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EU0701: A UK VTEC O157 risk assessment model for meat products

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FINAL REPORT

1 Executive Summary

To address the risk posed to human health by the consumption of VTEC O157 within contaminated pork, lamb, and beef products within Great Britain, a quantitative risk assessment model has been developed. This model aims to simulate the prevalence and amount of VTEC O157 in different meat products at consumption within a single model framework by adapting previously developed models. This framework allows investigation into the attribution of human VTEC O157, to estimate which products pose a higher human health risk. The model is stochastic in nature, enabling both variability (natural variation between animals, carcasses, products) and uncertainty (lack of knowledge) about the input parameters to be modelled.

Based on the model assumptions and data, it is concluded that the prevalence of VTEC O157 in meat products (joints and mince) at consumption is low (i.e., <0.04%). Beef products, particularly beef joints, present the highest estimated risk with an estimated four out of 20,000 servings on average resulting in human infection with VTEC O157. To consider case attribution; the model predicts that, among the products considered in the risk assessment, beef joints, account for 50% of human infections, lamb joints 25% and pork joints and beef mince 12.5% each.

A benefit of QRA is to provide indications of the effectiveness of potential interventions for risk management. The model currently predicts a significant amount of growth during retail and storage. This, combined with the subsequent consumption of additional raw products (i.e., minimal inactivation) lead to a higher risk of illness. Therefore, In terms of risk management, it is critical to relay to the consumers the importance of proper storage and cooking practices to minimize growth and maximize inactivation of bacteria. This would be combined with current measures in place to reduce the amount of VTEC O157 on the product prior to storage as part of a cross-cutting harmonized approach to controlling food-borne pathogens.

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2 Introduction

The aim of this study is to use quantitative risk assessment models to attribute human VTEC O157. This research forms a single work-package (WP5) within a larger European Union (EU) SafeFoodEra project (www.campec.net). Through previous Food Standards Agency (FSA) funded research, the Veterinary Laboratories Agency (VLA) has developed risk assessment models for VTEC O157 through a number of food sources (WRc-NSF, 2004). In particular, models for beef, lamb and pork were developed. However, as quantitative risk assessment (QRA) is still in it is infancy in terms of methods and approaches being used, it is important to revise risk assessments particularly as our understanding of the system being modelled is increased. In the last few years, additional studies have been undertaken that can assist in better parameterising QRA models for *E. coli* O157; the majority of these studies have been undertaken in Ireland to parameterise and validate an Irish QRA study in beef burgers (Duffy et al., 2005).

This work-package within the overall SafeFoodEra project focuses on the following aims:

- review the United Kingdom (UK) VTEC O157 risk assessments in the context of case attribution and, where possible, update with new, relevant, data
- assess each model in its applicability to attribute VTEC O157 infections for other EU countries
- assess each model on it is applicability to attribute to non-VTEC O157 infections
- make recommendations on how to approach case attribution of VTEC infections

The latter aim is a joint objective between the VLA QRA models and the DFVF feasibility study of attributing human VTEC O157 infections. Unfortunately, due to lack of data, the latter component of the project (Task 5.2) could not be achieved; the research undertaken is summarised in Appendix 1.

The UK VTEC O157 risk assessment was originally developed in 2002 with a minor revision in 2004 (FSA Project BO1019). The scope of the risk assessment was extensive covering meat products from cattle, pigs, sheep and chickens and milk products from dairy cattle and goats. Due to data restrictions, the chicken and goat risk assessments were qualitative; all other species and products were quantitative. For this project focus was paid to products and species that have been associated with or have greatest potential to be associated with VTEC O157 human infection: minced meat and joints from sheep, pigs and cattle. The models have been redeveloped since their original inception and utilise different approaches given currently available data.

At the time of model development of the FSA VTEC models, Liverpool University were developing a qualitative and quantitative risk assessment for pasteurised milk products from dairy cattle funded by the Department for Environment, Food and Rural Affairs (Clough *et al.*, 2006). This model has been revisited during this project.

2.1 Project report overview

This report aims to address each of the above objectives in turn. The main component of the research, the updating of the VTEC O157 risk assessments is summarised in Section 2 in the format of a scientific paper accepted for publication in the peer-reviewed literature (Kosmider *et al.*, 2009). Where appropriate, additional information relating to this QRA is included within Appendixes 2-3.

In Section 3 of the report, objectives 2 and 3 are addressed. Lastly, for information, the minutes of joint meetings between Work-package 5 and Work-package 4, whereby source attribution of *Campylobacter* is considered, are included in Appendixes 4 and 5.

3 UK VTEC O157 QRA Models

THIS RESEARCH HAS BEEN ACCEPTED FOR PUBLICATION IN THE JOURNAL Risk Analysis (Kosmider et al., 2009), published online, *DOI: 10.1111/j.1539-6924.2009.01317.x.*

Attribution of human VTEC O157 infection between meat productions: a quantitative risk assessment approach

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3.1 Introduction

The use of quantitative risk assessment (QRA) models to assess the risk of human infection from food-borne pathogens, particularly VTEC O157, is not new as is illustrated by the numerous models currently available (see for example Nauta *et al.*, 2001, FSIS, 2001, Cassin *et al.,* 1998, Cummins *et al.*, 2008). All of these models have focused on beef mince, which is considered the primary product associated with human food-borne infection. However, studies have isolated VTEC O157 at retail sale in other meat products (e.g. lamb). Further, it is not only cattle that harbour VTEC O157; the bacterium has been isolated from both sheep and pigs in a recent British abattoir survey (Milnes *et al.*, 2008). Therefore, there is potential for human infection from consumption of other meat products and species.

To address this latter potential, this paper focuses on the development of a QRA, which assesses the risk of human infection from consumption of joints and minced meat from cattle, sheep and pigs. Modelling more than one livestock species within a QRA is not a commonly used approach. However, in so doing, it is anticipated that the relative contribution to human infection from the three species can be assessed.

3.2 Materials and Methods

3.2.1 Model overview

The range of food products available in today's supermarkets is varied and extensive, including for example, fresh and frozen produce, ethnic and exotic food and ready-made meals. The available meat products can be broadly categorised into three main groups: intact raw meat, meat in which a process has taken place, and meat with other ingredients added. To consider a range of products from each category for cattle, sheep and pigs would require a varied understanding of the effects of processing on the presence of VTEC O157 in a contaminated product and be extremely data intensive. To address these data limitations, focus was paid to products considered 'high risk'. For cattle these products included raw intact retail cuts of meat and minced meat. For pigs and sheep raw intact meat was considered.

In order to be able to attribute VTEC O157 infection from three different species within a single overall QRA model, it is important to use comparable modelling approaches and, where possible, data for each species considered. Given a paucity of data before processing for pigs, in particular, the model commences at the slaughterhouse, and then follows the carcass post-chilling to mincing or jointing into retail cuts, retail and distribution, preparation and consumption. The prevalence (*P*) and counts of VTEC O157 (*N*) were modelled throughout this chain (Figure 1). Where possible similar modelling approaches have been adopted for each species to avoid additional output uncertainty due to variation in modelling of the inputs.

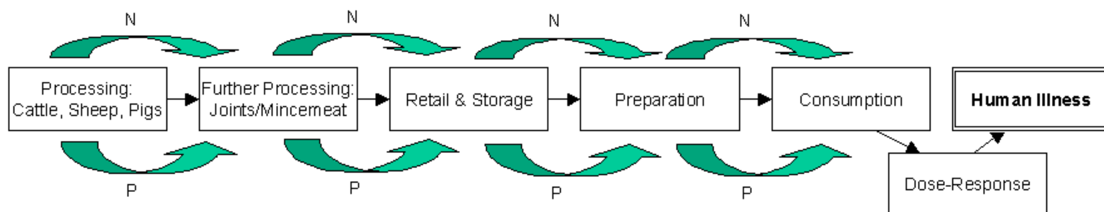

Figure 1: Pathway for assessing the human health risk from consumption of minced meat and whole cuts of meat from cattle, sheep and pigs

A species-specific model is developed for processing and further preparation. During retail and storage, however, as meat products are typically stored at the same temperature and for the same duration, the module encompasses all species and products considered. During preparation, the module is modelled depending upon the product (i.e. mince made into burgers or joints) as it is considered that these are generally cooked using different methods and finally consumption is also product-specific. This model is restricted to products that are fresh and are consumed in the domestic setting (i.e. home).

The model was developed within @Risk 4.5 (© Palisade Corporation), an add-on package within Microsoft Excel (© Microsoft Corporation). In doing so, where appropriate, inputs were described using probability distributions to represent either the variability between animals (carcasses, products) or uncertainty about the input parameter. The overall output was a distribution of the amount of VTEC O157 consumed in a single serving and the mean number of human infections resulting from this exposure to VTEC O157.

3.2.2 Model assumptions

As with many other QRAs and mathematical models, this model is a simplification of the processes under study. Accordingly, assumptions must be made. The main assumptions are as follows:

- Contamination levels on beef and lamb carcasses following rupture of the intestine are assumed to be the same as when the carcass is contaminated from the hide (or fleece). This assumption is mentioned in the work of Cummins *et al.*(1998)
- A carcass only contaminates itself and does not contribute to cross-contamination of other carcasses; cross-contamination from equipment is not considered.
- There is no strain-to-strain variation between VTEC O157 isolates found on beef, pork, and lamb.
- Storage temperature is constant throughout the duration of storage between further processing and retail, at retail, between retail and the home, and then in the home.

- Retailers in GB maintain the same temperature for refrigerators as those in the United States.
- The duration of time it takes a consumer to travel from the supermarket to the home is the same in GB as it is in Ireland.
- Infection occurs due to consumption of contaminated meat products; cross-contamination with other products is not included in this model.

3.2.3 Model development

<u>Processing</u>

Given the species-specific steps sheep, cattle and pigs undergo during processing, a different model was developed for each species. This was also required to accommodate differences in data available between the three species resulting in the application of two different modelling approaches. For sheep and cattle, the approach previously used by Cummins *et al.* (2008) and Cassin *et al.*, (1998) was adopted. For pigs, whereby raw data on *E. coli* counts were obtained from pigs processed at four English abattoirs (FSA study MO1040), data were fitted to probability distributions to estimate the change in concentration of *E. coli* for the main processing stages (See Appendix 2 for further details). In using the latter approach it was assumed that *E. coli* is a suitable proxy for VTEC O157, which may result in an over-estimate of the actual amount of VTEC on the carcass. The approach adopted for pigs is outlined in greater detail in Simons *et al.* (2009, in prep).

For cattle and sheep, the model by Cummins *et al.*, (2008) was adapted and re-parameterised, where possible, for the UK (see Appendix 3 for a diagram of the model framework). In applying this approach for sheep, several assumptions and modifications were made due to paucity in data. For example, it was assumed that the amount of VTEC O157 on the fleece of an animal was equal to the amount of faeces estimated on cattle at dehiding (10.1*Beta(3.95,2.473)*;* Nauta *et al.*, 2001) times the quantity of VTEC O157 observed in a gram of lamb faeces (Strachan *et al.*, 2001). This is likely to be an over-estimate and hence forms a worst-case scenario. The table of parameters and their values are given in Table 1. The output from this module is the amount of VTEC O157 on a random half carcass of a single animal slaughtered in the UK.

Table 1: Summary of the parameters, distributions and inputs used in the processing model for cattle and sheep

Parameter	Distribution/value for cattle	References	Distribution/value for sheep	References
Prevalence in gut (P_g)	Beta(121,2434)	Milnes *et al.*, 2007	Beta(21,2806)	Milnes *et al.*, 2007
Prevalence on hide/fleece (P_H)	Beta(131,107)	Mather *et al.*, 2008	Beta(6,86)	Small *et al.*, 2002)
Transfer ratio between hide/fleece and carcass (TR)	Beta(4,220)/Beta(123,101)*	Mather *et al.*, 2008	1	Assumption
Prevalence of contaminated carcass	$TR \times P_H / (1 - P_H + TR \times P_H)$	Cummins *et al.,* 2008	$TR \times P_H / (1 - P_H + TR \times P_H)$	Cummins *et al.,* 2008
Counts on hide/fleece ($\log_{10}$ CFU/100cm^2) (I_h)	Cumulative distribution fitted to data	O'Brien *et al.*, 2005	(10.1+Beta(0.395,2.43))x Cumulative distribution fitted to data	Assumption based on Nauta *et al.*, 2001 and data from Strachan *et al.*, 2001
Recovery factor (F_i)	Uniform(0.5,1.5)	Cummins *et al.,* 2008	Not applicable	-
True number on hide (H_{ht})	$Log(10^{(Ih+Fi)/}100)$	Cummins *et al.,* 2008	Not applicable	-
Count reduction from hide/fleece to carcass (*R*)	1.39	Based on data in Brichta-Harhay *et al.*, 2008	1.3	Assume same as for cattle
Number contaminating carcass during dehiding (I_c)	I_{ht}-*R*	Cummins *et al.,* 2008	I_h-*R*	Cummins *et al.,* 2008
Total contaminated surface area (cm^2) (*A*)	$10^{(Triangular(log(30),log(300),log(3000))})$	Ebel *et al.*, 2004	0.0143 x carcass weight	Assumption based on ratios of contamination to carcass weight in cattle
Counts on hide after	$Log((10^{Ic})X\ A)$	Cummins *et al.,* 2008	$Log((10^{Ic})X\ A)$	Cummins *et al.,* 2008

dehiding *(Bc,h)*				
Probability intestines ruptured during evisceration	0.001	WRc-NSF, 2004	0.001	WRc-NSF, 2004
Intestines ruptured? (E_f)	Binomial(1,0.001)	WRc-NSF, 2004	Binomial(1,0.001)	WRc-NSF, 2004
Gut colonised? (F_g)	Binomial(1,P_g)	WRc-NSF, 2004	Binomial(1,P_g)	WRc-NSF, 2004
Contamination event occur at evisceration?	If(E_f&F_g=0,0,1); *0=no, 1=yes*		If(E_f&F_g=0,0,1); *0=no, 1=yes*	
Amount of contamination during evisceration (*Bc,e*)	Assumed equivalence to hide contamination; *Bc,h*	Cummins *et al.,* 2008	Assumed equivalence to hide contamination; *Bc,h*	Cummins *et al.,* 2008
Amount of contamination after evisceration (*B*)	$Log(10^{(Bc,h)}+10^{(Bc,e)})$	Cummins *et al.,* 2008	$Log(10^{(Bc,h)}+10^{(Bc,e)})$	Cummins *et al.,* 2008
Amount of contamination after carcass splitting (*Bc,s*)	Binomial(*B*, 0.5)	Nauta *et al*., 2001	Not applicable	-
Amount of growth during chilling (GPC)	Pert(-2,0,5)	Cassin *et al*., 1999	Pert(-2,0,5)	Cassin *et al*., 1999
Amount of contamination after chilling	$Bc,s \times 2^{GPC}$	Cassin *et al*., 1999	$Bc,s \times 2^{GPC}$	Cassin *et al*., 1999

*It is stated in Mather *et al*. (2008) about 1% of carcasses are contaminated during processing given an initial hide prevalence of 55%

Further processing - joints

After chilling, the carcass is typically butchered into primal joints comprising a set percentage of the overall carcass side weight. In the UK, pigs, for example, are butchered into 5 main joints: head (9.5%), belly (9.9%), leg (30.6%), loin (20.4%) and shoulder (29.6%). Sheep are butchered into 7 primal joints: leg (23.6%), chump (9.6%), scrag (2.7%), shoulder (30.7%), breast (13.1%), loin (9.4%), and neck (74%). Cattle due to their size are cut into 11 main primal joints: leg (4.3%), topside and silverside (15.3%), rump (6.9%), sirloin (8.87%), flank (11.5%), fore-rib (4.8%), chuck and blade (13.8%), rib (8.5%), brisket (9%), neck and clod (9.1%) and shin (2.9%) (Figure 2).

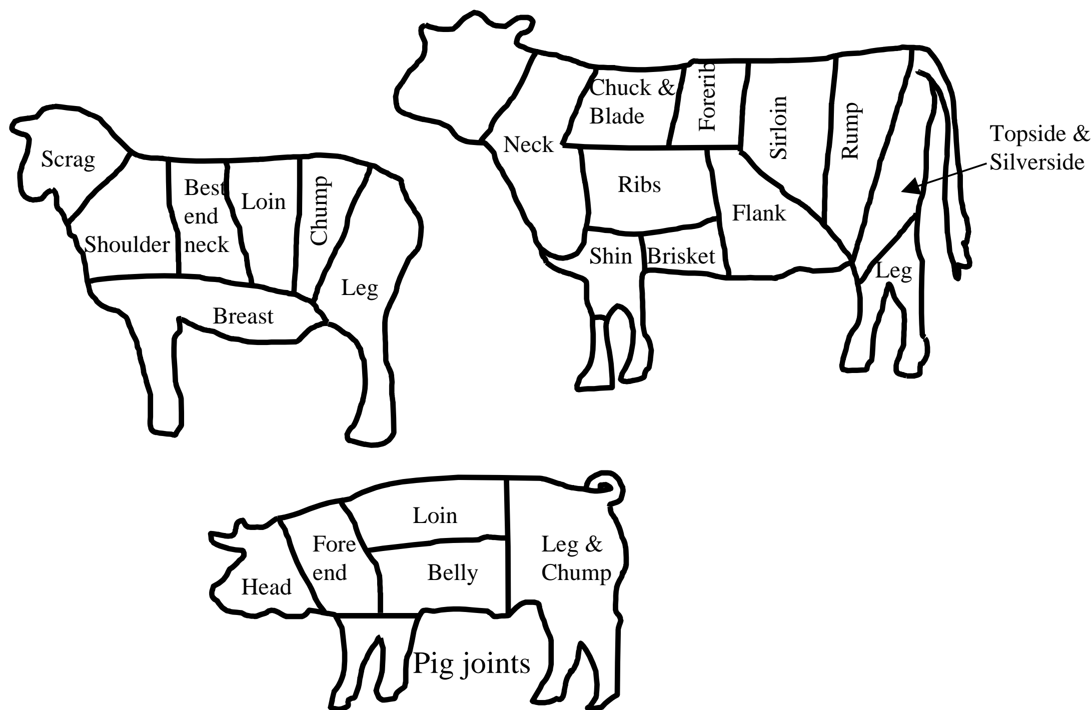

Figure 2: Primal joints for sheep, cattle and pigs

Each of these primal joints comprises a specific percentage of the overall weight of the half carcass. Using this information and the partitioning process outlined by Nauta *et al.*, 2001, the amount of VTEC O157 on each primal joint (*j*), assuming VTEC O157 is clustered on the carcass, is given by

$$N_{cut,s}(j) = Binomial(N_{chill}(i) - \sum_{k=1}^{k=i} N_{cut,k}(k), beta(b, b(\frac{W - \sum_{k=1}^{k=i} W_{cut,k}}{W_{cut,k+1}}) - 1)),$$

where W is the weight of the carcass half (or whole carcass for sheep), W_{cut} is the weight of a random joint i, $b=1$ is the clustering parameter, k is the number of joints and s is the species (s is either a cow, sheep, or pig). For carcasses in which the number of bacteria was large, the normal approximation to the binomial was used.

These primal joints are further partitioned into retail cuts of meat. These range from cutlets, steaks, and joints, for example, depending upon the primal joint from which it is derived. Surveying current weights in a major supermarket chain alongside information on how to partition a primal joint into a retail cut, the amount of VTEC O157 on a retail cut was given by

$$N_{retail,s}(j) = Binomial(N_{cut,s}(j), beta(b, b(\frac{W_{cut}}{W_{retail}}) - 1)),$$

where W_{retail} is the weight of retail joint as defined by a Uniform distribution of the minimum and maximum observed weights.

Further processing – mince

The mixing of trimmings from beef carcasses into combo boxes which are then combined into a grinder from which a retail serving of mince is produced. Using the approach outlined by Cummins *et al.*, (2008), it is assumed that trimmings from one or more carcasses are combined into a 27kg box. From here, the trimmings are combined into a 150kg grinder from which a retail portion of either 250 or 500 gram is obtained. The parameters for this module are summarised in Table 2.

Table 2: Summary of the model parameters used for the mincing module

Parameter	**Distribution/value**	**References**
Mass of a combo bin (M)	2700	Cummins *et al.*, 2008
Mass of trimming (M_m)	Cumulative distribution	Table 4 of Cummins *et al.*, 2008
Number of trimmings per carcass (N_c)	Triangular(5,6,7)	Cummins *et al.*, 2008
Number of trimmings carcass contributes to box	Uniform(4,N_c)	Cummins *et al.*, 2008
Surface area of trim (Sa_{trim})	Uniform(0.1,0.5)	Cummins *et al.*, 2008

Retail and Storage

After the product has been distributed to the retail market, there is a period of time before which the product is consumed. Specifically, there are three main opportunities, depending upon the conditions, for VTEC O157 to grow after further processing and prior to preparation namely, during transport from further processing, at retail, during transport from retail to the home and finally during home storage. Current research has shown that VTEC O157 can grow at temperatures exceeding 7°C (Palumbo *et al.*, 1995). Therefore, it can be assumed that if at any point in the chain from further processing to prior to preparation, the temperature exceeds 7°C, growth of VTEC O157 may occur.

Firstly, the probability that temperature abuse occurs during the four stages was estimated. During transport from further processing, it was assumed that no abuse occurred; expert opinion for a UK *Salmonella* in pigs risk assessment stated the maximum temperature was 4°C for cold stored products (Hill *et al.*, 2003) ($P_{abuse,transport1}=0$). Based on Audits International Data (1999) from the United States, during retail the temperature exceeds 7°C 14% of the time ($P_{abuse,retail}$=Beta(1429,8573)). Within this same data set, it was observed that the temperature change during transport was dependent upon the duration of time the product was out of the fridge. Therefore, linking the Audits International data on the mean temperature change per time out of the fridge with the time it takes to get home from the supermarket in Ireland (Kennedy *et al.*, 2005), the overall temperature of the product during transport was estimated. The number of times an abuse occurred out of 10,000 iterations of the latter sub-model was observed ($P_{abuse,transport2}$=1.00) At home, Irish data suggests that 39 out of 50 (77%) fridges are set to temperature exceeding 7°C (Kennedy, *pers. comm.*) ($P_{abuse,home}$=Beta(40,12).

Given that a temperature abuse occurs, the actual temperature the product is stored is derived by fitting a Cumulative distribution to the data from both Audits International (Retail) and Irish data (Home; Kennedy pers. comm..). For transport to the home, the temperature is calculated by noting the change per time out of the fridge (Audits International) given the duration of time between retail and home (Kennedy *et al.*, 2005).

The duration of storage at retail is assumed to be between a minimum of 1 day, a maximum of 5 days and a most likely value of 3 days based on expert opinion provided within Hill *et al.*, (2003). The duration of transport is outlined in Table 3.

Table 3: Summary of the distribution used for the duration of transport (based on data within Kennedy *et al.*, 2005)

Distribution of time (minutes)	Cumulative probability
U(10,29)	0.58
U(30,90)	0.93
U(91,180)	0.99
U(181, 300)	1.00

In the home, it is assumed that products are stored for a period of time, represented by an exponential distribution with mean 36 hours and truncated between 0 and 120 hours; the later is based on data in Mahon *et al.*, 2003.

The growth of VTEC O157 observed during the different stages and conditions defined above is modelled using Gompertz microbial growth equations (Marks *et al.*, 1998). At each stage the amount growth and time left in the lag phase is estimated. In this way, the remaining lag period from the previous step is compared to the time in current step and if the lag phase is less than time, no growth occurs. This is achieved within a specifically written macro in Visual Basic for Applications from which the probability of observing a total logs growth ranging from –3 to 20 logs is predicted. This is fitted to a General distribution.

Preparation

During preparation there is a risk that some bacteria may survive the cooking process, particularly if consumers do not cook their food adequately. Further, bacteria may spread via cross-contamination, as a result of poor hygiene practices, from contaminated products to ready-to-eat products. The latter route is of particular importance for poultry products and in this risk assessment it was assumed that cross contamination of bacteria from meat products would not occur due to the firm texture of the product. Consequently, focus was paid to the inactivation of VTEC O157 during an inadequately cooked food product.

For whole cuts of joints, it was assumed that if a product was well cooked that all the bacteria in the product became inactivated. In a study of Irish consumers,(Bolton *et al.* 2005) it was observed that between 3.2% and 15.8% of respondents cooked their meat medium-well to rare ($Pcook = U(0.032, 0.158)$. For products that were not thoroughly cooked ($Binomial(1, Pcook) = 1$), it was assumed that no inactivation of VTEC O157 occurred. This is considered a worst-case assumption as some inactivation is likely to occur on the surface of the joint given that VTEC O157 is only present on the surface.

For beef burgers the approach used by Duffy *et al.*, (2005) was adopted whereby the amount of VTEC O157 remaining in the burger depends upon whether it is well done (87% of time), medium (12% of time) or rare (1% of the time). Given the doneness of the burger, the temperature is ascertained: 68.3°C (well done), 62.7°C (medium) or 54.4°C (rare) (Cassin *et al.*, 1998; Jackson *et al.*, 1996). Using this information, the thermal inactivation from cooking is given by (Cassin *et al.*, 1998)

$$C = -10.165 + 0.211 * T,$$

where T is the temperature of the product. The amount of bacteria (N_{prep}) in the product post-cooking is then estimated by:

$$N_{prep} = N_{postretail} - 10^{C}$$

where $N_{postretail}$ is the amount of VTEC O157 in the product after retail and storage.

<u>Consumption</u>

At the end of preparation the product is further *partitioned* into a serving size. These serving sizes are variable between consumers and particularly between age groups. Recently, the UK Food Standards Agency funded a study to examine the food portion sizes for adults aged 19-64 (Wrieden & Barton, 2006). Using the raw data from this study, a Normal distribution was fitted to the portion sizes for beef, pork and sheep retail joints (e.g. shoulder, chops). The amount consumed, therefore, in the home was a further portioning process given by

$$N_{H,s} = Binomial\left(N_{prep,H,s}, beta\left(k, k * W_{H,s} - 1\right)\right).$$

Probability of illness – dose response

The probability of illness given exposure to VTEC O157 at consumption is modelled using the previously published Beta-Binomial model (Cassin *et al.*, 1998). The uncertainty in the dose response model as represented by the 5th and 95th percentiles of the model output is illustrated in Figure 3.

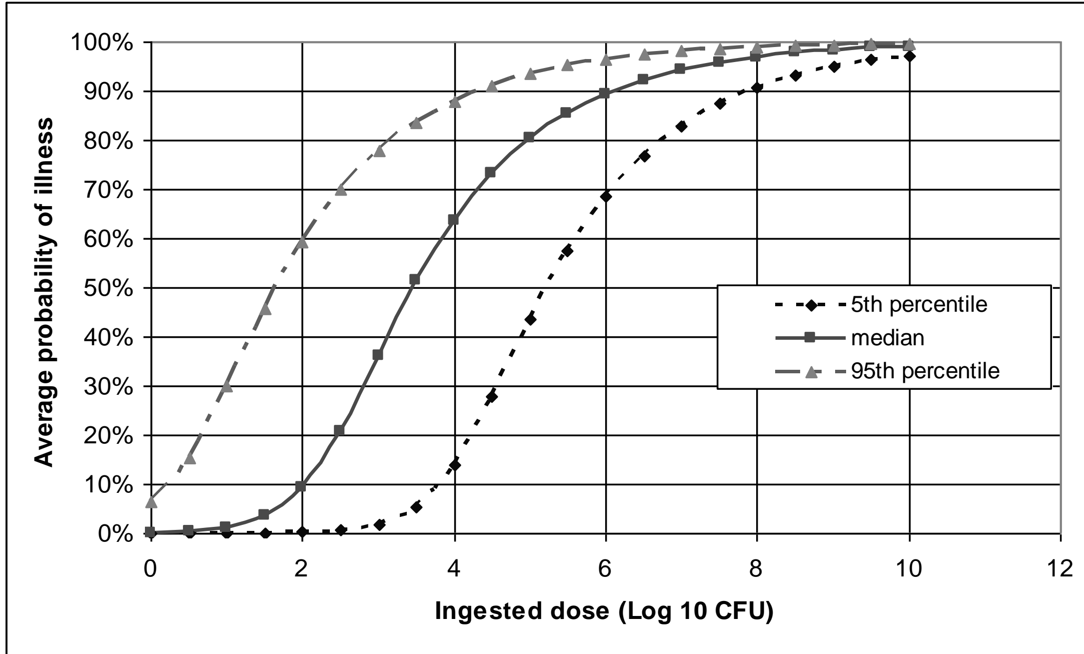

Figure 3: Illustration of the Beta-Binomial dose response model (Cassin *et al.*, 1998).

Given the probability of illness per serving exposure (P), an individual will either become ill or not. The variation in this process is given by a Binomial(1,P) where a 1 represents illness and 0 represents no illness.

Generation of results

In order to model the change in the prevalence and amount of VTEC O157 along the food chain, Monte-Carlo simulation was used. In running the model, the probability distributions characterising the model parameters are sampled numerous times, or iterations, such that each iteration represents a potential event of transmission along the food chain. The models were run for 20,000 iterations. This number was considered sufficient to allow convergence of all the probability distributions. The prevalence of contaminated units (i.e. carcasses, joints etc) was derived thus

$$\Pr = \frac{\sum_{i=1}^{50000} N = 0}{50000},$$

where *N* is the number of VTEC O157 per unit generated by each iteration. Given that a unit was contaminated the median, 5th% and 95th% values were obtained. The median value rather than the mean value was obtained due to the positive skew of the distributions.

3.3 Results

The median, 5th and 95th percentile for the amount of VTEC O157 during the various stages of the food chain for each species is outlined in Table 4.

Table 4: Summary of the prevalence and concentration of VTEC O157 during the various stages

Species & Product	Module	Mean prevalence of contaminated units	Amount of VTEC Median (5th%, 95th%)
Cattle - joints	End of processing	0.037	76 (1, 953)
	Further processing	0.012	2 (1,23)
	End retail & storage	0.012	4 (1,250)
	End preparation	0.007	1 (1,30)
	Consumption	0.002	1 (1,12)
Sheep - joints	End of processing	0.050	76 (1,)
	Further processing	0.007	13 (1,2150)
	End retail & storage	0.007	26 (1,4800)
	End preparation	0.004	2 (1,45)
	Consumption	0.002	1 (1,35)
Beef – mince	End of batch	0.17	14 (1,235)
	End of retail cut	0.014	1 (1,3)
	End of single burger production	0.004	1 (1,2)
	End retail & storage	0.004	2 (1, 650)
	End preparation	0.002	2 (1, 25)
	Consumption	0.008	2 (1, 25)
Pig – joints	End of processing	0.0008	
	Further processing	0.0002	1 (1,1)
	End retail & storage	0.0002	36 (1,75)
	End preparation	0.0001	1 (1,2)
	Consumption	0.0001	1 (1,1)

The variation in the amount of VTEC observed between the different stages and species as highlighted in Table 4 is illustrated in Figure 4.

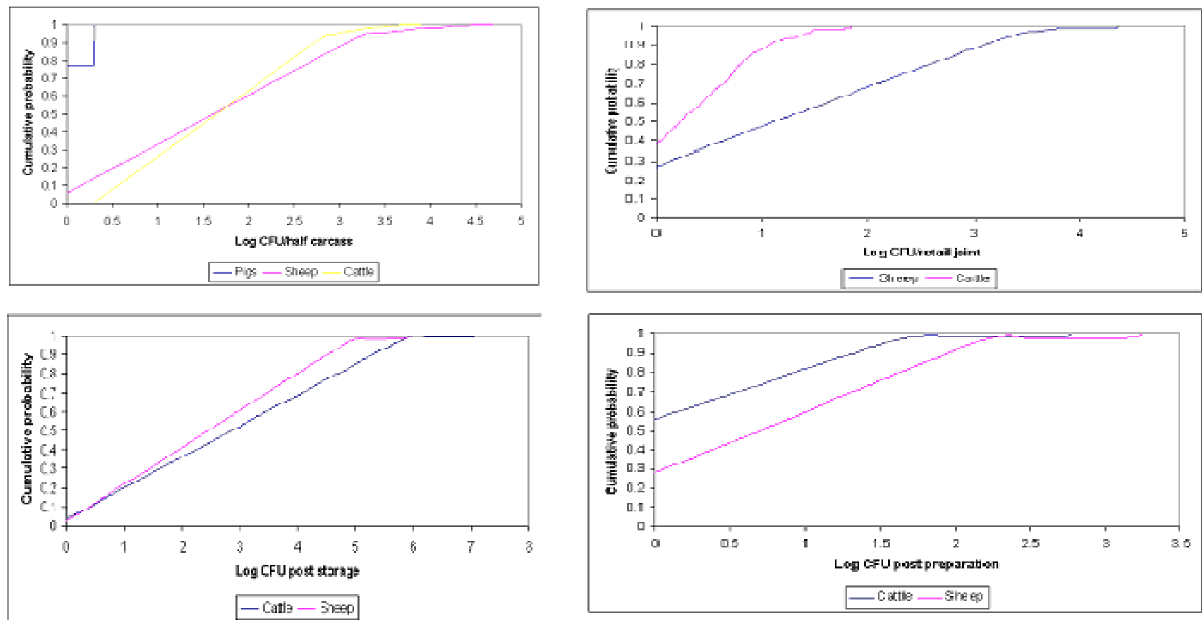

Figure 4: Illustration of the cumulative probability of VTEC O157 on a half carcass (top left), on a retail joint (top right), after storage (bottom left), and after preparation (bottom right) for cattle and sheep.

It can be seen from Table 4 that less than 1% of the 20,000 servings are contaminated and, further, these are contaminated at very low levels of around 1 to 12 VTEC O157 organisms. Given the model assumptions and data, it is observed that during retail and preparation significant amount of growth occurs. For sheep, for example, the median amount of VTEC O157 increases from 13 at the end of further processing to 26 at the end of retail and storage. Contrary to expectation is the increased amount of VTEC O157 on lamb joint products compared to beef products which are considered to be of higher risk than other species. This is due to the assumptions made as a result of the lack of available to realistically model the sheep-processing module. In particular, this stems from the assumption of the amount of VTEC O157 on a sheep carcass. This is an area that needs to be addressed in further research.

Number of infections

The number of infections resulting from the amount of VTEC O157 an individual is exposed to after consumption of 20,000 servings is estimated using the Beta-

Binomial dose-response model. The ranked number of infections by product type is outlined in Table 5. From this we can estimate the proportion of infections attributable to each product type ($P_{Inf}(i)= n_{inf}(i) / N_{inf}$ where $n_{inf}(i)$ is the number of infections from product *i* and N_{inf} is the total number of infections across all products).

Table 5: Number of human VTEC O157 infections arising from 20,000 servings

Product, *i*	**Number of infections, $n_{inf}(i)$**	**Proportion of infections, $P_{inf}(i)$**
Beef joints	4	50%
Lamb joints	2	25%
Pork joints	1	12.5%
Beef mince	1	12.5%

3.4 Model Validation

As with other models, it is important to validate the results in order to ensure that the model simulates, as accurately as possible, the processes understudy. There are limited points at which data are available for validation but one such point is at retail using data from retail surveys. Thus far, there have been several retail surveys conducted in GB, each focusing on a different region of the country and time period. For example, two studies have been undertaken in Sheffield, England (Chapman *et al.* 2000, Chapman *et al.* 2001). In the first study, from April 1996 to March 1997, between 400 and 430 samples were collected from raw processed meat products purchased from small butcher shops in south Yorkshire. Overall, VTEC O157 was isolated from 36 (1.1%) of 3,216 raw beef product samples and from 29 (2.9%) of the 1,020 lamb products. The second study examined the prevalence of VTEC O157 in raw lamb and beef from retail butchers during the period of April 1997–March 1998 (Chapman *et al.* 2001) *E. coli* O157 was isolated from 22 (0.44%) of 4,983 samples of raw meat products; slightly more lamb products than beef products (0.8% vs. 0.45%) were contaminated with the bacteria. Enumeration of the bacteria using the most probable number method yielded that products had mostly <3 *E. coli* O157 CFU per gram but could be as high as 90 CFU per gram in burgers (Chapman *et al.* 2001).

A later study was conducted in southeast Scotland (Coia *et al. 2001*) from April 1997 to March 1999 examining the prevalence of VTEC O157 within retail lamb and beef products. During the study, 829 beef and 233 lamb samples were collected from retail butcher shops and other retail outlets. No VTEC O157 was isolated from any of the 233 lamb products. However, the bacterium was isolated from 3 (0.36%) of the 829 retail beef products. A relatively recent study was conducted in Scotland between 2004 and 2006 in which 3% of samples of minced beef products and 3% of samples of minced lamb products from rural supermarkets tested positive for *E. coli* O157.(Solecki *et al., 2008*) Further, an Irish study identified VTEC O157 in 2.8% of minced beef products at retail sale.(Cagney *et al.,* 2004)

It can be seen from the above studies that the prevalence of VTEC O157 at retail sale varies from 0.36% to 3% for beef products and 0.8% to 3% for lamb products. This compares to a mean model prediction of 1.2% of beef joints, 0.4% of beef mince and 0.7% of lamb joints being positive for *E.* coli O157 on retail sale. The model estimates for beef, based on current model assumptions and data, are broadly in line with observed results. For sheep, the model slightly underestimates the observed prevalence; however, the model is restricted to lamb joints rather than lamb mince.

Currently, in the UK, the annual number of *E. coli* O157 infections (excluding those from food eaten abroad) is 1,035. This, however, is not categorised by food sources and includes data from outbreaks and sporadic cases. Therefore, it can not be concluded if the relative contributions predicted by the model are representative of real life.

3.5 Discussion

A quantitative exposure assessment of VTEC O157 in cattle, sheep, and pig products was developed by following a processing-to-consumption approach building upon models previously developed for BO1019. It is aimed to attribute the relative number of human infections (given 20,000 servings) between the products within a single framework. Using this approach, it was estimated that VTEC O157 human infection from beef products predominates.

There are several methods that can be used for source attribution, including microbial subtyping, exposure assessment, epidemiological studies, Bayesian methods, and expert opinion (Pires *et al.,* 2009). The method considered here has

the strength that, within the single model framework, many different potential sources of infection can be considered including, for example, both environmental and food sources (WRc-NSF, 2004). It is also able to consider risk management options for reducing the amount of VTEC O157 along the food chain. However, a disadvantage is that it is data intensive. It would be advantageous, given the limitations and strengths of different approaches, to be able to use more than one approach to answer the question of source attribution for VTEC O157. However, thus far, there are not enough data to estimate the attribution of human VTEC O157 infection from animal products using microbial subtyping approaches to link human infection data and animal data (Pires, 2008). Until such time that data are available, QRA is considered a useful approach to use.

The model developed is large in scope, covering the processing-to-consumption stages for beef, lamb and pork. It is not feasible (or indeed necessary) to include every possible risk factor in the model. Apart from time constraints, the added complexity to the modelling process and the need to develop similar models for every livestock species, every parameter that is included adds more uncertainty into the model. There is always some degree of uncertainty with any dataset, some more than others, and these all add up, leading to greater uncertainty about the final output. However, the main aim for this risk assessment was the ability to investigate source attribution (and inclusion of different sources) and prediction of the effects of changes to the current process by implementing interventions. Consequently, a parsimonious approach was adopted, i.e. to include the minimum number of factors required, while still producing a robust model that includes the critical points and can investigate interventions.

Therefore, we are aware that there are many factors not considered in the model that may be considered important, such as fat content or reduction in levels of VTEC due to antagonistic microbial growth. Smith *et al.* (2001) found that *E. coli* strain O157:H7 was more heat resistant in meat containing 19% fat than meat containing 4.8% fat, Ahmed *et al.* (1995) found that D values for *E. coli* were lower in low fat products than normal products. With regards antagonistic microbial growth, Roca *et al.* (1989) showed that *Lactobacillus jensenii* and *Streptococcus* 17SB could inhibit *Escherichia coli*. However a study by Saad *et al.* (1999) found that Non-pathogenic *E.coli*, *Pseudomonas putida* and *Leuconostoc* sp. did not affect growth of *E. coli* O157:H7 in ground beef, either during refrigeration or at room temperature. While these factors are not modelled, any significant effect that they may have will be present in the

variability of related parameters (e.g. if there is any reduction in levels of VTEC due to antagonistic microbial growth at any stage, then this will implicitly be included in the data used to parameterise the model).

The model aimed to simulate the changes in the prevalence and amount of bacteria along the food chain. By doing so, the variation in the amount of VTEC O157 on an average serving at the point of consumption was estimated. This was achieved by using traditional MRA techniques of fitting distributions to observed data and a more recent approach, that of the Modular Process Risk Model (MPRM). For beef processing, in particular, is based on that of the Irish quantitative risk assessment model for Ireland (Cummins *et al.*, 1998). This is supplemented with other modelling approaches as outlined in Nauta *et al.*, (2001) and Cassin *et al.*, (1998) in order to aim at representing the UK VTEC O157 situation as realistically as possible.

The prevalence at retail predicted by the model compares favourably with published retail figures, albeit slightly lower. Unfortunately it is not possible to validate the numbers of VTEC 0157 on the product types predicted by the model, due to lack of data. If such data were to become available this would be an invaluable validation point, particularly as despite slightly underestimating the prevalence at retail the model appears to be overestimating the number of illnesses.

Assumptions have been made given the paucity of currently available data and can be re-examined upon further information being made available as part of the iterative process of QRA.

As with other QRAs and mathematical models, the outputs are dependent upon the quality and availability of the input data and model assumptions. Currently, research has focused predominantly on cattle where a greater amount of quantitative data is available, particularly at processing. There are, however, several important data gaps and uncertainties for sheep, including, the total contaminated surface area of the carcass, the counts of VTEC O157 on the fleece, and the count reduction from the fleece to the carcass. For the retail to preparation modules, there are data gaps on storage and cooking practices that individuals undertake within their home that inevitably impact on the presence (or absence) of VTEC O157 on a product. The data from the U.K. Food Standards Agency study MO1040 used for the pig abattoir model were of an ideal format for QRA. However, this study was very intensive, following individual animals and only relatively few data were collected. Similar

studies of this nature for cattle and sheep would be most beneficial to aid in the accuracy of parameterising risk models. Further, it could enable in-depth investigation of potential intervention measures during processing to reduce the burden of VTEC O157 (or other bacteria) at further processing.

These data uncertainties and the model assumptions made to account for them are considered to be the main reason that the model currently underestimates the observed prevalence of VTEC O157, in particular on lamb products in current retail studies. It is therefore prudent that further research is conducted on VTEC O157 in sheep, particularly during processing, in order to refine the model parameters for this species, as part of the iterative process of risk assessment. It is acknowledged that availability of these data may change these assumptions and thus the model estimates and conclusions.

3.6 Recommendations

The results of this risk assessment suggest that the prevalence of VTEC O157 infection among livestock populations is non-negligible throughout the food chain. As a result of this work the following recommendations are suggested:

- The model suggests that there is a fair degree of growth of organisms during the retail and storage phase. This, combined with the subsequent consumption of additional raw products (i.e., minimal inactivation) lead to relatively high risk of illness
    - Therefore, in terms of risk management, it is critical to relay to the consumers the importance of proper storage and cooking practices to minimize growth and maximize inactivation of bacteria.
    - This would be combined with current measures already in place to reduce the amount of VTEC O157 on the product prior to storage as part of a cross-cutting harmonized approach to controlling food-borne pathogens
- Intervention strategies at the start of the pig abattoir process may not be the most effective method to control carcass contamination at the end of the slaughter line and thus a method to reduce the increase in contamination at evisceration may be more beneficial. This would be beneficial to not only VTEC O157 but also other organisms such as *Salmonella*.

- There are many data gaps and further research should be done to effectively parameterise the model, particularly with relation to
  - Human dose response parameters
  - Estimation of concentrations of organisms throughout the food chain (e.g. on carcasses at slaughterhouse, at retail and at consumption, including on products that may have been cross contaminated)
  - Storage and cooking practices of individuals in the home
- The results suggest that the majority of human cases come from beef products and so to reduce human illness intervention measures should first focus on cattle.
  - The risk from pork and lamb was not negligible, with the model showing the potential for human infection, so these routes, although a lesser risk to human health , should not be ignored.

3.7 Conclusion

Based on the model assumptions, it was deduced that the mean prevalence of contaminated meat products at consumption was low. In particular, for pork, the mean prevalence of contaminated products at consumption was 0.01%. Given that they are contaminated, the expected levels of VTEC O157 were less than one organism (with 95% certainty). The mean prevalence of contaminated cattle and sheep products (both retail cuts) at consumption was higher than pork. Specifically for beef and sheep joints, it was estimated that a mean of 0.2% of servings are contaminated with VTEC O157. With regards to case attribution; the model estimated that, among the products considered in the risk assessment, 50% of infections were attributable to beef joints, 25% to lamb joints and 12.5% to both pork and beef mince. The amount of VTEC O157 consumed in these contaminated servings was estimated to be lower for beef products (≤12 with 95% certainty) than sheep joints (≤35 with 95% certainty). However, it needs to be borne in mind that due to data gaps, several assumptions were made for input parameters describing the processing of sheep.
For beef mince, it was estimated that 0.8% of products were contaminated whereby a single contaminated serving would contain ≤25 VTEC O157 with 95% certainty.

4 Other EU Countries and VTEC O157 QRA

There are already VTEC O157 risk assessments that have been developed for the Netherlands (Steak Tartare) (Nauta *et al.*, 2001) and Ireland (Beef burgers) (Duffy *et al.*, 2005). Indeed, these risk assessments have been reviewed and adapted in developing the model for the UK (see Section 2).

In adapting a UK model to other EU countries there are numerous considerations to be made. These relate to the country specific aspects of the food chain and are considered below:

Processing –

- Prevalence of VTEC O157 in gut of livestock species (cattle, pigs, sheep)
- Prevalence of VTEC O157 on hide
- Transfer rate of VTEC O157 from hide to carcass
- Weight of livestock carcasses
- Processing stages included
- Effects of any decontamination procedures
- Duration of chilling

Further processing –

- Size of retail joints of meat
- Mincing process – how many trimmings contribute to batch, batch size

Retail and Storage –

- Probability of temperature being greater than 7°C during transport from further processing to retail, at retail, during transport from retail to home and at home.
- Duration and temperature of storage prior to retail, at retail, during transport home and at home.

Preparation –

- Frequency consumers cook beef burgers rare, medium well, and well done.
- Duration consumers cook meat joints

- Temperature of food product during cooking

Consumption –

- Amount consumed per serving (grams)

Further, depending upon the data available, the modelling approach undertaken for the UK model may not be applicable. There are several different approaches that have been used within QRA for food safety problems. The two main categories of approaches are either empirical (whereby data are fitted to distributions) or mechanistic. The modular process risk model approach (MPRM) originally developed and proposed by Nauta *et al.*, (2001) is an example of a mechanistic approach whereby risk assessments which utilise estimation of the log change in concentration of a bacteria through the food chain often use an empirical approach (see Appendix 2).

Consequently, in addition to the specific country specific factors that need to be considered, attention needs to be paid to the quality and type of available data in order to accurately assess whether the UK model could be adapted or modified for another EU country.

5 Non-VTEC O157 and QRA

In developing the UK QRA model, it was assumed that all strains/clones of VTEC O157 behaved the same. This is a simplification as Avery (2003), for example, identified that different clones of VTEC O157 predominated in vivo after consumption of pre-biotic sugars. Further it is known that in the UK, Phage type 28/44 dominates. Therefore, the situation in terms of VTEC O157, specifically, is more complex than is currently accounted for within current QRA models. However, in principle taking these caveats into account, the UK QRA could be adapted to consider other non-VTEC O157 serotypes. The limiting factor is available data. In particular data are required on the prevalence of gut colonised animals with non-O157, the prevalence of hide contaminated animals, the amount of non-O157 on the hide and in the gut, and the impact of processing, retail and storage and cooking on the survival of non-O157 serotypes. A description of UK data for each of these aspects is briefly outlined below.

On-farm studies: gut prevalence

In the UK, it is only recently that studies have begun to focus on other non-VTEC O57 serotypes that have been associated with human infection (e.g. O26, O111, O103); the main reason being that VTEC O157 is still the dominant serotype causing human infection. In other parts of the world, non-VTEC serotypes have started to take precedence to VTEC O157 in causing human VTEC infection. In Germany, for example, non-O157 VTEC serotypes have replaced O157:H7 as the VTEC most commonly isolated in haemolytic uraemic syndrome(HUS) (Bonardi *et al.*, 2002)

The focus of research into non-VTEC O157 serotypes in the UK has taken place mainly in Scotland dating back to 2000. Jenkins *et* al., (2002), for example, made 4 visits over a period of 8 months to a beef cattle farm in Scotland during which time 45 different serotypes of VTEC strains were isolated. These included O128ab:H8, O26:H11 and O113:H21 (Jenkins *et al.*, 2002). Later, Pearce *et al.*, (2006) conducted a further study between March 2002 and February 2004 to determine the prevalence of *Escherichia coli* O26, O103, O111 and O145 in faeces of Scottish cattle. The weighted mean percentage of farms on which shedding was detected were 23% for *E.coli* O26, 22% for *E.coli* O103 and 10% for *E.coli* O145. The results for *E.coli* O26

and O103 are similar to the mean percentage of farms with cattle shedding *E.coli* O157 (22.8%). No *E.coli* O111 was detected (Pearce *et al*., 2006).

A further study was held investigating the temporal shedding patterns and virulence factors of *E.coli* groups O26, O103, O111, O145 and O157 in a cohort of beef calves and their dams was held over a 5-month period (Pearce *et al*., 2004). *E. coli* O26 was shed in 94% of calves, *E.coli* O103 in 51% and for O145 and O157 shedding was rare. Once again no shedding of *E.coli* O111 was detected. *E.coli* O26 was detected in three times as many samples as *E.coli* O103, and the rate at which calves began shedding *E.coli* O26 for the first time was five times greater than that for *E.coli* O103 (Pearce *et al*., 2004).

Retail and storage: Validation

A study was carried out in Ireland on 800 minced (ground) beef samples (352 pre-packed and 448 loose mince beef) for *E.coli* O26 and O111. Two minced beef samples (0.25%) tested positive for *E.coli* O26 but none contained O111.(Murphy *et al*., 2005)

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Appendix 1: Summary of DVFV Attribution Model

Attribution of human VTEC infections: feasibility study of the use of the Microbial Subtyping approach

By Sara Monteiro Pires (Fødevareinstituttet)

Background

To identify and prioritize food safety interventions, it is important to quantify the burden of human illness attributable to the responsible sources. A defining variety of "human illness attribution" methods are used worldwide, including the microbial subtyping approach. The principle of this method is to compare the subtypes of isolates from different sources (e.g. animals, food) with those isolated from humans. The approach requires intensive monitoring of the pathogen in all major sources and of human cases, and involves characterization of the isolates by different pheno- or genotypic typing methods (e.g. serotyping, phage typing, antimicrobial susceptibility testing, pulsed-field gel electrophoresis and sequence-based subtyping).

Objective

The objective of this study was to assess the feasibility of the use of the microbial subtyping approach to attribute human cases of verocytotoxin-producing *E. coli* (VTEC) infection to specific sources.

Method

The considered method attributes the number of domestically acquired human infections caused by different pathogen subtypes as a function of the prevalence of these subtypes in animal and food sources and the amount of each food source consumed, using a Bayesian framework with Markov Chain Monte Carlo simulation (Hald et al., 2004).

Attribution of human VTEC cases to the reservoir source (production level) requires data on the number of human cases caused by each subtype, the distribution of the

subtypes in all potential sources and the use of similar subtyping methods for both human and animal data. Typing approaches that can be used include serotyping, phagetyping, virulence factor profiling, PFGE typing or combination of serotype and virulence factors of the isolate. (VTEC isolates from certain serotypes with different combination of virulence factors have been linked to human cases with different frequency and pathogenicity, e.g: O117 - vtx1; O158 - (vtx1) vtx2 eae; O26 - vtx1, eae; O26 - vtx1 vtx2 eae (highly pathogenic)).

The most common sources of human exposure to VTEC are cattle, sheep, goats, deer, different sources within the same reservoir (e.g. milk and beef) and environmental – e.g. imported vegetables/salad). The microbial subtyping approach attributes human cases to the reservoir level and does not consider the different transmission routes from the reservoir until human exposure; thus, environmental and direct contact transmission are not considered.

Progress

The progress of the study was limited by the lack of available subtyping data. Frequently, only VTEC O157 is investigated in both animals and humans. In addition, we observed subtyping differences between human and animal data. As an example, the data available in Denmark for human cases is very detailed: human isolates are serotyped, phagetyped, tested for virulence factors and DNA finger-printed using PFGE. On the contrary, surveillance in animal reservoirs only investigates the presence of VTEC in cattle, and isolates are classified uniquely by the serogroup. There is also a lack of prevalence data for other sources than cattle.

Our literature search and expert elicitations revealed that, at current stage, no country has sufficient data available for a successful assessment of the feasibility of the method. A detailed report with the theory of the approach, methods and required data will be produced.

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Appendix 2: Pig Processing model

Model developed by Robin Simons, Susan Cheung, Andrew Hill

Report by Robin Simons

Data used to develop this model is still pending approval from the FSA – related project FT5069

1. Aim of the pig processing risk assessment

The aim of this risk assessment is to produce a processing model for *E.coli* in pigs. This has been achieved by adapting the existing VLA processing model for *Salmonella* in pigs (Simons *et al.* 2008). The development of the latter model was part of project FT5069, funded by the FSA, which incorporated data provided by Nottingham University on prevalence/concentration of *Salmonella* (or related organisms). The data provided by Nottingham university also included similar data on *E.coli* and we used this data here; this is still pending approval from the FSA.

2. Brief overview of current model

The original *Salmonella* risk assessment model is being developed as part of a Defra-commissioned project OZ0323 (An integrated risk based approach to the control of E.coli in UK pig farms). A diagram of the model framework is shown in Figure 2. A batch of pigs, *j,* enter the abattoir from transport and lairage. At this point, the prevalence of infected pigs is converted into the prevalence of contaminated carcasses, by relating the ratio of infected pigs/contaminated carcasses to the prevalence of infection at the end of lairage (Davies *et al.*, 1999). The carcasses then pass through the production line of the abattoir, where we estimate the prevalence and concentration of *Salmonella* contamination on the carcasses at each processing stage *k* (p_k) and the concentration of *Salmonella* on each contaminated pig *i* from a random batch *j*, $c_k(i,j)$. After the chilling stage, the carcasses move onto the cutting.

The most important parameters within the model are the *change* in prevalence and contamination between each stage, F_k and $\chi_k(i)$ respectively, as these parameters

will be the ones to be changed when investigating intervention strategies. Both the change in prevalence and contamination are represented as proportional changes to the previously estimated prevalence and concentration of the carcass at the previous processing step, p_{k-1} and $c_{k-1}(i,j)$.

We now describe how the FT5069 data have been analysed and used to adapt the *Salmonella* risk assessment for *E. coli*.

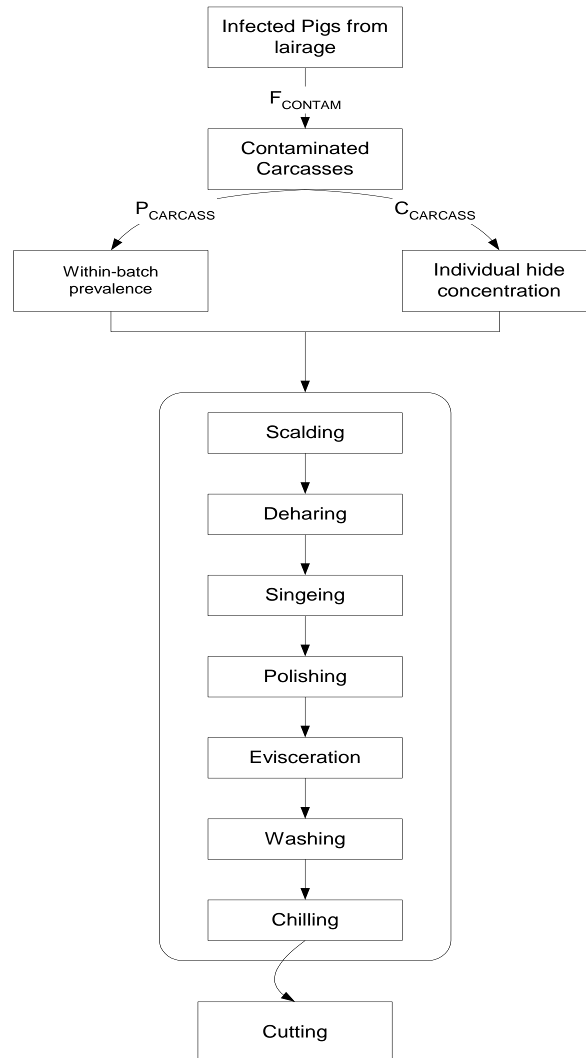

Figure 2: Schematic of the slaughter module showing the different stages modelled in the risk assessment.

3. Parameter estimation

3.1 Overview of how FT5069 data have been used

The data collected from four slaughterhouses over the sampling period is presented in more detail in the MO1040 FSA report (Dodd, *et al.* 2008). In this study counts were collected for *E. coli*, enterobacteriaceae, TAC and *Salmonella*. While these data were for all *E. coli*, not just VTEC O157, **it is assumed that that proportion change of *E.coli* between stages is an adequate proxy for the proportion change of VTEC**. Similarly the presence/absence of *E.*coli is used as a proxy for the change in prevalence. We acknowledge that as this data are for all *E. coli* it may overestimate the prevalence. Where possible, we used the *E. coli* data to define a probability distribution for the variability between carcasses of the proportional change in concentration of *E. coli* at each processing stage *k*, $\chi_k(i)$.

Unfortunately, data were not collected for every stage at every abattoir, as sometimes it was not possible to safely access the carcasses. This was particularly true at the scalding stage where data could only be collected from 2 of the 4 abattoirs. These omissions naturally reduced the amount of data available to work with: in order to retain a reasonable sample size a judgement was made that if we had observations from only one abattoir for a particular stage this stage was omitted from the model (i.e. washing of the carcass). Therefore, we consider changes in concentration for the following processes (number of observations): bleeding – scalding (20); scalding to de-hairing (20); de-hairing – singeing (40); singeing – polishing (30); polishing – evisceration (30); evisceration – pre-chill (40).

3.2 Initial prevalence

To initiate the model an initial prevalence of VTEC among pigs entering the abattoir was required. A 12-month abattoir study starting in January 2003 (Milnes *et al.* 2007) found that VTEC 0157 faecal carriage in pigs was 0.3%. This was not considered to be significantly different to a previous study undertaken in 1999-2000 (Paiba *et al.* 2002). Thus we assume that the prevalence of VTEC entering the abattoir is 0.3%. To allow for variation in this prevalence among batches of pigs, we fit a binomial distribution to this so that the actual number of infected pigs in a batch, *j,* entering the abattoir, $N_{INF}(j)$, is defined as

$$N_{INF}(j) = Binom\left(N_{BATCH}(j), 0.3\right),$$

where $N_{BATCH}(j)$ is the number of pigs in batch *j.*

3.3 Estimating change in prevalence

The presence/absence of *E.coli* at each stage was used to determine the change in prevalence. This data is shown in Table 1. We combine the data from all 5 slaughterhouses (A,B,C,D,E and the extra data collected after the refit of slaughterhouse D) to get the total number of positive results (*s*) and the total number of samples (*n*). We then calculate the proportion positive (*p*) by the calculation $p=s/n$.

Table 1: Summary of studies where the prevalence (recorded as a percentage) of *E.coli* was reported at specific stages in the abattoir.

Abattoir stage (k)	Total Positive (s)	Number of samples (n)	Proportion positive (p)	Average proportion change (p^c)
Post-Bleed	50	50	1	-[a]
Post-scald	4	20	0.2	0.2
Post-dehair	50	50	1	5
Post-Singe	7	40	0.175	0.175
Post-polish	30	50	0.6	3.4286
Post-evisceration	18	40	0.45	0.75
Post-chill	19	50	0.38	0.8444

[a] a proportion change at post-bleed is not calculated as there is no previous stage.

We can account for the variability in the data by using a beta distribution. Thus the proportion positive at each stage in the model is

$$p_k = Beta(s_k + 1, n_k - s_k + 1)$$

Consequently, the estimated proportion change in concentration for an individual pig at stage *k* is

$$p^c(k) = \frac{p_k}{p_{k-1}}.$$

3.4 Fitting concentration data to probability distributions

The simplest way to incorporate the *E.coli* data into the risk assessment model is to take the average proportional change in concentration between stages. i.e.:

Average proportional change at stage *k* = $\frac{\text{concentration at stage } k}{\text{concentration at stage } k - 1}$.

However, this method, used in previous risk assessments (e.g. Hill et al., 2003), has the disadvantage of losing information contained within the dataset on the variability between carcasses. Including the variability is considered extremely important in risk assessment, as it is the tails of the distributions where most risk lies (e.g. when a carcass is heavily contaminated with *E.coli*, it is more likely that some of those *E.coli* will survive to infect a human at consumption).

Therefore, another method is to create an empirical distribution based on the sample data from each carcass before and after stage *k*. However, two issues affect this simple empirical analysis of the data: i) where counts are reduced to zero at one stage, but have a positive non-zero number at the next stage, what is the proportional increase?; and ii) censored data, in the form of high counts at certain stages, where it is only possible to state > 10,000 cfu/cm^2, rather than a value.

The first issue is resolved by assuming any point that has zero concentration is equal to 1 cfu. This allows a conservative but quantitative estimate of $\chi_k(i)$. The second issue is resolved by applying survival analysis methods to the censored data and is described in more detail below.

The *E.coli* counts at the post-bleeding stage were very high, often so high that it was not possible to accurately count them all (this was also true for a few counts at other

stages). In these cases a value of 10000 was used, which was equivalent to twice the maximum countable limit. However, sometimes it was possible to count more, in which case the actual counts were used.

In order to use survival analysis methods, we must specify some minimum proportional change for those censored data points (such that we are confident that the proportional change is greater than some value x). Therefore, for the case where the "before" stage (k-1) is censored, but the "after" stage (k) is uncensored, we should determine the maximum possible value of the censored data (i.e. the maximum concentration of *enterbacteriaecae*). However, where the "after" stage is censored, but the "before" stage (k) is uncensored, we must determine the minimum possible value of the censored data. (In the case of both points being censored, we must use the maximum value for the "before" stage, and the minimum value for the "after" stage). The maximum concentration was hypothesized to be 1,000,000cfu/cm^2 (P. Richards, Nottingham University, *personal communication*). Given the much lower values for most samples, this maximum was considered to be too restrictive and it was decided to use a 99% value instead (such that you would expect 99% of the concentrations to be less than that value). This value was estimated to be around 100,000cfu/cm^2. The minimum concentration that could not be reliably counted was estimated to be **800 cfu/cm^2** (P. Richards, Nottingham University, personal communication).

Therefore in the extreme case where subsequent stages are censored, the minimum proportional change would be 800/100,000, and so $x = 8*10^{-4}$. In this case, we can only say that the proportional change is greater than $8*10^{-4}$. However, this double-censored situation only occurs once throughout the dataset.

As our data is censored such that we do not know the maximum count, only that it is greater than a certain number, we have type I censored data (also know as right censored data) (Krzanowski, 1998) and so we can employ survival analysis methods as described by Andersen & Vaeth (1988). The likelihood function, *L,* for type I censored data from a probability distribution *f(t)* with cumulative distribution function *F(t)* is

$$L = C\prod_{i=1}^{r} f(t_i)\prod_{i=1}^{n-r}\left(1 - F(t_i^{+})\right),$$

where t_i, is the i'th uncensored observation, t_i^+ is the i'th censored observation, *r* is the number of uncensored data points, *n* is the total number of observations (hence *n-r* is the number of censored data points) and *C* is a constant. From this we can calculate the maximum likelihood estimate (MLE) using standard methods. For these data the most appropriate distributions to fit are either an exponential distribution

$$f(t) = \lambda e^{-\lambda t},$$

where λ is the reciprocal of the mean (such that the mean of the exponential distribution is $1/\hat{\lambda}$), or a Weibull distribution

$$f(t) = \frac{\alpha}{\beta^{\alpha}} t^{\alpha-1} e^{-\left(\frac{t}{\beta}\right)^{\alpha}},$$

where *α* and *β* are constants that govern the shape and scale of the function. (Note that the exponential distribution is just a particular form of the Weibull distribution when α approaches 1). We choose to use the Weibull fitted distribution where possible as this distribution can produce more biologically-plausible distributions that take into account variability between individual bacteria.

For the exponential distribution the MLE estimate, $\hat{\lambda}$, for type 1 censored data is:

$$\hat{\lambda} = \frac{r}{\sum_{i=1}^{r} t_i + \sum_{i=1}^{n-r} t_i^{+}}.$$

For the Weibull distribution the MLE estimate for $\hat{\alpha}$ can be found by solving:

$$\frac{r}{\alpha} + \sum_{i=1}^{r} \ln(t_i) - \frac{r\left(\sum_{i=1}^{r} (t_i)^{\alpha} \ln(t_i) + \sum_{i=1}^{n-r} \left(t_i^{+}\right)^{\alpha} \ln\left(t_i^{+}\right)\right)}{\sum_{i=1}^{r} \ln(t_i)^{\alpha} + \sum_{i=1}^{n-r} \ln\left(t_i^{+}\right)^{\alpha}} = 0.$$

This estimate can then be used to calculate $\hat{\beta}$

$$\hat{\beta} = \left[\frac{1}{r}\left(\sum_{i=1}^{r} (t_i)^{\hat{\alpha}} + \sum_{i=1}^{n-r} \left(t_i^{+}\right)^{\hat{\alpha}}\right)\right]^{\frac{1}{\hat{\alpha}}}.$$

Following this method we were able to fit either Weibull or exponential distributions to the proportion change in concentration between each stage in the abattoir (in the case of the Weibull distribution the equations were solved using Microsoft Excel's SOLVER routine), which are shown in Table 2. The SOLVER routine is an iterative process, which continually re-estimates the parameter values until the relative difference between the previous five estimates is less than a pre-defined convergence value (we used 0.0001). As such it is not always guaranteed to work as the parameter estimates may well diverge instead of converge in which case a solution would not be reached. In these cases we used the MLE estimate for the exponential distribution (which does not require SOLVER).

Table 2: MLE parameter estimates for proportion change in concentration at each abattoir stage. Distribution are either Weibull(α,β) or exponential(λ).

Abattoir Stage (k)	**Fitted exponential parameter (**λ)	**Fitted Weibull parameters (α,β)**
Post-Scald	2.353039	(0.6844, 0.1290)
Post-Dehair	0.002832	(0.4514, 150.7530)
Post-Singe	205.7326	-[a]
Post-Polish	0.4700	(0.7809, 1.5809)
Post-evisceration	0.0321	(0.3540, 1.6305)
Pre-chill	0.9264	(0.5880, 0.3438)

[a] Excel SOLVER failed to fit a Weibull distribution to the data for this stage

As an example Figure 3 shows the cumulative distribution functions for different fitted distributions to the change in concentration data between scalding and de-hairing. The red line is the distribution fitted by the censoring method, which is an exponential with parameter λ=0.0034. The green line is the distribution fitted by the computer package @Risk (Palisade Corp. ©) using standard methods, which is an exponential distribution with parameter λ=0.0040. The blue line represents the Weibull-fitted distribution. It can be seen that the non-censored exponential distribution has a lower probability of producing high proportional increases than the Weibull censored fit. Hence taking into account censored data allows for higher values, which may be missed through restrictions in sampling.

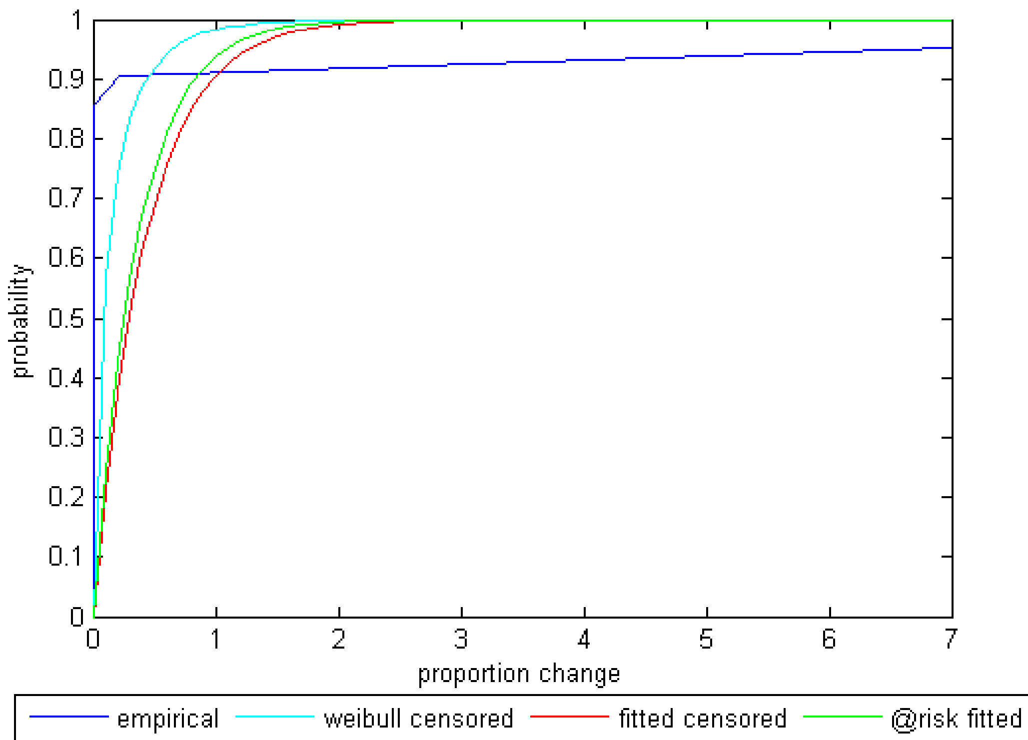

Figure 3: Comparison of fitted distributions to the censored data for proportion change between bleeding and scalding.

3.4.1 Initial concentration

To estimate the initial carcass concentration of VTEC O157 we use the concentration of *E.coli* post-bleed from the Nottingham. As this is the first stage in the abattoir and we know the concentrations here, it is not necessary to know the concentration before this. While this data is for all *E.coli* not just VTEC O157, it is possible that it may overestimate the concentration of VTEC O157.

To model this we fitted distributions to the post-bleed counts using the censored method described above. The fitted exponential parameter was λ= 0.000251. The fitted Weibull parameters were α=1.0407, β=3981.7187. The plotted distributions along with the empirical distribution and exponential and gamma distribution fitted using @Risk are shown in Figure 4.

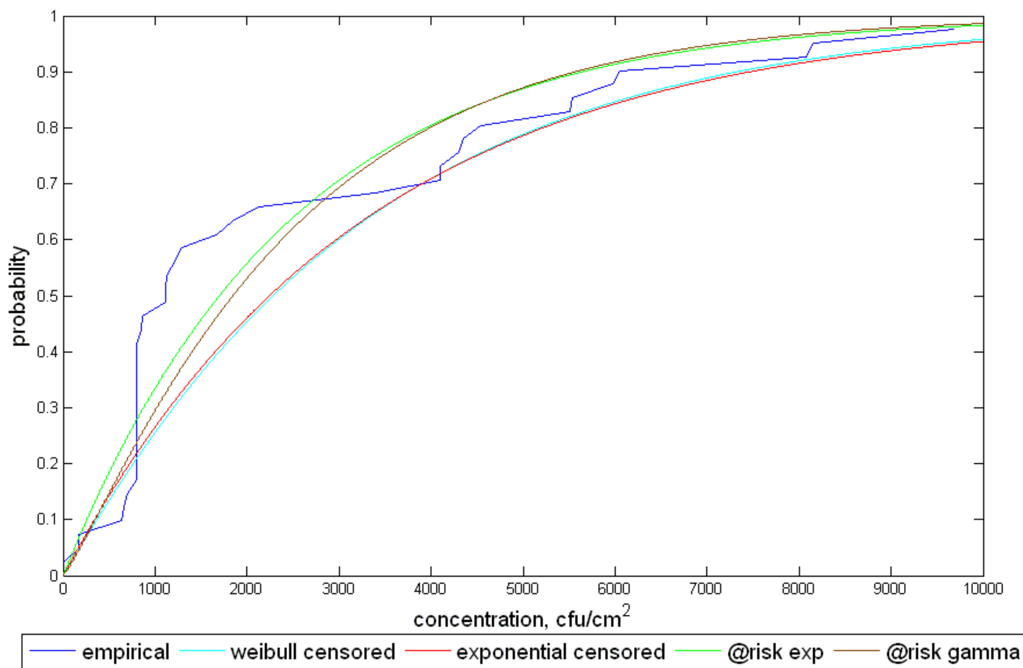

Figure 4: Comparison of fitted distributions to the censored data for counts of *E.coli* post-bleed.

It can be seen that there is very little difference in the fit between the exponential and Weibull distributions fitted using the censored approach (which is to be expected as the Weibull distribution approaches the exponential as α tends to 1). However we can see that there is a big difference in the fit compared to the distributions fitted that do not account for the censoring, as would expected they give less chance of higher values. From Figure 4 we can see that there is a sharp rise in the empirical distribution at 800, which is represents the abundance of censored values. This highlights the need for fitting a distribution to the data so that we can estimate the actual variability in numbers without the sharp increase at the censored values.

As there is little difference in the fitted distributions we use the exponential distribution (λ= 0.000251) as the analysis on the sample data suggests that the data is well approximated by this distribution.

3.5 Interventions and further investigations

3.5.1 Post-De-hairing

One intervention thought to be efficient at the abattoir is the inclusion of a washing and drying measure after the de-hairing stage. Nottingham University collected data on this measure. Over the course of two days, they collected data on the concentrations of bacteria on pig carcasses at different stages of the abattoir (post-bleeding, post-de-hairing, post-singeing and pre-chilling). The experiment consisted of a control run, a run with a washing measure and a run with a washing and drying measure. Data were collected from 8 pigs on each of the 2 days.

The relevant part of these data for the intervention measure is the concentrations post-de-hairing. The proportional change in concentration between post-bleeding and post-de-hairing between the different runs is shown in Figure 4. It can be seen that washing alone does not tend to increase the efficiency of the removal of enterobacteriaceae, although washing and drying does tend to increase removal efficiency to a small degree.

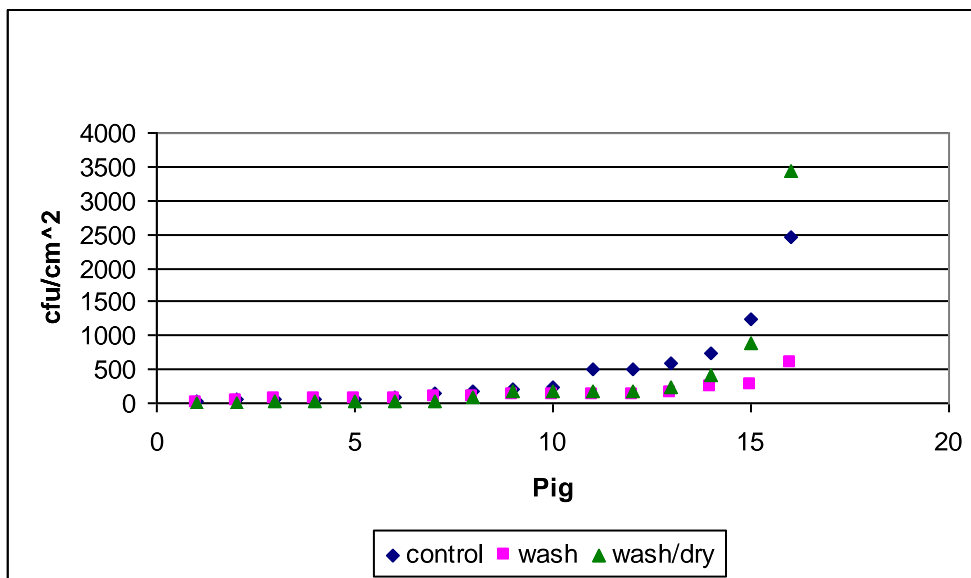

Figure 5: Comparison of proportion change of *e.coli* between post-bleed and post-dehair.

It should be noted that this is only a small sample of data, which includes one case in the control study where the concentration on a carcass went up. From such a small study it is difficult to tell how often this is likely to happen and consequently if the washing and drying intervention will effectively stop this. It can be seen that the washing and drying intervention has little effect in the cases where the concentration is reduced to less than 10% of its original value. However, washing and drying

appears to be more consistent in reducing concentration than washing alone. Again, this is only a small study and further research should be undertaken before it can safely be assumed that applying the washing and drying measure will always have this effect.

To model this intervention we ran a simulation using a distribution fitted to the values for proportion change between post-bleed and post-dehair for the control data and then run simulations with the wash only and wash/dry values to investigate the effect this has on the prevalence and concentration of *E.coli* at the end of the abattoir process (and later the model will be run to determine the effect it has on the number of human cases). Exponential distributions were fitted using @Risk (Palisade Corp. ©). The parameter estimates are shown in Table 3.

Table 3: MLE exponential distribution parameter estimates (λ) for proportion change in concentration between post-bleed and post-dehair

Intervention	Fitted exponential parameter (λ)
Control	8.1162
Wash	33.4102
Wash/Dry	63.8447

4 Results

4.1 Baseline results

The model was run for 5000 iterations. In each iteration, 80 batches of pigs go through the abattoir. The average batch prevalence at each modelled stage is shown in Figure 2 and the distribution of concentrations between batches is shown in Figure 3. It can be seen from these figures that both the prevalence and concentration increase at the dehairing stage (the concentration increases from the order of 0.01 cfu/cm^2 post-scald to the order of 10 cfu/cm^2). The average concentration of *E.coli* on the carcass after singeing is generally quite low; however, the concentration between batches is variable and there and a few batches are more highly contaminated than the average concentration (from the order of 10^{-4} cfu/cm^2 post-singeing to the order of 0.01 cfu/cm^2 post-evisceration).

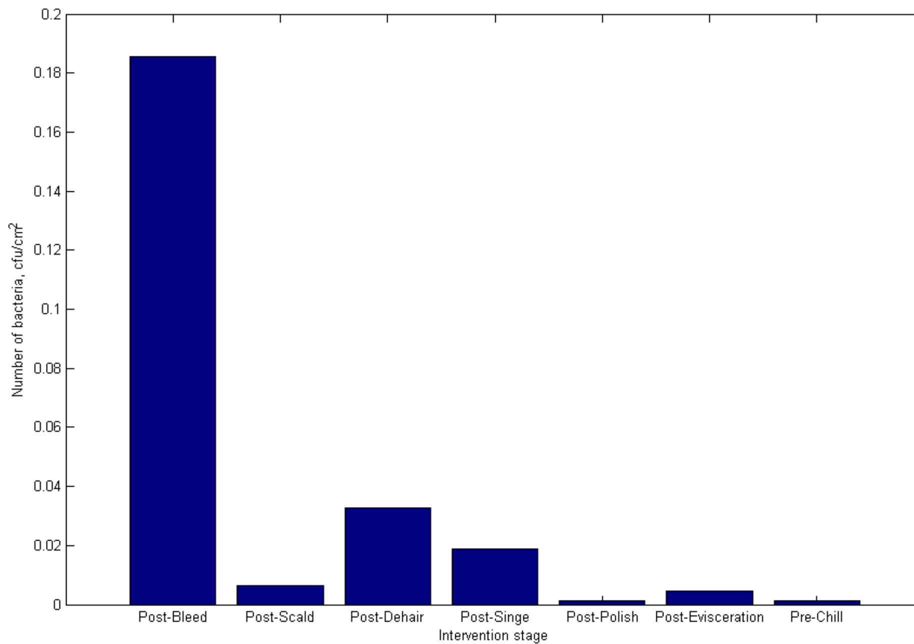

Figure 6: Average prevalence of carcass contamination at different stages of the abattoir.

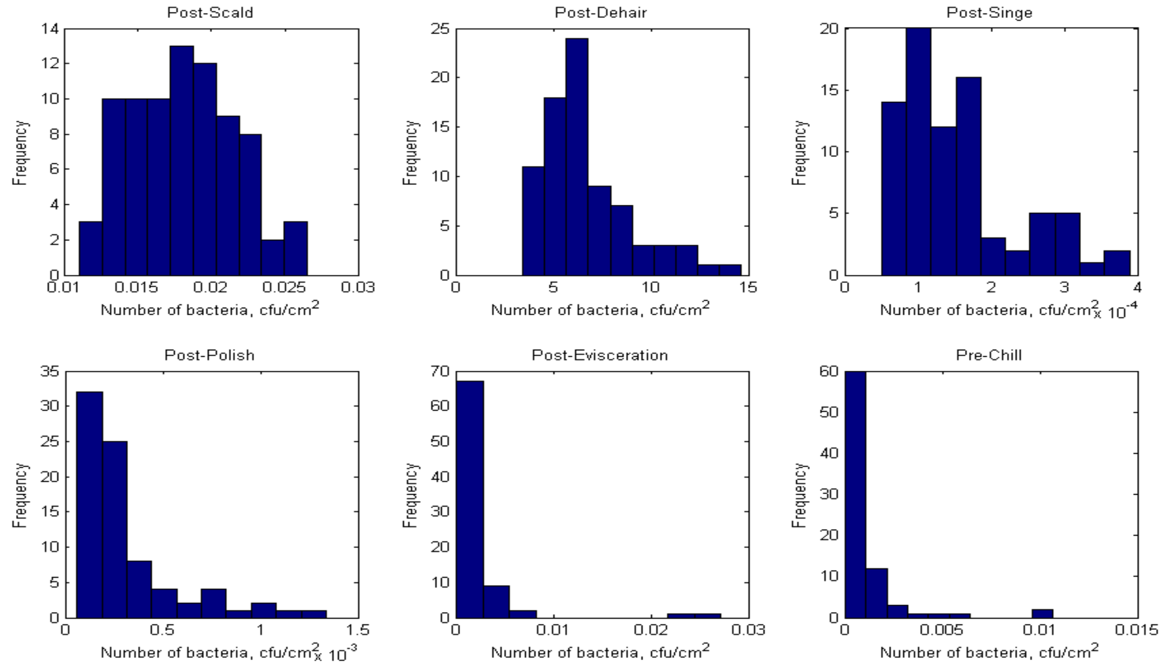

Figure 7: Distribution of concentration (cfu/cm^2) of *E.coli* on carcasses between batches at different stages of the abattoir.

4.2 Post-dehair intervention

Table 4 and Figure 8 show the average concentrations of *E.coli* at the different stages in the model, for the different interventions. Calculating the proportion change in concentration between post-bleed (before the intervention) and post-dehair (just after the intervention) we see that the control gives a 55% reduction. The wash intervention gives an 89% reduction and the wash/dry intervention is the best giving a 94% reduction. However looking at the concentrations immediately prior to chilling we that there is a 53.772% reduction from the average baseline concentration pre-chill from the baseline to the wash only intervention, but the wash/dry intervention only reduces the concentration by about a further 2% (55.498%). However, much of this reduction is achieved by eliminating high concentrations present on carcasses pre-scald; the effect on lower concentrations is less prominent. The first graph in Figure 8 shows the concentrations at all stages, where it can be seen that the concentrations after singeing are much smaller than at bleeding and dehairing. The second graph shows just the stages after dehairing so it can be seen more clearly

that the wash and wash/dry interventions still result in a reduction in average concentration at these stages, when compared to the control run.

Table 4: Average concentrations, cfu/cm^2, of *E.coli* at different stages of the slaughterhouse for interventions at the de-hairing stage

	Post-Bleed	Post-dehair	Post-singe	Post-polish	Post-evisceration	Pre-chill
Control	11.9250	5.3275	1.4476e-008	2.0156e-008	1.3694e-007	5.3530e-008
Wash	11.8828	1.2857	3.3404e-009	6.6159e-009	6.0312e-008	2.4746e-008
Wash/Dry	11.9255	0.6744	1.7651e-009	2.9839e-009	3.3583e-008	2.3822e-008

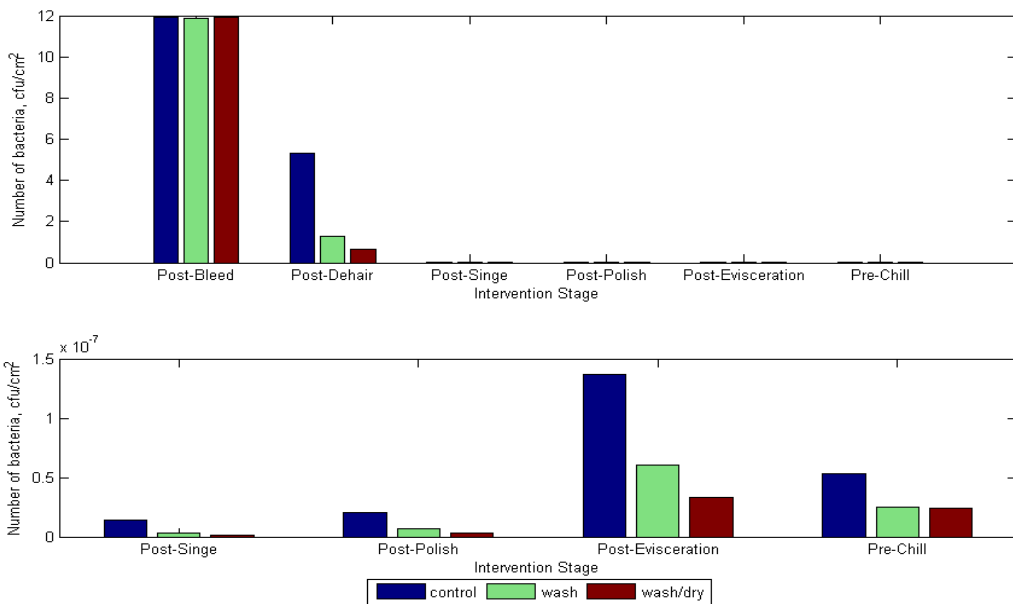

Figure 8: Average concentrations of *E.coli* at different stages of the slaughterhouse for interventions at the de-hairing stage

As the parameter for the proportion change post-dehair had to be estimated as the change between bleeding and de-hairing, rather than between scalding and deharing, the results for the post-dehair interventions should not be compared with the results from the main model.

5. Discussion

The data provided from project FT5069 has allowed us to update the current *Salmonella* pig risk assessment model to estimate the variability of concentration of *E.coli* on contaminated pig carcasses at different stages in the abattoir.

The results of the model show that the prevalence and concentration of *E.coli* can vary significantly between different stages of the abattoir. It shows that even though the singeing stage is very effective at reducing both prevalence and concentration, both can increase again by the time the carcasses reach the chilling stage. This is thought to be due to faecal leakage and subsequent cross contamination. The model shows that while the concentrations are generally low (<0.005 cfu/cm^2), a small number of carcasses may be contaminated with higher concentrations (> 0.01 cfu/cm^2) at the post-evisceration and pre-chill stage. It is these more heavily contaminated carcasses that are likely to be responsible for human cases of *E.coli.*

Due to practical and financial restrictions, the data provided was of a smaller sample size than ideal for risk assessment. While the dataset was incomplete to do everything intended at the outset, we were able to achieve the main intended output. We were able to use the *E.coli* data to estimate probability distributions for the proportional change in concentration levels contaminating carcasses for the following stages: bleed to scald; scald to de-hairing; de-hairing to singe; singe to polish; polish to evisceration; evisceration to pre-chill. These probability distributions represent a significant improvement in the modelling of the processing stage, where the variation in the change in concentration between carcasses has been accurately captured for the first time. This is significant because it is the rare occasions, e.g. where a carcass is heavily contaminated but receives an inefficient singe, which will contribute most to the risk of human infection.

While we have been able to use these data, there are significant caveats that should be noted. With only 10 samples at each abattoir and inconsistent data collection points between abattoirs, the parameter estimates, which aim to be representative of the real life data for the whole of the UK, will have significant error margins. Due to the small sample size the censoring of data points also becomes a significant problem as it reduces further the number of accurate values.

The data provided for intervention modelling was limited. The washing and drying data collected at de-hairing provided useful data for modelling interventions. Unfortunately, no data were collected at the scalding stage. Scalding is potentially a significant step to have data for as bacteria counts tend to increase between scalding and de-hairing. Missing out the scalding stage means that the distributions have to be fitted between bleeding and de-hairing and thus do not take account of this increase. Therefore, the results of the de-hairing interventions and the results of the main model should not be compared, particularly because the small sample sizes for the intervention study means that there are large error margins associated with all estimated distributions.

The results from the dehair intervention suggest that while the proportion decrease in *e.coli* carcass concentration post-dehair from the wash/dry intervention is 5% more than the wash only intervention, this gap is reduced to less than 2% by the time the carcasses reach the chiller. This is likely due the fact that the singeing stage removes a large proportion of bacteria from the carcass anyway, but then contamination occurs again at processing and evisceration, which is largely independent of what happens at dehairing. This suggests that focussing on intervention strategies at the start of the abattoir process may not be the most effective method and a method to reduce the increase in contamination at evisceration may be more beneficial.

6. References

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Appendix 3: Processing model framework for cattle and sheep

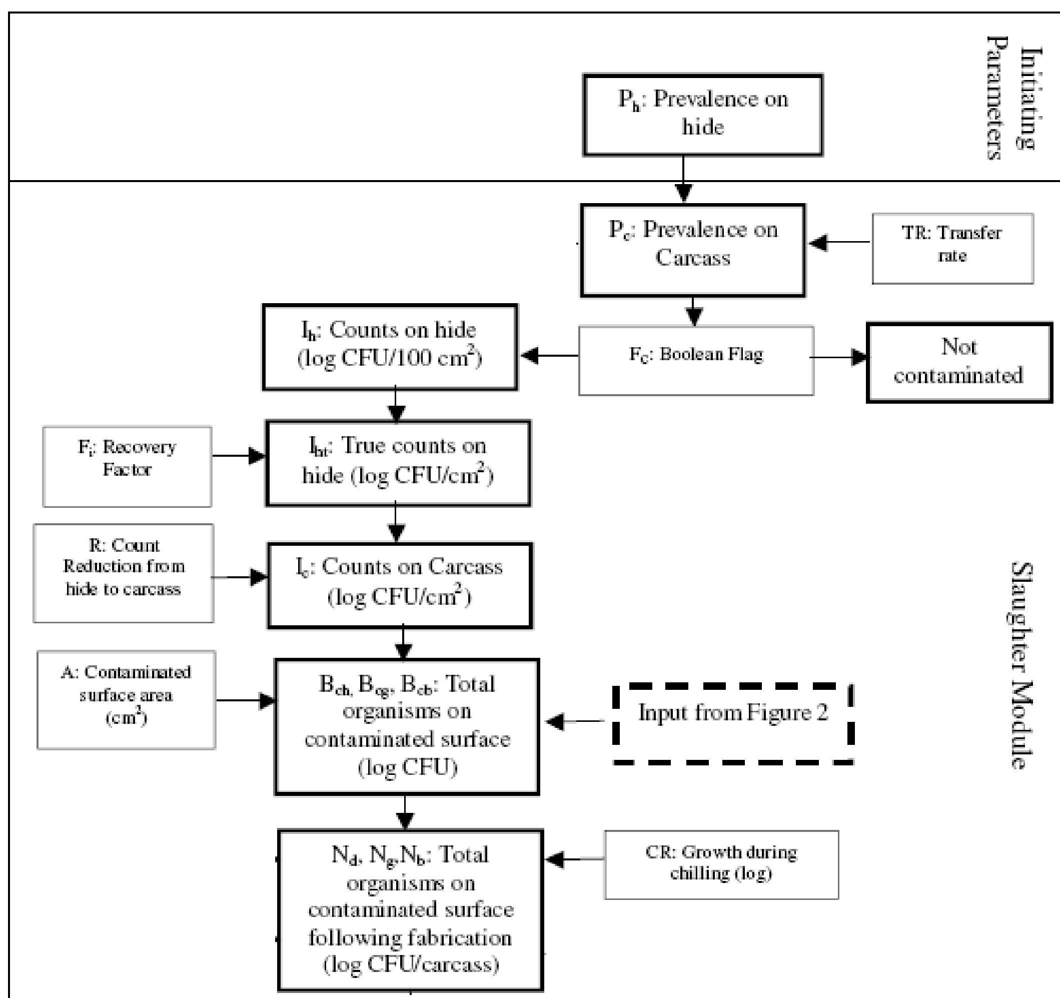

Figure 1: Representation of the model framework for processing of cattle and sheep (Adapted and copied from Cummins et al*.,* 2008)

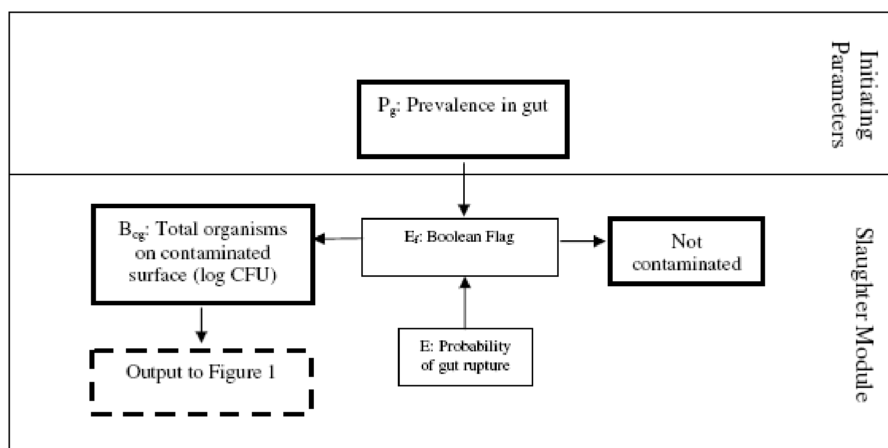

Figure 2: Module pathway for contamination of carcass during evisceration (Copied from Cummins *et al.*, 2008)

Appendix 4: Minutes of WP 4 and 5 First Meeting

WP4 and 5 kick-off meeting

VLA Weybridge

23-24th July 2007

Attendees

Andy Hill (AH), VLA, UK
Anne Margrete Urdahl (AMU), Norwegian Zoonosis Centre, National Veterinary Institute, Norway
Emma Snary (ES), VLA, UK
Franklin Georgsson (FG), Matís, Iceland
Hildegunn Viljugrein (HV), National Veterinary Institute, Norway
Jukka Ranta (JR), Evira, Finland
Line Vold (LV), Norwegian Institute of Public Health, Norway
Pádraig Nally (PN), VLA, UK
Sara Monteiro Pires (SMP), Danish Zoonosis Centre, Danish Technical University, Denmark
Telmo Pina Nunes (TPN), Faculty of Veterinary Medicine, University of Lisbon, Portugal
Terhi Virtanen (TV), Evira, Finland
Tine Hald (TH), Danish Zoonosis Centre, Danish Technical University, Denmark

Welcome and introductions

ES welcomed attendees to the VLA and gave a brief account of its role and history. JR and ES described the aims of WP4 and WP5 and explained what the objectives of the kick-off meeting were. Attendees then introduced themselves and ES passed on apologies from Helen Clough (HC), University of Liverpool, UK.

Attribution of human VTEC infection - a feasibility study - Tine Hald

TH gave a presentation on the feasibility of attributing human VTEC infections using the Danish *Salmonella* attribution model. It emerged that estimates of consumption are not necessarily required for this model. The most important assumption behind

this model is that there is a heterogeneous distribution of subtypes between species and that discriminatory, definitive and repeatable microbiological methods (e.g. serotyping) exist for identification of these subtypes. These methods need to be discriminating without being too discriminating because too many subtypes can cause problems for the model. A discussion of typing methods for VTEC followed. Typing of VTEC is more problematic. It emerged that some countries only look for VTEC O157 and don't look for other VTEC strains. Also, while O-typing is sufficient for VTEC O157, H-typing is required for non-O157 VTEC. For example, a strain can have the same O-type but different H-antigens in farm animals on the same farm. Some of the typing methods considered were PFGE, phage typing (as practiced in Denmark) and antibiotic resistance profiling. It was agreed that this project could be seen as a proof of principle if suitable data for a particular country was available.

TH also gave a brief outline of *Campylobacter* attribution in New Zealand It was agreed this was a useful study since there is no importation of meat into NZ. It was also agreed that MLST was a suitable method for the typing of *Campylobacter* strains.

Action point 1 (AP1): PN to send contact information for people involved in investigating VTEC in Scotland (Chris Low, Scottish Agricultural College (SAC) and John Cowden, Health Protection Scotland) to TH.

AP2: PN to provide link to UK abattoir survey and contact information for Geraldine Duffy, Teagasc, Ireland (regarding antimicrobial resistance testing) to TH.

Source attribution & *Campylobacter*, some notes about modelling - Jukka Ranta

JR outlined some issues that arise when attributing cases of *Campylobacter* infection. In Finland, on an annual basis, outbreaks may have a minor role in the total number of *Campylobacter* infections – but this may occasionally be distorting the picture in monthly records. Also, information on the role of travel abroad is missing from 25% of human cases in the Finnish infectious disease register. This creates uncertainty but can still be accounted for in the model. Finland practices monthly reporting of cases in both humans and animals. However, weekly data is sometimes available and when this is studied, cases for a particular subtype may appear in humans before they appear in animals as pointed out in a Welsh study (R.J.

Meldrum, et al: The seasonality of human *Campylobacter* infection and *Campylobacter* isolates from fresh, retail chicken in Wales. *Epidemiol. Infect.* (2005), 133, 49-52). Animal sampling in Finland is carried out on a risk basis and since there is a lower risk in the winter, fewer samples are taken at this time. This has consequences on statistical inference regarding prevalence, leading to wider confidence intervals for the winter months. JR also explained that including subtyping information in the model causes difficulties partially because some subtypes can be found in more than one animal species. TH agreed saying she didn't recommend including subtype information but that this could be discussed in the final report and that the approach used in New Zealand should be described. Therefore, JR suggested attributing cases in humans from animal sources without taking subtype into account since it is currently not clear how the subtyping information should be interpreted towards source attribution. At the simplest level, this would involve looking at (all *Campylobacter*) cases from broilers versus all other sources. If a MRA model is used to attribute cases of *Campylobacter* infection in humans, temporal rather than survey data is required as this allows the calculation of the percentage of cases attributed to a particular source. Validation of JR's model could come from e.g. outbreak studies, case-control studies of sporadic cases and the results of PFGE analysis, if available. It was suggested that time series analysis might be a useful modelling approach and that this would allow the incorporation of other variables, such as temperature, in the model. Another issue discussed was the modelling of zero samples from the surveillance data. TH recommended treating these as true zeros because the posterior would then be equal to the (uninformative) prior which is e.g. Uniform(0,1)-distribution, leading to unrealistically wide CI's. JR suggested that a Markovian model of prevalence would allow borrowing strength from neighbouring time steps which enables better estimation of prevalence for those steps with zero samples. (Likewise, hierarchical Bayesian modelling in general could be used in cases of missing data). There was also some discussion regarding the use of quantitative data (i.e. bacterial counts as well as prevalence) in the model (TH has quantitative data for *Campylobacter* in Danish and imported poultry, while FG said similar Icelandic data may be available) and whether the model can attribute cases of human infection for different groups, for example, age brackets. The starting point of the attribution model was then discussed. Two starting points could be used, source (i.e. retail) data or reservoir (i.e. primary production) data. It was suggested that using primary production data allows the influence of control measures to be assessed, for example the effect of a particular measure on the summer peak of *Campylobacter* infections. Thus the starting point as such may have no effect on the model structure

(since the model parameters implicitly represent remaining effects, and any variables can be taken as explanatory variables in a statistical model that looks at statistical associations). However, the choice of starting point can matter if risk management interventions at specific points in production chain are to be assessed. These should then be represented by appropriate model structures.

Human *Campylobacter* infections in Finland - Terhi Virtanen

TV presented a synopsis of Finish human *Campylobacter* infections. She explained that most of the infections are sporadic, more than half of them are in travellers and that in recent years waterborne cases were very common. Large outbreaks are usually waterborne. There is a large peak in infection during the summer and *C. jejuni* is the most common cause of infection.

A discussion of *Campylobacter* data availability in other countries ensued following the description of the Finnish situation. It was agreed that, ideally, time series data from humans, broilers and other species (exposures) was required and that the sampling and testing method used should be taken into consideration. LV said that Norway compiled monthly statistics on human *Campylobacter* infections. FG said that Icelandic data going back 20 years in humans and 6 in broilers was available and that quantitative data would be available from research work since 2000. AMU said that Merete Hofshagen should be contacted for Norwegian data and poultry (including turkey) data is available. ES explained that until recently the UK did not routinely collect poultry data. Therefore it would be necessary to contact each poultry company separately for historical data. However, there was a study conducted in Wales (Meldrum *et al.*, 2004 J. Food Prot. 67(6): 1226-1228) that may be useful. PN said that a retail survey for *Campylobacter* in poultry in Ireland had been proposed but wasn't sure if it had been completed. TH said that Denmark gathers information on broilers and humans but that little information on other species (apart from some turkey data) was available. She also said that there were some (disjointed) yearlong surveys conducted and that random sampling of imported food was carried out. TPN said that no human *Campylobacter* data is available in Portugal but that a small number of food studies were available. There was some discussion of the nature of the data in each country. In particular, JR asked if the data was broken down by subtype, and whether this should be looked at, and FG said it was in Iceland at least for human cases while TH said speciation of *Campylobacter* strains does not routinely occur in Denmark. A discussion was held regarding the inclusion of

outbreak data in the model. It was agreed that the model would most likely have to be adapted for each country because of data availability issues although it might be studied if it was possible to define some parameters through hierarchical modelling based on several countries.

The availability of consumption data from various countries was discussed. For example, Finish consumption is known but a polling company owns the data and the raw data would need to purchased. TH said that consumption and production data was available in Denmark and that the production data may be better as it is then possible to differentiate between domestic and imported food. FG said that Icelandic production data was available and it was agreed that this would be good data since there are very limited raw meat imports into Iceland. LV suggested that the raw data should be obtained as this allows the breakdown of consumption by age group. PN said that consumption data from the then Ministry of Agriculture, Fisheries and Food (MAFF) had been used in previous risk assessments and ES said that a nutrition surveys were now carried out in the UK but that the data obtained is often difficult to analyse.

JR said that he will email workshop participants over the summer with the aim of obtaining the data by the end of September and that a statistician will commence work on the Finnish data in September before progressing data from other nations. HV said she has experience of time series analysis and is available to analyse the data if required, which was a welcomed suggestion.

AP3 JR to email workshop attendees with a formal request for Campylobacter *infection data in both humans and animals and consumption information. Workshop attendees will provide any available data by the end of September. The deadline for the provision of data will be the end of September.*

AP4 PN to see if Irish retail survey went ahead.

Risk assessment for VTEC O157 in meat - Pádraig Nally

PN outlined a QRA model for the attribution of VTEC from pork, beef and lamb products. He explained that the model was originally developed for the UK and used a modular approach. WP5 will update and adapt this model to attribute cases of VTEC infection in the UK. However, the feasibility of using the model to attribute

O157 and non-O157 infection in other countries will also be investigated and key data requirements will be identified. The updated model will start with animals presenting for slaughter and will model the production of whole and minced beef, whole and minced lamb, whole pork and sausages. ES cautioned that pork products consumed in the UK might not be produced using UK pork and the same may be true for beef and lamb products. PN outlined the model and suggested some adaptations (for example, home storage and cooking practices) based on previous experience with an Irish risk assessment for minced beef. The model is currently a first-order one but consideration will be given to making it into a second order one. The model currently uses the FSIS dose-response equation. ES suggested that the dose-response section of the WHO/FAO inception document for VTEC risk assessment be considered and a discussion about dose-response modelling ensued. The suitability of using the same dose-response model for different countries was raised. TPN asked about the problem of attributing sources relatively versus attributing human cases relatively, saying that the relative shares of dose exposures resulting from different food types may not be the same as the relative proportions of corresponding human cases due to nonlinearities in dose response curve and so on. ES explained that use of a dose-response model allowed estimation of the relative importance of different meat products as a source of VTEC infection and there was agreement that human exposure to VTEC should also be an output of the QRA. Other papers focussing on dose-response modelling, including those of Teunis, Strachan and some Japanese studies, were identified. PN was asked if growth is modelled in the existing model and he explained that a first order kinetics model was used. LV suggested that pH might be an important parameter to include in the growth model.

As regards attribution of VTEC infections in other European countries using the UK attribution model, contact with members of WP2 was suggested. Also information on surveillance of non-O157 VTEC is required (including methods used) and it was suggested that EnterNet contacts might be able to help with this. Finally, it was suggested that the UK model be described on a module-by-module basis so that it would easy for other countries to use the modules relevant for their situation.

AP5 PN to study other dose-response approaches

Risk assessment for VTEC O157 in milk - Helen Clough/Emma Snary

ES presented HC's risk assessment for VTEC from on and off farm pasteurisation of milk. Comments and questions arising from the presentation were collated for forwarding to HC. These include:

- For the on-farm model, can HC clarify whether bottling occurs on the same farm as pasteurisation or whether the pasteurised milk is bottled off-site?
- In response to HC's request for information regarding the prevalence of on-farm pasteurisation in other countries, it was reported that only a tiny amount of milk, if any, in Norway, Portugal, Iceland and Finland is pasteurised on-farm. However, TH related a case in Denmark where a small organic dairy receives milk from the farmer's own milk production as well as from other farmers. A couple of years ago, the dairy was implicated in an outbreak related to pasteurised milk, which may have been triggered by a sudden increased in consumer demand for the dairy's products
- Does HC know if there are differences in VTEC shedding and prevalence between different breeds of cows?
- Is the collection of milk from farms understood? How many farms contribute to a tanker of milk and how far does the tanker travel from the dairy?
- How are sporadic and outbreak cases incorporated into the model and is it possible to calculate the number of cases resulting from one pasteurisation failure?
- Is recontamination of pasteurised milk included in the model?
- Does the model use prevalence in cows to model prevalence in milk? If so, information on the prevalence of non-O157 VTEC in cows could be used to calculate the prevalence of VTEC in milk. Otherwise, it may be difficult to estimate the prevalence of non-O157 VTEC in milk.
- TH asked whether the model could be used/adapted to assess the risk from cheese made with raw milk. ES commented that the FSA conducted a review of *Listeria* spp. in European cheeses, which included the gathering of information regarding processing methods, and that she could provide this data if asked.
- Finally, details on the time and temperature combination used to pasteurise milk in the UK was requested.

AP6 PN to send list of questions and comments to HC

AP7 HC to respond to questions by Friday, August 10th, 2007

Any other business

It was agreed that the presentations from the meeting would be made available on the private, and therefore password protected, project website. The presentations will be converted into *.pdf files before being uploaded.

AP8 PN to convert presentations and contact Danica Grahek-Ogden regarding password access to the project website

Appendix 5: Minutes of WP4 and 5 Second meeting

CampEc-NET, WP4-5, 2nd workshop, Evira/Helsinki. April 17-18, 2008

Presentations

Invited quest speaker: Aamir Fazil, Health Canada:
(1) Campylobacter risk modelling,
(2) Canadian risk attribution activities: C-EnterNet.

Invited quest speaker:
Marja-Liisa Hänninen, Helsinki University:Molecular typing and source attribution

Hanne Rosenquist, DTU: Danish Campylobacter data

Jukka Ranta, Evira: Source attribution modelling
Different approaches for source attribution problem were discussed: (1) the approach starting from reported human cases, attributing them all to source groups, (2) the approach starting from a specific food chain or exposure path, calculating the case burden for each described pathway, (3) observations from direct population experiments (e.g. withdrawal of all broiler foods, thus eliminating one pathway and observing the result), (4) detailed studies of patients. These can all be applied either in a cross sectional or temporal setting. The approaches can exploit bacterial (sub)typing data and/or temporal changes over time, obtained from regular surveillance data. It was then explained that the data in this project were collected from Finland, Norway, Denmark & Iceland, and consisted of existing time series (monthly data) for both human cases and monitored source(s) only, regarding Campylobacter spp., or at most with species level information and some consumption data. The statistical approach could then study the temporal patterns, distinguishing between Campylobacter species where possible. The number of source groups would be mostly limited to 'broiler' vs. 'all other', or at most using partially similar data from cattle, pigs and turkey (e.g. partial time series from Finland). The simple model of competing risks with Poisson intensities was then explained, parameterising the monitored sources and discussing a 'noise' model for

the unmonitored remaining sources. Preliminary results with Finnish data were shown and some more examples with an even more simplified toy experiment were given in a separate handout

Hildegunn Viljugrein, NVI:
Norwegian Campylobacter data

Rowena Kosmider, VLA:
An outline of progress on WP5 was presented including an overview of the two tasks involved (task 1: quantitative risk assessment, task 2: sub-typing approach within a Bayesian framework). The first task was outlined by a presentation on the VLA Quantitative risk assessment models for pigs, sheep and cattle in which each risk module (e.g. farm, processing, retail, preparation and consumption) were described. A different approach was being used for each species' processing models: for pigs, data on generic E. coli was fitted to a distribution to estimate the log change in concentration through the processing steps; for cattle and sheep, a modular process risk model approach was being used. All species will feed into a single retail to consumption model in which the risk of consuming VTEC directly and indirectly from the meat products is estimated. An update on the University Liverpool milk model was also presented. For Task 2 of WP5, it was noted that Tine Hald's group had ascertained that, at the moment, it was not possible to assess the feasibility of using the sub-typing approach to attribute human cases of VTEC infection to specific sources. In summary, the presentation outlined that the work for Task 1 was still in progress. Within the work-package, two approaches for attribution were being used namely farm-to-fork quantitative risk assessment (QRA) and estimating the source of human cases linked to animal data within a Bayesian framework. For the QRA approach, several points of discussion were raised including: there were few points in the farm-to-consumption chain in which to validate the model (e.g. slaughter prevalence, retail products prevalence, comparison with human cases), the model is currently single order but could be 2nd order, there is an on-going issue with data quality for inputs, which dose-response model to use would impact on the number of human cases estimated by the model and lastly, the preparation to consumption chain can be considered a "black-box" describing highly variable habits.

Discussion (summary of both days)

-Data limitations

Campylobacter: a review of current Campylobacter situation in Nordic countries has been written and this can be evaluated in the context of source attribution. However, this review does not cover all data available in Nordic countries, focusing on published literature and data sent from project partners. Basically, data concerning other sources than broiler is very limited, as well as (sub)typing information, and data from the retail level are scarce. Even if data were available on common food pathways, data related to other exposure routes such as recreational waters and pets would be hard to collect. It is implausible that extensive data on all exposure pathways would be obtainable representatively. Therefore, modelling methods need to be developed to account for the biased, flawed or otherwise sparse and limited data, choosing the model scope appropriately with only such details that allow sound estimation. Concerning Nordic countries, the role of broiler import is negligible except for Denmark, which complicates the analysis since there are two categories of broiler: imported and domestic, both affecting the human case incidence. VTEC: The main data limitation highlighted within WP5 was the lack of prevalence data for sources other than cattle for which to link human cases with the animal sources. This data gap has led to the conclusion that, at the moment, the feasibility of the microbial sub-typing approach can not be assessed. Other data limitation specific to the QRA are the lack of scientific studies which follow the same carcass through the processing chain in order to estimate the change in concentration/prevalence of VTEC during processing.

-Use of models in different contexts

Campylobacter: statistical models are less detailed but easily adapted to other countries when based on similar data structures. Detailed pathway models are tailored to describe one ‘system’ and therefore difficult to apply to a different system. Statistical model based on reported human case time series data as well as broiler (and possibly other food animals, or retail) surveillance data can describe Nordic countries since such data are available. It may be explored if some of the parameters could be defined as common for a group of countries, or as a hierarchical structure (hyper priors) exploiting assumptions of exchangeability over or within populations. This would allow taking advantage of ‘cross information’ when part of the data contain good information about one parameter and a different part about another parameter. This information synthesis can use formal probabilistic methods such as Bayesian hierarchical modelling building on the concept of exchangeability and conditional exchange ability of VTEC: The use of the QRA models for other VTEC

serotypes and for other European countries has yet to be explored within WP5. This is on-going research.

-Source attribution

Campylobacter & VTEC:

Definition and purpose of source attribution need to be considered. The goal could be either to identify the original sources and quantify their relative contribution, or to estimate the role of different (not necessarily all) pathways. The approach can be either to build from the pathways to human cases, or to start with the human cases which are then attributed to sources. Either way, methods need to combine both sources of existing information in a coherent way, to take full advantage of both human data and food production chain data. If it is also required that an intervention effect is to be evaluated, this can influence how the source attribution problem is formulated for that goal. Eventually, factors operating at the time of exposure are the most causal ones, and the last points where prevention can occur, whereas reservoirs of bacteria are enabling the exposures. Before prevention can be planned, source attribution should be quantified, but the scope of 'sources' needs to be defined in a way that is most effective for further evaluation, and also respecting the realistic limits of available data. Modelling and analysis methods that could be applied to routine data in several countries could be developed whereas tailored models for targeted analysis of specific details could be useful as case studies albeit not easily repeatable in different contexts. The distinction between 'source' and 'causality' should be made carefully since a 'source' in itself does not cause anything, and human cases can be merely associated with some sources whereas the exact single cause of cases may be impossible to identify (compare: bad weather does not cause traffic accidents, it is the drivers, but accidents may be associated with bad weather). Therefore, it can be too easy to identify simplified 'sources' as causal factors, albeit reducing the exposure risk can clearly cause a reduced risk, if all other factors remain unchanged (compare: reduce speed limits during bad weather).

-Further research needs, regarding source attribution:

Campylobacter:

The use of MLST typing methods in larger scale could provide data that allows more detailed source attribution, based on several sources at retail and in other exposure pathways, based on differences in ST distributions between sources and humans (single 'key types' not likely to exist).This may require further research to enable such typing methods in large quantities, yet this may not be currently economical enough

to be feasible in large scale. In this regards, development of rapid detection methods could be useful. It is also of interest to study how testing results from primary production and those from retail are related and whether sampling could be best targeted to one or the other. There is a need to develop statistical methods to deal with infrequent, biased, flawed or otherwise missing and limited data. This includes methods to combine temporal surveillance data with cross sectional studies from the same time period, and methods for individual level data (from humans and animals) accounting for time lags such as delay until consumption, incubation periods, etc. Modelling methods are also needed to account for multiple pathways of the same source, but with limited data. Methods for formal validation and comparison of models would be useful, especially when the quantity of interest cannot be directly observed for validation purposes. Also methods for studying the role of 'third' factor affecting simultaneously both human exposure and broiler exposure to campylobacter (e.g. climate) would be useful as well as methods for exploiting the information in situations when some causal factor in the system has changed (e.g. freezing, heat treatment) and the population outcome (human cases) then observed, and studies comparing models with different causal assumptions. There is also a need for more automated data base of surveillance results and more automated retrieval of existing data. Moreover, consumer behaviour and other social factors should be studied, also focusing on non-food related exposures, in randomized prospective studies in targeted groups. While interviews of diagnosed cases may not provide reliable answers about what the source was in each case, these might be used to question what the source was *not*, since it may be easier to rule out some causes. This information could still be used in statistical analysis. E.g. a vegan rules out direct meat exposure, (comparison with non-vegan population), but it would still be difficult to assess cross contamination without further research on that.

VTEC:

The main research need in the area of source attribution for VTEC is acquiring similar data for both animals and humans so that a sub-typing approach can be applied. Other research needs include ensuring that studies collate information on the same animal as it passes through the slaughterhouse and, where possible, to quantify the concentration of bacteria within a sample.