slaughterhouse decontamination interventions are effective in reducing risk; reducing prevalence of infection in slaughter pigs is also an effective risk reduction strategy and low contamination rates at retail. In summary, despite a much more complicated scope and framework, the generic EU MS

QMRA model predicts similar results to the national QMRA models, but incorporates a much wider selection of interventions, and in turn these interventions can be implemented for a wide range of production systems across the EU.

During the development of the QMRA, many data gaps/deficiencies were identified. These were investigated as part of an uncertainty analysis; where we assessed the effect that parameters (with a particular lack of information) have on the model output and, in particular, the probability of illness. From this analysis, it is concluded that the following parameters were both highly uncertain and influential on the probability of illness. It is therefore recommended that further data generation is undertaken in order to provide improved estimates for these parameters. The identification of such data gaps is a positive feature of any risk assessment model and many risk managers utilise such information to direct future research.

Farm:

- Prevalence of feed contamination (MS1)
- Prevalence of infection within the breeding pig herd (MS1 & MS2)
- Maximum mass of faeces ingested per day (finishers) (MS2)

Transport & Lairage:

- Probability of pigs being stressed during transport (MS1 & MS2)*
- Dose-response parameter α (MS1)

Slaughter & Processing:

- Amount of faeces spilled while dehairing (MS1 & MS2)

Preparation & Consumption:

- Minced meat storage time in fridge (MS1 & MS2)*
- Portion sizes of pork cuts, minced meat patties and fermented sausages (MS1 & MS2)
- pH of fermented sausage (MS1 & MS2)

Those marked with an asterisk (*) were also identified as important in the sensitivity analysis, where the impact of the variability associated with the model parameters that are described as distributions is investigated

Although not able to be included in the uncertainty analysis, in order to obtain more reliable and quantitative estimates for the importance of different source to human salmonellosis in the EU, it is recommended to develop an EU model for the attribution of human salmonellosis based on the microbial subtyping approach. This will require MS-specific data on the distribution of *Salmonella* subtypes in the most important sources and in humans. The latter data have been particularly difficult to obtain, which is considered most unfortunate as these data are essential for understanding the trends and sources of human salmonellosis.

Finally it is important to recognise that, at this current time, this has been one of the most ambitious QMRAs ever developed in terms of both the complexity of the model (which has been built to maximise the potential for the consideration of current and future interventions) and also the requirement to produce a model that can represent all MSs within the EU.

Capturing variability within a single MS is in itself a challenge; however by trying to capture variability between MSs we believe that the area of QMRA has been taken to a new level.

In conclusion, a fully mechanistic farm-to-consumption QMRA has been developed by the consortium, which estimates the probability of illness and number of cases for pork cuts, minced meat and fermented RTE sausage. The model can, provided the appropriate data is available, be parameterised for any EU MS and can be used to assess the impact of interventions at the farm, during transport and lairage and at the abattoir. We therefore believe that we have: achieved the aims and objectives set by EFSA; produced a useful tool for assessing the effect of farm and abattoir interventions in reducing both slaughter pig prevalence and number of human cases attributable to pig meat consumption; and finally produced a QMRA model applicable for all EU MSs to use.