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Attribution of Human VTEC O157 Infection from Meat Products: A Quantitative Risk Assessment Approach

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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. 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 modeled. 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 burgers, present the highest estimated risk with an estimated eight out of 100,000 servings on average resulting in human infection with VTEC O157.

KEY WORDS: Attribution; meat products; risk assessment; VTEC O157

1. INTRODUCTION

Verocytotoxingenic *Escherichia coli* (VTEC) O157 has gained publicity in Great Britain (GB) following two-large scale outbreaks of human infection in Scotland in the mid to late 1990s[1,2] and more recently in 2005 in south Wales. Following investigation of these and other large-scale outbreaks elsewhere in the world, human infection with VTEC O157 has been closely linked to the consumption of contaminated meat and dairy products.

To address the risk posed to human health by consumption of food-borne pathogens, including VTEC O157 in meat, a quantitative risk assessment (QRA) can be developed. Risk assessment is a transparent and logical approach for assessing the risk of an unwanted outcome and has been used in the field of food safety by governments, in particular, over the last 10 years. This is highlighted by the numerous risk assessment models for VTEC O157, in particular, currently available from a range of countries including the United States,[3] the Netherlands,[4] Canada,[5] Australia,[6] and the Republic of Ireland.[7,8] All of these models have focused on beef mince, which is considered to be the primary product associated with human food-borne infection.

However, cattle are not the only livestock species to harbor VTEC O157; the bacterium has been isolated from both sheep and pigs in a recent British abattoir survey.[9] Furthermore, studies have isolated VTEC O157 at retail sale in other meat products (e.g., lamb).[10] Therefore, there is potential for human infection with VTEC O157 from consumption of meat products from other species. In GB, where the incidence of VTEC O157 infection is relatively high compared to the rest of the world, human

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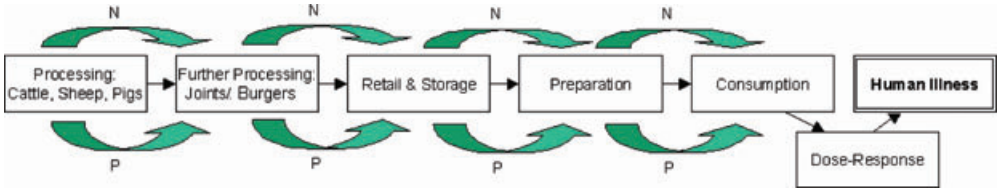

Fig. 1. Pathway for assessing the human health risk from consumption of whole cuts of meat from cattle, sheep, and pigs and burgers from cattle contaminated with VTEC O157.

exposure to VTEC O157 may occur via consumption of pork, lamb, or beef. To address this issue, a QRA has been developed that assesses the risk of VTEC O157 infection from consumption of meat products from cattle, sheep, and pigs within a single framework. In doing so, the attribution of each product to the overall human health risk can be assessed. The issue of attributing human infection to different sources is becoming a commonly adopted approach.[11,12] Typically, however, it is undertaken by using Bayesian methods, for example, to link data on human illness with the prevalence of strains in various animal sources. The approach outlined here is similar to that used previously for Campylobacter[13] in which more than one source (i.e., livestock species) is incorporated within a single farm-to-consumption QRA framework.

2. MATERIALS AND METHODS

2.1. Model Overview

The range of food products available in supermarkets today is varied and extensive, including fresh and frozen produce, ethnic and exotic food, and ready-to-eat meals. Furthermore, food products may be produced entirely within one country or be imported from a range of countries. However, in general, meat products can be broadly categorized into three main groups: intact raw meat, meat on which a process has taken place without additional ingredients, and meat with other ingredients added. To consider the full range of products from each main group for cattle, sheep, and pigs within a QRA framework would require knowledge of the varying effects of processing on the presence of VTEC O157 in the products and would be extremely data intensive. To address the data limitations, focus was paid to products considered "high risk" from a human health point of view (i.e., they are considered to be those most likely to contribute to VTEC O157 human infection). For cattle these products included raw intact retail cuts of meat and minced meat (e.g., burgers). 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 framework, it is important to use comparable modeling approaches and, where possible, data for each species considered. Given a paucity of data for pigs, in particular, on the prevalence of VTEC O157 in GB at the farm level, the QRA model commences at the slaughterhouse (processing) for all species, and then follows the carcass postchilling to jointing into retail cuts (or mincing), retail and storage, preparation, and finally consumption. The prevalence (P) and counts of VTEC O157 (N) were modeled throughout this chain (Fig. 1).

A species-specific model was developed for processing and further processing. During retail and storage, as meat products are typically stored at the same temperature and for the same duration, the model is generic to all species and products considered. The preparation module is product specific (i.e., minced meat or joint), as it is considered that these products are cooked using different methods. Finally, the consumption module is also product and species specific to account for the variation in the amount of beef, lamb, and pork eaten by British consumers. The entire model framework is restricted to products that are fresh and are consumed in the domestic setting (i.e., at home). It is further assumed that the products are produced within GB. Imported products are considered to be beyond the scope of this assessment.

The overall output of the model was a distribution of the amount of VTEC O157 consumed in a single contaminated serving and the mean number of human infections resulting from exposure to VTEC O157.

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*.[8]
- 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.

These assumptions have been made given the paucity of currently available data and can be reexamined upon further information being made available as part of the iterative process of QRA. It is acknowledged that availability of these data may change these assumptions and thus model estimates and conclusions.

2.3 Model Development

2.3.1. Processing

A different processing model was developed for each species to account for the species-specific steps that animals undergo after slaughter. This was necessary to accommodate differences in data available between cattle and sheep compared to pigs, resulting in the application of two different modeling approaches. For pigs, raw data on *E. coli* counts were obtained from pigs processed at four English abattoirs (Food Standards Agency (FSA) study MO1040). The study followed individual carcasses through the slaughter process, during which time the concentration of *E. coli* at specific stages (postscalding, postdehairing, postsinging, postpolishing, posteviscer ation, postchilling) was recorded. The proportional change in *E. coli* counts between stages was estimated by fitting these data to a Weibull or exponential distribution; the Weibull distribution was fitted to all but one processing step for which an exponential distribution was deemed a better fit. In using the latter approach, it was assumed that *E. coli* is a suitable proxy for VTEC O157, which may result in an overestimate of the actual amount of VTEC on the carcass. By inputting an initial concentration of *E. coli*, postbleeding, based on the raw data from the FSA study, an initial prevalence of 0.3%,[9] and using the fitted distributions for the change in concentration for each stage, an estimate of the prevalence and final concentration of VTEC O157 postchilling was obtained. The approach is outlined in greater detail in the work of Simons *et al* .[14]

For sheep and cattle, an approach previously used[8,5] was adapted and reparameterized, where possible, for GB. The model framework is outlined in Fig. 2. In applying this approach for sheep, several assumptions and modifications were made due to paucity in data. For example, it was assumed that the area from which the sheep carcass is contaminated with VTEC O157 is proportionally equivalent to area of contamination on a beef carcass by weight per unit surface area. The parameters and their values are given in Table I. The output from this module, as for pigs, is the amount of VTEC O157 on a random carcass (or half carcass for cattle) postchilling.

2.3.2. Further Processing—Joints

After chilling, the carcass is typically butchered into primal joints comprising a percentage of the overall carcass side weight. In GB, pigs, for example, are butchered into five main joints: head (9.5%), belly (9.9%), leg (30.6%), loin (20.4%), and shoulder (29.6%).[24] Sheep are butchered into seven primal joints: leg (23.6%), chump (9.6%), scrag (2.7%), shoulder (30.7%), breast (13.1%), loin (9.4%), and neck (74%).[24] Last, 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%).[24]

Each of these primal joints comprises a percentage of the overall weight of the carcass (or half carcass) as indicated in the percentages noted above.

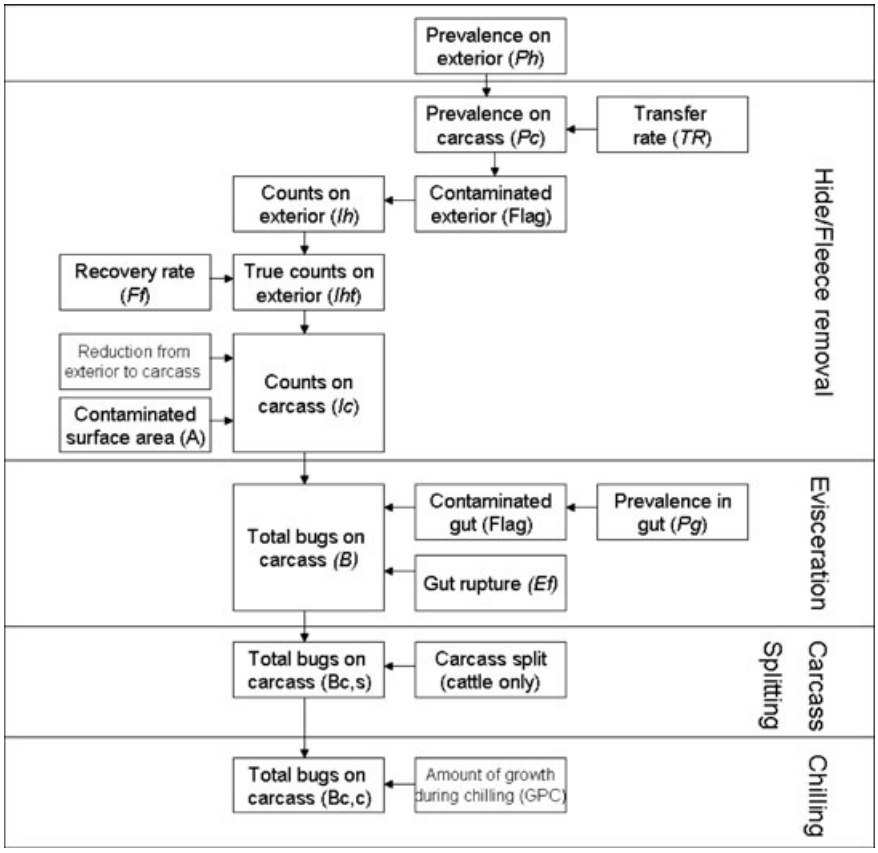

Fig. 2. Model framework for processing of cattle and sheep adapted from Cummins *et al.*[8]

Using this information, and the partitioning process outlined in the work of Nauta,[4] the amount of VTEC O157 ($N_{cut,s}$) on each primal joint (j), assuming VTEC O157 is clustered on the carcass, is given by:

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

where $N_{chill}(i)$ is the amount of VTEC O157 on a joint i after chilling, W_s is the weight of the carcass half (or whole carcass for sheep), $W_{cut,s}$ 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). The clustering parameter (b) in Equation (1) is unknown and set to 1 as undertaken in a previous VTEC O157 risk assessment[25] that represents a relatively low tendency for the bacteria to cluster (see Nauta *et al.* [25] for illustration). For carcasses in which the number of bacteria was large, the normal approximation to the binomial was used and rounded to a whole number.

These primal joints are then further partitioned into retail cuts of meat. These include cutlets, steaks, and joints, for example, depending upon the primal joint from which it is derived. Combining data from a survey of retail cuts on sale in a major supermarket chain with information on how a primal joint is partitioned into a retail cut, the amount of VTEC O157 ($N_{retail,s}(j)$) on a retail cut derived from joint j of species s was given by:

$$N_{retail,s}(j) = Binomial\Big(N_{cut,s}(j),$$
$$beta\left(b, b\left(\frac{W_{cut,s}}{W_{retail,s}}\right) - 1\right)\Big), \quad (2)$$

where $W_{retail,s}$ is the weight of a retail joint as defined by a uniform distribution of the minimum and

Table I. 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)[a]	9	Beta(21,2806)[a]	9
Prevalence of exterior(P_H)	Beta(131,107)[a]	15	Beta(6,86)[a]	16
Transfer ratio between exterior and carcass (TR)*	Beta(4,220)/Beta(123,101)[a]	17	Beta (7,395)/Beta(24,378)[a]	18
Prevalence on carcass (Pc)	$TR \times P_H /(1 - P_H + TR \times P_H)$	8	$TR \times P_H /(1 - P_H + TR \times P_H)$	8
Counts on hide/fleece ($\log_{10}$ CFU/100 cm^2) (I_h)	Cumulative distribution fitted to data	19	(Beta(0.395,2.43)) × Cumulative distribution fitted to data	Assumption based on (4) & data from (20)
Recovery factor (F_i)	Uniform(0.5,1.5)[a]	8	Not applicable	–
True number on hide (H_{ht})	$\text{Log}(10^{(\text{Ih+Fi})}/100)$	8	Not applicable	–
Count reduction from hide/fleece to carcass (R)	1.39	Based on data in 21	1.39	Assume same as for cattle
Number contaminating carcass during dehiding (I_c)	$I_{ht} - R$	8	$I_h - R$	8
Total contaminated surface area (cm^2) (A)	$10^{(\text{Triangular}(\log(30),\log(300),\log(3000))}$	22	0.0143 × carcass weight	Assumption based on ratio of area of contamination to carcass weight in cattle
Counts on hide after dehiding *(Bc,h)*	$\text{Log}((10^{\text{Ic}}) \times A)$	8	$\text{Log}((10^{\text{Ic}}) \times A)$	8
Probability intestines ruptured during evisceration	0.001	23	0.001	23
Intestines ruptured? (E_f)	Binomial(1,0.001)	–	Binomial(1,0.001)	
Gut colonized? *(F_g)*	Binomial(1, P_g)	–	Binomial(1,P_g)	
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 equivalent to hide contamination; *Bc,h*	8	Assumed equivalent to fleece contamination; *Bc,h*	Based on 8
Amount of contamination after evisceration (B)	$\text{Log}(10^{(\text{Bc,h})} + 10^{(\text{Bc,e})})$	8	$\text{Log}(10^{(\text{Bc,h})} + 10^{(\text{Bc,e})})$	8
Amount of contamination after carcass splitting (*Bc,s*)	Binomial(B, 0.5)	4	Not applicable	–
Amount of growth during chilling (GPC)	Pert(−2,0,5)	5	Pert(−2,0,5)	5
Amount of contamination after chilling	$B_{c,s} \times 2^{\text{GPC}}$	5	$Bc,s \times 2^{\text{GPC}}$	5

*It is stated in Small *et al.*[16] that about 1% of carcasses are contaminated during processing given an initial hide prevalence of 55%.
[a]Denotes an uncertain parameter.

maximum observed weights or a discrete distribution (see Table II for details).

2.3.3. Further Processing—Burgers (Beef Only)

Minced meat is produced by mixing trimmings from beef carcasses into combo boxes that are then combined into a grinder. From the grinder, a retail packet of beef burgers is produced from which it is assumed a single burger may be consumed. To model this process, the approach outlined by Cummins *et al.*[8] is adopted. In summary, it is assumed that trimmings from one or more carcasses are combined into a 27 kg box. From here, the trimmings are

Table II. Summary of the Variable Retail Weights of Joints per Species

Species	Retail Joint	Weight (g)
Sheep	Roast–leg half	500, 700, 900, 1100, 1300, 1400[a]
	Roast–leg whole	1400, 1600, 1800, 2000, 2200, 24000[a]
	Roast–part boned	903, 1003, 1103, 1203, 1303[a]
	Cutlets	U[b](225, 400), U(408, 502), U(200,320), U(400,640)
	No bone joint	307
Cattle	Steak	U(210, 470), U(180, 330), U(240, 550), U(220, 630), U(280, 520), U(170, 430), U(400, 690)
	Roast	U(1280, 1800), U(1280, 1550), U(1800, 2800), U(700, 1800),
	Roast	U(500, 1500), U(2500, 3200)
		900, 1000, 1100, 1200, 1300[a]
	Stew	U(400, 690)
Pigs	With bone	U(300, 500), U(500,900), 850, 1000, U(1100, 1800)
	Fillet	330, U(395, 565)
	Shoulder	330
	Chops	U(280, 440)
	Spare-rib	U(340, 650)

[a]Fitted to a discrete distribution.
[b]Represents the uniform distribution.

combined into a 150 kg grinder from which a retail packet of either two or four burgers is obtained. From this packet, a single burger (113 g) is selected.

2.3.4. Retail and Storage

After the product has been produced for retail distribution, there is a period of time before which the product is consumed. Specifically, there are four main opportunities, depending upon the conditions, for VTEC O157 to grow after further processing and prior to preparation, namely, during transport from further processing to retail, 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.[26] Therefore, it can be assumed that if at any point in the chain from further processing to preparation the temperature exceeds 7°C, growth of VTEC O157 may occur.

The approach applied to model this potential growth during retail and storage is that first developed by Ebel *et al.*[22] Using this approach, first the mean probability that temperature abuse occurs during each of the four stages was estimated. During transport from further processing, it was assumed that no abuse occurred; expert opinion for a U.K. *Salmonella* in pigs risk assessment stated the maximum temperature was 4°C for cold stored products[27] ($P_{abuse,transport1} = 0$). During retail, based on Audits International[28] data from the United States, the temperature exceeds 7°C 14% of the time ($P_{abuse,retail} = 0.14$). Within this 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. By linking the Audits International[28] data on the mean temperature change per time out of the fridge with the mean time it takes to get home from the supermarket in Ireland,[29] it was observed that the probability the temperature exceeded 7°C was 1 ($P_{abuse,transport2} = 1.00$). At home, Irish data suggest that 39 out of 50 (77%) fridges are set to a temperature exceeding 7°C[30] ($P_{abuse,home} = 0.77$). The probabilities for each stage are combined to provide the probability of a temperature abuse occurring in a total of 16 different pathways (e.g., no abuse, abuse at retail only, abuse at all stages, etc.).

Table III. Summary of the Distribution Used for the Duration of Transport (Based on Data in Reference 28)

Distribution of Time (minutes)	Cumulative Probability
Uniform (10, 29)	0.58
Uniform (30, 90)	0.93
Uniform (91, 180)	0.99
Uniform (181, 300)	1.00

Given that a temperature abuse occurs for a given pathway, the actual temperature (T) at which the product is stored, at each stage, is derived. This was achieved by fitting a cumulative distribution to Audits International[28] data (for retail) and Irish data (for home).[30] For transport to the home, the temperature was calculated by noting the change per time out of the fridge[28] given the duration of time between retail and home[29] (Table III).

In terms of bacterial growth, it is also important to note not only the temperature but also the duration of storage. At retail, it was assumed the storage duration (h) to be between a minimum of 1 day, a maximum of 5 days, and a most likely value of 3 days based on the expert opinion of Hill *et al.*[27] for storage of pig products in GB. The duration of storage during transport home is summarized in Table III. In the home, it is assumed that products are stored for a time period represented by an exponential distribution with a mean of 36 hours and truncated between 0 and 120 hours, based on results of a survey of Irish consumers.[31]

The growth of VTEC O157 observed during the different stages and conditions defined above is modeled using Gompertz microbial growth equations.[32] The Gompertz model predicts the growth that will occur in a bacterial colony over a time period τ at a storage temperature T using the statistical parameters a, b, c, d, and f that are estimated using regression techniques on data from *E. coli* O157 growth experiments.[32] The model consists of three key estimates: the lag phase, the generation time, and the maximum population density. The duration of the lag phase (LPD) is the time that elapses before the bacteria start to grow and is calculated by Ebel *et al.*:[3]

$$Ln(LPD) = Normal(a + b * Ln(T), 0.27), \quad (3)$$

where $a = 9.998$, and $b = -2.69$.[32]

The generation time (GT) is the time taken for bacterial numbers to double and is calculated as follows:

$$Ln(GT) = Normal(c + d * Ln(Ln(T)), 0.16), \quad (4)$$

where $c = 7.03$ and $d = -6.31$.[32]

Last, the maximum population density (MPD) is the maximum number of VTEC O157 present in a product and is given by:

$$MPD = Normal(TMD + f * T, 0.15), \quad (5)$$

where $TMD = Triangular(5, Uniform(5,10),10)$[3] is the theoretical maximum density (the maximum VTEC O157 growth achievable in a product) and $f = -0.014$.[32]

Equations (3) to (5) are estimated for each stage using the stage-specific temperature values (T) described above. Next the percentage of time available in the lag phase prior to and after each stage is estimated using the approach outlined in the work of Ebel *et al.*[22] Given this, the amount of possible growth that could occur for a stage (i) is estimated if the remaining lag phase is less than the time in the current stage using the following equation:[22]

$$G_i = \log_{10}\left(2^{\frac{T_i - LPD_i}{GT_i}} + 1\right). \quad (6)$$

The amount of actual growth is assumed to be the minimum of G_i and the MPD for that stage. For each possible pathway (e.g., temperature abuse in stages 1 and 2), the amount of actual growth is summed over all stages. The described equations are modeled within a specifically written macro in Visual Basic for Applications (VBA) and run 1,000 times, from which the probability of observing growth ranging from −3 to 20 $\log_{10}$ CFU is predicted for all pathways. This range was fitted to a general distribution.

2.3.5. Preparation

During preparation, there is a probability 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. However, in this risk assessment, due to data limitations, focus is paid to inactivation of VTEC O157 during inadequate cooking of the food product and consequently cross-contamination of bacteria from meat products is assumed not to occur.

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,[33] 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, an approach used previously by Duffy *et al.* [7] 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 cooking level of the burger, an associated temperature is ascertained: 68.3°C (well done), 62.7°C (medium), or 54.4°C (rare).[5,34] Using this information, the thermal inactivation (log CFU) from cooking is given by

Cassin *et al.*:[5]

$$C = -10.165 + 0.211(T), \tag{7}$$

where T is the final internal temperature of the product. The amount of bacteria (N_{prep}) in the product postcooking is then estimated by:

$$N_{prep} = N_{postretail} - 10^{C}, \tag{8}$$

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

2.3.6. Consumption

At the end of the preparation the product is further partitioned into a serving size. These serving sizes are variable between consumers and particularly between age groups. Recently, the U.K. Food Standards Agency funded a study to examine the food portion sizes for adults aged 19–64 years.[35] Using the raw data from this study, a truncated normal distribution was fitted to the 25th and 75th percentiles of portion sizes for beef, pork, and lamb retail joints (e.g., shoulder, chops). The amount of VTEC O157 consumed in the home was modeled by a further portioning process given by:

$$N_{H,s} = Binomial(N_{prep,H,S}, beta(k, k * (W_{H,s} - 1)), \tag{9}$$

where H is the portion type (mince, joints), s is the species (cattle, sheep, pigs), $N_{prep,H,s}$ is the amount of VTEC O157 on the joint or in the minced meat after preparation, $k = 1$ is the clustering parameter, and $W_{H,s}$ is the weight of the joint divided by the weight of the portion.

2.3.7. Probability of Illness—Dose Response

The probability of illness given exposure to VTEC O157 at consumption was modeled using a previously published beta-binomial model.[5,36] Specifically, the probability of illness (P) is given by:

$$P = 1 - (\exp(\ln\Gamma(Ncon + \beta) + \ln\Gamma(\alpha + \beta) - \ln\Gamma(\alpha + Ncon + \beta) - \ln\Gamma(\beta))), \tag{10}$$

where Γ is the gamma function, and α and β are susceptibility parameters ($\alpha = 0.267$; $\beta = \exp(Normal(5.434,2.47))$.

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 1 represents illness and 0 represents no illness.

2.4. Generation of Results

The cattle and sheep processing model and the further processing to consumption models were developed within @Risk (version 5.0.1, Palisade Corporation, Newfield, NY, USA), an add-on package to Microsoft Excel (Version 2003, Microsoft Corporation, Redmond, WA, USA). In doing so, where appropriate, inputs were described using probability distributions to represent either the variability (natural variation between animals, carcasses, products) or uncertainty (lack of knowledge) about the input parameter (Table I). The pig processing model was developed in Matlab (Version 7.4, The Mathworks, Nattick, MA, USA). The results from this latter model were then used within the further processing-to-consumption models developed in @Risk.

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 characterizing the model parameters are sampled numerous times, or iterations, such that each iteration represents a potential event of transmission along the food chain. The model was run for $\alpha = 100{,}000$ 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:

$$\Pr = \frac{\sum_{i=1}^{\alpha} N(\alpha) > 0}{\alpha}, \tag{11}$$

where $N(\alpha)$ is the number of VTEC O157 per unit generated by each iteration α. Given that a unit was contaminated, the median, 5th–95th percentile values for $N_{c,s}$, and the amount of VTEC O157 per stage c and species s were obtained. The median value rather than the mean value was obtained due to the positive skew of the distributions.

As mentioned above, the distributions in the model describe either variability or uncertainty about a parameter. To assess the impact that the uncertain parameters (Table I) have on the model output, the model was run twice. First, the model was run with all the distributions as described above to provide an overall estimate of both uncertainty and variability. Second, the model was run with all the

Species & Product	Module	Mean Prevalence of Contaminated Units (%)	Amount of VTEC Median (5th%, 95th%)
Cattle—joints	End of processing	3.8	87 (22, 1198)
	Further processing	1.3	2 (1, 20)
	End retail & storage	1.3	4 (2, 310)
	End preparation	0.12	4 (2, 90)
	Consumption	0.04	2 (1, 200)
Sheep—joints	End of processing	2.1	3 (1, 39)
	Further processing	0.05	1 (1, 29)
	End retail & storage	0.05	3 (2, 306)
	End preparation	0.03	22 (5, 39)
	Consumption	0.03	9 (1, 14)
Beef—mince	End of batch	17.5	18 (2, 221)
	Retail packet	1.65	1 (1,3)
	Single burger	0.65	1 (1,2)
	End retail & storage	0.65	2 (1, 301)
	End preparation	0.03	5.6×10^4 (1.74×10^3, 5.11×10^6)
	Consumption	0.03	2.1×10^5 (1.43×10^4, 5.75×10^6)
Pig—joints	End of processing	0.86	1 (1,2)
	Further processing	0.02	1(1,1)
	End retail & storage	0.02	2 (1, 27)
	End preparation	0.003	2
	Consumption	0.001	1

Table IV. Summary of the Prevalence and the Amount of VTEC O157 During the Various Stages Given Both Uncertainty and Variability Modeled

uncertain parameters set to their mean value; thereby only variability was simulated.

3. RESULTS

3.1. Prevalence and Amount of VTEC O157

The median, 5th and 95th percentile for the amount of VTEC O157 on contaminated units during the various stages of the food chain for each species is outlined in Table IV given both uncertainty and variability. The median and percentiles are reported rather than the means and variances due to the positive skew of the model distributions.

It can be seen from Table IV that for meat joints less than 1% of the 100,000 servings are contaminated and, furthermore, are contaminated at low levels of between 1 and 200 (with 95% certainty) VTEC O157 organisms. For beef burgers, it can be seen that in 0.03% of contaminated burgers, the number of VTEC O157 organisms after preparation and at the point of consumption is large (i.e., >500 CFU/portion). This is because there is, on occasion, significant growth during retail and storage in a few burgers that are subsequently consumed rare (and hence there is no inactivation of bacteria), resulting in significant amounts of bacteria at consumption. The resulting distribution of the amount of VTEC O157 per contaminated burger is consequently heavily skewed to the right. It is acknowledged that significant growth may occur, on occasion, on joints during retail and storage. However, during preparation of joints there is always inactivation of the bacteria due to the differences in the modeling approaches for joints compared to burgers (Section 2.3.5) resulting in an overall reduction in the amount of bacteria on joints compared to (rare) burgers at consumption.

The results from simulating only variability are outlined in Table V. It can be seen from Table V that the prevalence predominantly remains the same for both scenarios. An exemption to this is the prevalence of VTEC O157 on consumed servings of lamb joints whereby the prevalence has decreased to 0% from 0.03% with both uncertainty and variability simulated. In general, the confidence intervals for the amount of VTEC O157 for the various stages have decreased, indicating that the amount of uncertainty in the model parameters impacts on the model results. For example, at the end of processing, the amount of VTEC O157 on a beef half carcass is 87 (22, 1198) with both uncertainty and variability simulated. This is reduced to 86 (11, 804) when simulating only variability.

3.2. Number of Infections

The number of infections resulting from the amount of VTEC O157 an individual is exposed to

Species & Product	Module	Mean Prevalence of Contaminated Units (%)	Amount of VTEC Median (5th%, 95th%)
Cattle—joints	End of processing	3.8	86 (11, 804)
	Further processing	1.3	2 (1, 12)
	End retail & storage	1.3	4 (2, 247)
	End preparation	0.12	3 (2, 57)
	Consumption	0.04	1 (1, 28)
Sheep—joints	End of processing	2.0	3 (1, 40)
	Further processing	0.02	1 (1, 63)
	End retail & storage	0.02	4 (1, 201)
	End preparation	0.002	2
	Consumption	0	0
Beef—mince	End of batch	17.5	18 (2, 177)
	Retail packet	1.48	1 (1,2)
	Single burger	0.56	1 (1,2)
	End retail & storage	0.56	2 (1, 443)
	End preparation	0.01	2.05×10^6 (1.79×10^4, 7.38×10^{12})
	Consumption	0.01	2.05×10^6 (3.81×10^4, 9.39×10^{12})
Pig—joints	End of processing	0.86	1 (1,2)
	Further processing	0.02	1(1,1)
	End retail & storage	0.02	2 (2, 28)
	End preparation	0.003	2
	Consumption	0.001	1

Table V. Summary of the Prevalence and Amount of VTEC O157 During the Various Stages Given Only Variability is Simulated

Table VI. Number of Human VTEC O157 Infections Arising from 100,000 Servings

Beef mince	8
Beef joints	2
Lamb joints	0
Pork joints	0

assuming consumption of 100,000 servings was estimated using the beta-binomial dose-response model. The ranked number of infections by product type is outlined in Table VI.

It can be seen from the number of infections per 100,000 servings that minced meat (burgers) presents the highest risk to human health followed by beef joints. No infections were estimated by the model as a result of exposure to VTEC O157 in lamb or pork joints. In terms of attribution, therefore, based on the current model assumptions and data, the model predicts that beef is the sole contributor to human infection.

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 under study. 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.[37,38] 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.[38] *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.[38]

A later study was conducted in southeast Scotland[39] 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.[10] Further, an Irish study identified VTEC O157 in 2.8% of minced beef products at retail sale.[40]

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 1.65% retail packets of beef burgers, 0.65% of beef burgers, 1.3% of beef joints, and 0.05% 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 underestimates the observed prevalence; however, the model is restricted to lamb joints rather than lamb mince.

Another validation point would be to consider the number of *E. coli* O157 human infections reported in GB. Currently, the annual number of *E. coli* O157 infections identified (excluding those from food eaten abroad) is 1,035.[41] This, however, is not categorized by food sources and includes data from outbreaks and sporadic cases. Therefore, it cannot be ascertained whether the number of human infections and relative contributions predicted by the model are representative of the clinical situation.

5. DISCUSSION

The model described here is a QRA exposure assessment that aims to assess the risk of human illness with VTEC O157 due to consumption of beef, lamb, and pork joints and beef burgers. In doing so, it is aimed to attribute the relative number of human infections (given 100,000 servings) between the products within a single framework. Using this approach, it was estimated that VTEC O157 human infection from beef products predominates, particularly beef burgers, and no infections (out of 100,000 exposures) arose from lamb or pork joints.

There are several methods that can be used for source attribution, including microbial subtyping, exposure assessment, epidemiological studies, Bayesian methods, and expert opinion.[11] 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.[23] 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.[42] Until such time that data are available, QRA is considered a useful approach to use.

Based on the model assumptions, it was deduced that the mean prevalence of contaminated meat products at consumption was low. In particular, for pork and lamb joints the mean prevalence of contaminated products at consumption was 0.001% and 0.03%, respectively. Given that these products were contaminated, the expected levels of VTEC O157 were less than 14 organisms (with 95% certainty). The prevalence of VTEC O157 at consumption was highest among cattle products with 0.04% of joints and 0.03% of beef burgers. These products accounted for an estimated 10 human infections (out of 200,000 exposures) in total, predominantly from beef burgers (8 infections) due to the large number of bacteria present in the serving as a result of significant growth at retail and storage and limited inactivation during preparation.

As with other QRA 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 data gaps for sheep. For example, there is limited knowledge on the area of the carcass that is contaminated with bacteria and the quantity of VTEC O157 on the contaminated 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. These data gaps can be addressed as further research is undertaken enabling the model to be readdressed as part of the iterative process of risk assessment.

There are several important data gaps and uncertainties for sheep specifically, including, for example, 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. 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 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.

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 parameterizing 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.

A benefit of QRA is to provide indications of the effectiveness of potential interventions for risk management. The model currently predicts that beef burgers pose the highest risk of human infection compared to the other products modeled. The reason for this increased risk in burgers is the significant amount of growth during retail and storage in a few burgers combined with the subsequent consumption of a further few raw (i.e., minimal inactivation). In terms of risk management, therefore, 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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