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Quantitative risk assessment for verocytotoxigenic *Escherichia coli* in ground beef hamburgers in Argentina

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ABSTRACT

A quantitative risk assessment was developed for verocytotoxigenic *Escherichia coli* (*E. coli* VTEC) following hamburger consumption. The assessment considers initial contamination levels, cross-contamination and decontamination events during the cattle slaughter process and the distribution, storage and consumption patterns in Argentina and in similar countries in Latin-American. The model predicted an infection risk of 8.12×10^{-7}, a probability of Hemolytic Uremic Syndrome (HUS) of 4.6×10^{-8} and a probability of mortality of 5.9×10^{-9} per meal for adults. For children, the estimates per meal were 3.23×10^{-7}, 1.8×10^{-8} and 6.31×10^{-10} for infection, HUS and mortality, respectively. The risk of infection and HUS, were sensitive to the type of storage at home ($r=-0.416$), slaughterhouse storage temperature ($r=0.240$) and bacterial concentration in the cattle hide ($r=0.239$). There was an association between home preparation of hamburgers ($r=-0.116$) and the risk of illness, although this was a result of the type of storage at retail ($r=-0.110$) and at home and not their intrinsic characteristics. The most sensitive stages of the process were identified through the risk assessment and these can be used as a basis for measures of risk management.

1. Introduction

Verocytotoxigenic *Escherichia coli* (VTEC), serogroup O157, is an important cause of uncomplicated or bloody diarrhoea (BD) and its most important sequelae, haemolytic uremic syndrome (HUS) in humans. *E. coli* O157:H7 was first identified as a human pathogen in 1982 and was associated with two major outbreaks of BD in the US. It is the most prevalent VTEC serotype. However, other VTEC serotypes have been reported to cause outbreaks or sporadic cases of BD and HUS in many countries (Oteiza et al., 2006).

A variety of foods have been implicated in those outbreaks, but raw or undercooked foods of bovine origin are primarily responsible. *E. coli* VTEC is present in the faeces and intestines of healthy bovines and can contaminate meat during slaughter. Due to the importance of meat products in food outbreaks, cattle have been the focus of many studies to determine their role in the transmission of the disease to humans (Meichtri et al., 2004; Omisakin et al., 2003; Padola et al., 2002).

In Argentina, HUS is endemic and approximately 400 new cases are reported each year by hospital nephrology units. The majority (95%) of HUS cases are identified in children less than 5 years old, and in 2002, the annual incidence rate reached 12.2/100,000. This rate is higher than those reported in Oregon (2.6/100,000), Washington (3.0/100,000), Canada (3–4/100,000), Chile (4.2/100,000) and Uruguay (5.0/100,000) (Padola et al., 2004). In children, HUS is the leading cause of acute renal failure and the second leading cause of chronic renal failure. Approximately 30% of children receiving kidney transplants suffered from HUS (Ministerio de Salud y Ambiente de la Nación, 2005). Argentina not only has one of the highest recorded incidence rates of HUS in the world (Padola et al., 2004), but it also registers the greatest consumption of bovine meat per inhabitant (>69 kg/year) (Rearte, 2007).

Risk analysis is now widely accepted as the preferred means to assess possible links between hazards in the food chain and actual risks to human health (FAO-WHO, 2006). The quantitative risk assessments of acquiring *E. coli* VTEC following hamburger consumption published thus far have not considered the storage conditions, distribution and consumption patterns in developing countries. For these reasons, they have not been useful to the risk managers in developing countries for the identification or implementation of appropriate measures to control the risk of acquiring *E. coli* VTEC.

The objective of this study was to develop a quantitative risk assessment to model *E. coli* VTEC contamination of beef hamburgers in Argentina. This is the first study in Argentina that has used a farm-to-table risk approach.

2. Materials and methods

2.1. Model development

The prevalence and counts of *E. coli* VTEC bacteria were modelled at various stages along the agri-food beef chain. The conceptual model upon which the mathematical model was based is depicted in Fig. 1.

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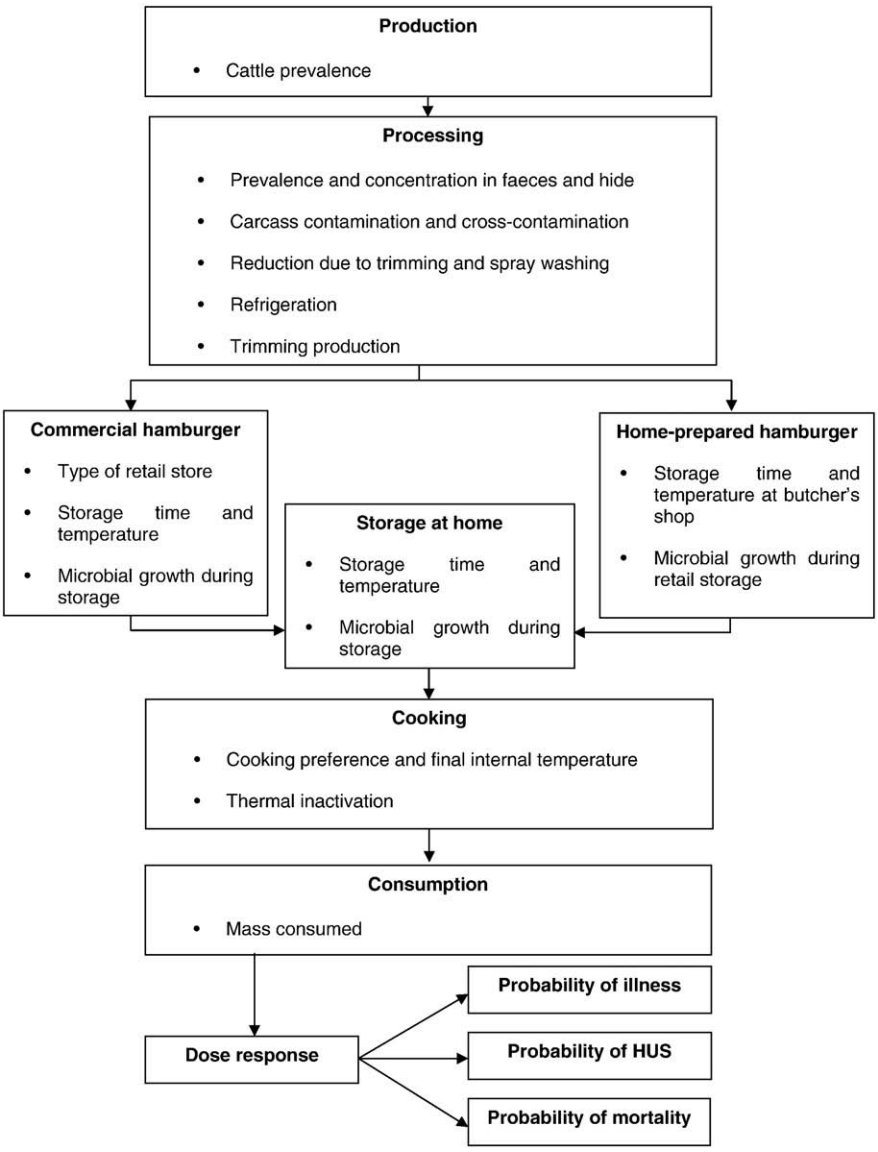

Fig. 1. Flow diagram of the mathematical model of exposure assessment and the dose–response model for *E. coli* VTEC in hamburgers.

The model was created in Microsoft Excel 2007 with the add-on package @Risk (version 4.0, Palisade Corporation, New York, USA).

The model was developed using inputs derived from Argentinean data, information and expert opinion, whenever possible. However, where Argentinean-specific data were not available, international data and scientific literature were consulted to improve the basis for the model.

2.2. Exposure assessment

The potential exposure to *E. coli* VTEC in a single-meal serving was estimated to assess the risk to human health associated with the consumption of beef hamburgers. The exposure model was divided into four modules: production, processing (slaughter), post-processing and consumption. Each module yielded one or more output distributions that served either as inputs to the next module or as final outputs of the estimation of the probability and number of viable organisms present in a single-meal serving at the time of consumption.

2.2.1. Production

This module estimates the prevalence of *E. coli* VTEC-infected cattle (P_f) entering Argentinean slaughterhouses. This module used previous studies in Argentina that concerned prevalence of *E. coli* VTEC in cattle. There are a number of studies that suggest differences in pathogen prevalence in bovine species according to feeding practices, cattle age and season of the year. However, there is no scientific information in Argentina regarding the impact of these factors on *E. coli* VTEC prevalence and so they were not incorporated into the model. The previous studies in Argentina that were used to estimate the cattle-prevalence distribution are shown in Table 1. The distribution parameters were estimated using the method of

Table 1
Detection rates for *E. coli* VTEC in Argentinean cattle.

Reference	Location	Year	No. positive/No. tested (%)
Meichtri et al. (2004)	Buenos Aires, Santa Fe, Córdoba y San Luis (Argentina)	1999–2000	78/200 calves (39)
Sanz et al. (1998)	Argentina	No details	24/83 calves (28.9) 40/91 cows (43.9)
Padola et al. (2004)	Buenos Aires	2000	37/59 cattle (62.7)
Notario et al. (2000)	Santa Fe, Buenos Aires y Córdoba (Argentina)	No details	30/68 cattle (44.1)

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Table 2
Model input parameters: production and processing.

Variable	Description	Units	Distribution/Model
P_f	Prevalence of *E. coli* VTEC in cattle faeces		Beta(209 + 1501 − 209 + 1) fitted from Table 1
$P_{f\text{-}HUS}$	Prevalence of *E. coli* VTEC-associated with HUS		$P_f \times$ Beta(44 + 1.86 − 44 + 1)
P_{fe}	Prevalence of *E. coli* in cattle faeces		Beta(91 + 1327 − 91 + 1) based on Elder et al. (2000)
P_{ce}	Prevalence of *E. coli* in cattle carcass based on Elder et al. (2000)		Beta(148 + 1341 − 148 + 1)
F_{cc}	Factor for cross-contamination		P_{ce}/P_{fe}
P_c	Probability of infected carcass		$F_{cc} \times P_{f\text{-}HUS}/(1 - P_{f\text{-}HUS} + F_{cc} \times P_{f\text{-}HUS})$
I_h	Initial levels of bacteria in animal hides	$\log_{10}$ CFU/100 cm^2	Cumulative distribution based on O'Brien et al. (2005)
R_f	Recovery factor		Uniform(0.5, 1.5)
I_{th}	True number on hide	$\log_{10}$ CFU/cm^2	$\log(10^{(Ih + Rf)}/100)$
R	Reduction factor from hide to carcass		Cumulative distribution fitted from Bacon et al. (2000)
I_c	Initial number in carcass introduced during de-hiding	$\log_{10}$ CFU/cm^2	$I_{th} - R$
TSA	Total surface area	cm^2	$10^{\text{Triangular}(\log10(30),\log10(300),\log10(3000))}$
I_{ch}	Initial organisms on contaminated carcass after de-hiding	$\log_{10}$ CFU/carcass	$\text{Log}((10^{Ic}) \times \text{TSA}$
R_{dec}	Decontamination reduction	$\log_{10}$	Triangular(0, uniform(0.3,0.7), uniform(0.8,1.2))
Ref	Refrigeration	$\log_{10}$	Normal(uniform(−0.5,0.5),1)
G_{cd}	Growth during cutting and deboning	$\log_{10}$	Triangular(0, 0.33, 2.00)
N_{acd}	Number of organisms per carcass after cutting and deboning	$\log_{10}$ CFU/carcass	$I_{ch} - R_{dec} + \text{Ref} + G_{cd}$
C_{CA}	Concentration of *E. coli* per contaminated area	$\log_{10}$CFU/cm^2	$\log_{10}(10^{Nacd}/\text{TSA})$

moments (Vose, 1996) and assumed that the prevalence could be characterised with a beta distribution.

The ability of VTEC strains to cause severe disease is related to the production of several proteins: one or more Shiga toxins (Stx1, Stx2, and variants of Stx2) that are encoded by lysogenic bacteriophages; intimin (encoded by the chromosomal *eaeA* gene), which is responsible for the attachment and effacement lesions in the intestinal mucosa; a 60 MDa megaplasmid that encodes an enterohaemorrahagic haemolysin (Padola et al., 2004). However, only some variants of stx2 were associated with HUS. Meichtri et al. (2004) observed that 51.2% of the *E. coli* VTEC strains isolated from Argentinean cattle belonged to 16 serotypes already associated with bloody diarrhoea or HUS in several countries. These data were used to adjust the P_f to calculate the prevalence of *E. coli* VTEC associated with HUS ($P_{f\text{-}HUS}$) cattle (Table 2).

2.2.2. Processing

In general, Argentinean slaughterhouses follow the same procedures. The prevalence and counts of *E. coli* VTEC were modelled at various stages along the slaughter line from the arrival of live cattle to carcass storage in the refrigerator. An "average slaughter line" is depicted in Fig. 2.

Transmission of *E. coli* VTEC from infected to susceptible cattle may occur during cattle transportation from the farm to the slaughterhouse. However, evidence suggests that there is no significant difference in faecal prevalence between the farm and slaughter plant (Rice et al., 1997) and the duration of transportation was not associated with faecal positive status (Cornell Collaborative Project, 1998). Additionally, it has been demonstrated that faecal prevalence could be a better predictor of carcass contamination than hide prevalence (Elder et al., 2000). For those reasons, the transportation effect was not incorporated in the present model. Another study demonstrated that pre-slaughter washing for 3 min did not statistically reduce the numbers of *E. coli* O157:H7 transferred from the hide to the carcass during slaughter (Byrne et al., 2000); therefore, it was not included in the model.

E. coli is not naturally present in meat from healthy cattle, but it may be deposited by faeces at one or several points between slaughter and packaging, especially during de-hiding. In this step, the carcass can be contaminated by direct contact with the hide or by cross-contamination. The transfer of contamination from hide surface to carcass is considered unavoidable. Information reported by Elder et al. (2000) was used to model the cross-contamination process (F_{cc}) by generating two Beta distributions for *E. coli* prevalence in faeces (P_{fe})

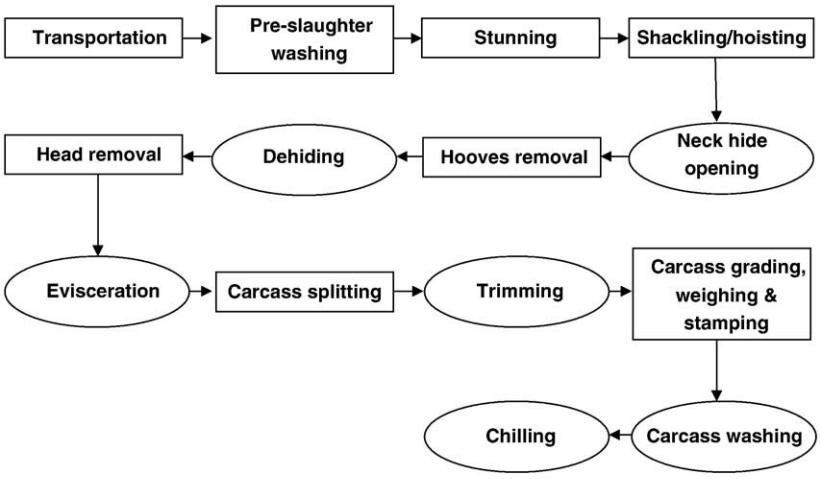

Fig. 2. Flow diagram of the stages involved in Argentinean cattle slaughter. Ovals denote steps that may either increase or decrease contamination. Rectangles denote steps with little or no increase in concentration.

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and on the carcass (P_{ce}) before the evisceration and finally calculating their relationship (P_{ce}/P_{fe}).

To model the number of *E. coli* VTEC on carcass (C_f), we used the approached of Cummins et al. (2008). No information is available regarding the initial levels of bacteria on animal hides (I_h) from Argentina. We therefore used the information reported by O'Brien et al. (2005) to generate a cumulative frequency distribution. Because the number of cells recovered are not the total bacteria in the carcass (I_{th}), the model introduced a recovery factor (R_f) to consider this aspect with an uniform distribution ranging from 0.5 to 1.5 $\log_{10}$, using the information reported by O'Brien et al. (2004). Bacon et al. (2000) reported a reduction in the level of the microorganisms on the carcass compared to the hide level. Following the approach of Cummins et al. (2008), the initial number of bacteria introduced to the carcass during de-hiding (I_c) was modelled by applying an R_f that fitted a continuous empirical cumulative distribution based on the information reported by Bacon et al. (2000). The lack of Argentinean data about the *E. coli* VTEC concentration on the surfaces of the hide and carcass is an important limitation of this model. A significant amount of uncertainly was introduced to the model with the use of information generated in other environmental conditions and this is identified as a simplification.

Because there is no evidence regarding the total contaminated surface area (TSA) on carcasses, this model used the approach suggested by USDA-FSIS (2001) that considers the minimum area that contamination might be spread across to be 30 cm^2 (based on the measurable detection threshold), the most common level to be 300 cm^2 and the maximum area to be 3000 cm^2.

If the gut is nicked during the evisceration process, there is a possibility of contamination, but no data are available to quantify this risk. McEvoy et al. (2003) reported that evisceration did not appear to contribute to carcass contamination with *E. coli* O157:H7. Others authors (Cassin et al., 1998; Roberts et al., 1999) neglected to consider the possible impact of evisceration in their models, and instead relied on the low influence of this step in carcass contamination. Argentinean slaughter processes consider the risk to be negligible if the animal has been slaughtered after at least 12 h of rest. Therefore, the present study did not model the effect.

Following hide removal, the Argentinean slaughterhouses remove visible spots of faecal contamination with knife trimming and spray washing after evisceration. The effectiveness of trimming as a decontamination treatment is highly variable and cross-contamination can occur through knife cuts if inadequate knife sterilisation methods are used (Cassin et al., 1998; USDA-FSIS, 2001). In Argentina, the carcasses are typically washed with potable water at room temperature (Rosmini, 2006). The prevalence of pathogens at particular carcass sites can increase or decrease during a potable water wash at a temperature of 35–40 °C, and this may be a result of the redistribution of the carcass contamination during the washing process (McEvoy et al., 2003). Another study (Gill and Landers, 2003) suggested that washing is effective when initial numbers are relatively high, but ineffective when numbers are relatively low. This is likely because the bacteria are probably associated with particles when numbers are relatively high and particles are easily washed off the meat by the large volumes of water applied in automatic washing operations. When numbers are relatively low, most of the bacteria are probably directly associated with tissues and may be refractory to removal through washing. Even if the effectiveness of decontamination treatment depends on the initial bacterial load, there is insufficient information to establish a mathematical relationship between the two parameters. Therefore, the reduction in bacterial numbers caused by washing was considered independent of the initial charge for this study. Additionally, Gill (1999) reported that carcass rinses reduced generic *E. coli* counts by 0.32 log CFU/cm^2, while Dorsa (1997) showed a 0.70 log CFU/cm^2 reduction of *E. coli*. In the present study, a triangular distribution modelled the reduction through decontamination (R_{dec}) with a minimum value of 0 logs, an uncertain "most-likely" value ranging from 0.30 to 0.70 logs (uniform distribution) and an uncertain "maximum" value ranging from 0.80 to 1.20 logs (uniform distribution).

After the sides of beef are decontaminated, they go into a chiller for at least 24 h. McEvoy et al. (2003) reported that chilling reduced the prevalence of *E. coli* O157:H7 on the carcass. Another author (Sheridan, 2000) reported that carcass contamination could be increased, decreased or unchanged following chilling and depended upon parameters such as temperature, air speed and relative humidity. Gill and Bryant (1997) reported that generic *E. coli* counts increased by 0.25 logs in one slaughterhouse and decreased by 1.34 log CFU/cm^2 in another slaughterhouse. Dorsa (1997) found a 1.2 log CFU/cm^2 increase in *E. coli* O157:H7 on carcasses stored at 5 °C for 48 h, although Gill et al. (1996) reported a reduction between 0.5 and 2.0 log CFU in coliforms and *E. coli* on carcasses following the cooling process. Due to the uncertainty present in this step (Ref), the model included a normal distribution with an uncertain mean ranging from −0.5 to 0.5 logs (uniform distribution) and a standard deviation of 1 log. Therefore, the model predicts that the most likely effect is that there is no effect from chilling, but it does allow for some variability.

During the cutting and deboning operations, contamination is possible from many sources including the environment, contaminated sides of beef, equipment and operators. Minimal data is available on the frequency and amount of *E. coli* VTEC contamination during the cutting and deboning process, but it has been suggested that it might result in an increased pathogen population on the meat trim (USDA-FSIS, 2001). In four abattoirs, Gill (1999) identified an increase in generic *E. coli* from 0 to 2.00 logs. In the present model, microbial growth during the cutting and deboning process (G_{cd}) was modelled using a triangular distribution with parameter values for the minimum, most likely and maximum growths of 0.0, 0.33 and 2.00 logs, in accordance with Cummins et al. (2008) and USDA-FSIS (2001).

Trimmings collected from the deboning process are used for the manufacture of ground beef. They are commonly between 50 and 500 g in size (m_{TRM}), and each trimming is likely associated with a different carcass. It was necessary to correlate the concentration of the pathogen on the carcass with the mass of the trim, as it was modelled per unit area, and an assumption of 0.1–0.5 cm^2/g of trim was considered (A_{APG}). The packages of trimmings were assumed to be ground by the retailer and set out for display in packages from 20 000 to 30 000 g (m_{PKG}). The occurrence of the organism in packaged meat was modelled using a Poisson process (C_{FGB}).

The hamburgers that are consumed in Argentina are manufactured both industrially and at home. A study carried out in Argentina by Quinteros et al. (2008) showed that approximately 50% of the consumed hamburgers were produced directly in homes from fresh ground beef and the rest were manufactured in processing plants ($Prod_{HAM}$). The growth of *E. coli* VTEC in home-prepared hamburgers was modelled using the development in ground beef dispensed in the butcher's shop. *E. coli* growth in commercial hamburgers was modelled using the type of the retail store (supermarkets, grocery store and butcher's shop) (R_{STO}), the type of storage (STO_R) and the storage temperature (T_{rs}), based on data published by Quinteros et al. (2008) (Table 3).

2.2.3. Post-processing

The elapsed time between processing plant departure of fresh ground beef/hamburger and the time of consumption was divided into two phases. Phase one began when the product left the processing plant and ended at the time of sale in the retail store (supermarkets, grocery store or butcher's shop) (T_r). Storage temperatures between 4 and 10 °C with a most likely value of 8 °C (T_{rs}) and maximum time of 96 h before sale were used for this phase (Quinteros et al., 2008). The second phase began at the time the product was bought at the retail store and ended at consumption. The storage temperature was

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Table 3
Model input parameters: post-processing and consumption.

Variable	Description	Units	Distribution/Model
m_{TRM}	Mass of a trimming	g	Normal(300,100; truncate 50)
A_{APG}	Surface area per gram of trimming	cm^2/g	Uniform(0.1;0.5)
A_{TRM}	Average trimming surface	cm^2	$m_{TRM} \times A_{APG}$
m_{PKG}	Mass of trimmings packaged	g	Uniform(20 000, 30 000)
N_{TRM}	Number of trimmings in a package		m_{PKG}/m_{TRM}
N_{CTRM}	Number of contaminated trimmings in a package		Binomial(N_{TRM}, P_c)
C_{PCT}	Concentration of *E. coli* in a package of trimmings	$Log_{10}CFU/g$	$(N_{CTRM} \times A_{TRM} \times C_{CA})/m_{PKG}$
C_{FGB}	Concentration of *E. coli* in contaminated FGB	$log_{10}CFU/g$	$\log_{10}(N/m_{PKG})$ where $N \sim Poisson(m_{FGB} \times 10^{CPCT})$
P_{FGB}	Probability of *E. coli* VTEC in fresh ground beef.		$P_{pct} \times (1 - e^{-mFGB \times Cpct})$
$Prod_{HAM}$	Hamburger production		Discrete({1;2}, {463;537}) Where 1 = house and 2 = processing plant production
R_{sto}	Type of retail		Discrete({1;2;3}, {34;13;4}) Where 1 = supermarket; 2 = grocery store; 3 = butcher's shop
Sto_r	Type of retail storage		Supermarket: Discrete({1;2}, {11;20}) Grocery store: Discrete({1;2}, {2;5}) butcher's shop: Discrete({1;2}, {3;1}) Where 1 = refrigeration; 2 = freezing; using Quinteros et al. (2008) data.
T_r	Time on retail display	Hours	Triangular(4, 48, 96)
T_{rs}	Refrigeration retail storage temperature	°C	Triangular(4, 6, 10)
Sto_{house}	Type of house storage		Discrete({1;2}, {9;35}) Where 1 = refrigeration; 2 = freezing; using Quinteros et al. (2008) data.
T_{house}	Refrigeration temperature in house	°C	Triangular(5, 8, 15)
$Ps_{initial}$	Initial concentration of *Pseudomonas* spp.	$log_{10}CFU/g$	Triangular(1, 1.5, 2) Lasta and Gimeno (1993)
μ_{Pseudo}	*Pseudomonas* spp. specific growth rate	$Hours^{-1}$	$Exp^{(Normal(-0.4863 + (0.1155 \times (\ln(T_{house}));0,12)}$
Λ_{Pseudo}	*Pseudomonas* spp. lag phase	Hours	$Exp^{(Normal(1.568 + (-0.33 \times (\ln(T_{house}));0,32))}$
Max_{TC}	Maximum time consumption	Days	$((Uniform(8,9) - Ps_{initial})/\mu_{Pseudo}) + \Lambda_{Pseudo}$
TC_{house}	Time to consumption	Days	$Uniform(0, Max_{TC})$
μ	Specific growth rate	$Hours^{-1}$	$Exp^{(Normal(-9258 + (7,155 \times (\ln(\ln(Trs))));0,25))}$
Λ	Lag phase	Hours	$Exp^{(Normal(10,169 + (-2,768 \times (\ln(Trs)));0,147))}$
TMD	Theoretical maximum density	log_{10}	Triangular(5,uniform(5,10), 10)
G_{rs}	Growth during retail storage	log_{10} CFU/g	$C_{FGB} + (TMD - C_{FGB}) \times e^{(-e(((e(1) \times \mu)/(TMD - CFGB))*(\lambda - Tr) + 1))}$
Freezer	Effect of freezing	$log_{10}CFU/g$	If $Sto_{house} = 2$; $G_{rs} = C_{FGB} -$ Cumulative(0, 3, {0;0.5;1;1.5;2;2.5;3}, {0;0;0.19;0.77;0.94;0.968;0.996})
K_0	Thermal inactivation model: regression coefficient		−10.165
K_1	Thermal inactivation model: regression coefficient		0.211
$Cons_{Pref}$	Cooking preference of the consumers		Home: Cumulative(54.4, 68.3, 1,2,3; 0.039,0.0039,0.922) Commercial hamburger Cumulative(54.4, 68.3, 1,2,3; 0,0.136,0.864) Where 1 = medium-rare; 2 = medium; 3 = well-done.
T_{ckh}	Internal temperature of cooked hamburger	°C	Medium-rare = Uniform(54.4,58.6) Medium = Uniform(62.7,65.6) Well-done = 68.3
I_{ckh}	Thermal inactivation from cooking	log_{10} CFU/gr.	$K_0 + K_1 T_{ckh}$
C_{ckh}	Concentration in cooked hamburger	log_{10} CFU/gr.	$G_{rs} - I_{ckh}$
D	Ingested dose of *E. coli* VTEC	CFU	$D \sim Poisson(10^{Cckh} \times m_{hm})$
m_h	Mass of hamburger ingested	g	$m_h \sim PERT(60,83,105)$
n_h	Number of hamburgers ingested in a meal		Adult: Discrete({0.5;1;2;3;4}, {2;7;27;7;1}) Child: Discrete({0.5;1}, {1;1})
m_{hm}	Mass of hamburger ingested in a meal	g	$m_h \times n_h$

dependent upon the type of home storage (Sto_{house}). A range of 5 to 15 °C was used to simulate storage under refrigeration (T_{house}) (Quinteros et al., 2008). The time to consumption (TC_{house}) was modelled using a uniform distribution, where the minimum time to consumption was 0 days (assuming consumption within the same day as purchased), and the upper limit (Max_{TC}) modelling was dependent upon the organoleptic characteristics of the product stored. The Max_{TC} in the home was modelled as the time required by *Pseudomonas* spp. to grow to a level at which it would generate changes in meat organoleptic characteristics (colour and off-odours) at home refrigerator temperatures, selecting values for the maximum growth rate (μ_{Pseudo}) and lag-phase (λ_{Pseudo}) based on data published by Coll Cárdenas et al. (2008).

However, some ground beef and hamburgers may be frozen during storage and transportation (Quinteros et al., 2008). A decline in *E. coli* VTEC levels between 0 and 3 logs per gram of frozen ground beef was modelled (Freezer) using laboratory studies on the effects of freezing on the pathogen level in ground beef (Ansay et al., 1999; Sage and Ingham, 1998; USDA-FSIS, 2001).

2.2.3.1. Microbial growth. To model the microbial growth during storage (G_{rs}), four mathematical models (two equations and two predictive microbiological models) were used for *E. coli* VTEC in ground beef under various storage conditions. The equations were reported by Marks et al. (1998) and by Tamplin et al. (2005) and the predictive microbial models were the Pathogen Modeling Program (PMP) and the Growth Predictor (GP). PMP was developed by the United State Department of Agriculture using one set of equations reported previously (Buchanan and Klawitter, 1992; Buchanan and Bagi, 1994, 1997; Buchanan et al., 1993). GP was built by the Food Standards Agency and the Institute of Food Research in the United Kingdom, using the equations developed by Baranyi and Roberts (1994). For each set of equations, the lag phase period (λ) and the specific growth rate (μ) in a pH range from 5.60 to 6.50 (USDA-FSIS, 2001) and temperatures from 5 to 34 °C (to consider storage at refrigeration and abuse temperatures) were calculated. After that, the linear relationship between temperature and the growth parameters (μ and λ) was calculated. The two linear equations were incorporated into normal distributions.

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A triangular distribution was used to model the theoretic maximum density (TMD) at refrigeration temperatures, where the minimum TMD was assumed to be 5 logs, the maximum TMD to be 10 logs and the "most likely" TMD to be uncertain but ranging uniformly from 5 to 10 logs, using the data reported by Lebert et al. (2000), Marks et al. (1998), and Powell et al. (2004). Finally, the parameters (μ, λ and TMD) were incorporated into the Gompertz growth equation, which is a commonly used mathematical model for predicting the growth of microorganisms at a constant temperature.

2.2.3.2. Thermal inactivation. The log reduction in concentration due to cooking was modelled as a function of the final internal hamburger temperature (T_{ckh}). *E. coli* VTEC thermal inactivation was estimated using a linear model provided by Juneja et al. (1997). The internal temperature of a cooked hamburger was assumed to depend upon the cooking preference of the consumers and was based on consumer survey data in Argentina (Quinteros et al., 2008). These authors reported the proportion of consumers who preferred hamburgers cooked medium rare, medium and well done in the Province of Santa Fe (Argentina). Each cooking preference was related with an internal temperature, using the approach reported by Jackson et al. (1996) (Table 3).

2.2.4. Consumption

The ingested dose (D) is a function of the *E. coli* VTEC in the hamburger at the time of consumption and the mass of hamburger ingested in a meal (m_{hm}). The hamburger mass (m_h) was assumed to be PERT distributed with a "most-likely" value of 83 g and minimum and maximum values of 60 g and 105 g, respectively. The amounts of hamburger ingested (n_h) by adults and children were modelled using data reported by Quinteros et al. (2008).

2.3. Dose–response assessment

Children under 5 years old and elderly people have an increased probability for severe outcomes, including HUS and mortality following infection. However, our study considered that the susceptible population had a similar vulnerability to illness following ingestion of *E. coli* VTEC due to the lack of specific dose–response equations for each subpopulation. Our study incorporated a Beta-Poisson dose response model reported by Strachan et al. (2005), which uses *E. coli* O157 outbreak data and data from published studies; it assumes a non-threshold level of illness (Table 4).

The severe outcome probability was assumed to be some fraction of the probability of illness. For children under 5 years of age, Noris and Remuzzi (2005) estimated that the HUS probability (P_{HUS}) is 3–9% and using information provided by the Argentinean Epidemiology Surveillance System, Rivas (2006) reported an HUS-induced mortality of 2.2–4.8% (P_{mort}) in Argentina between 1995 and 2004. For the elderly, the HUS mortality rate was assumed to be 12% (Cassin et al., 1998).

Table 4
Dose–response assessment.

Variable	Description	Distribution/Model
P_e	Probability of exposure to *E. coli* VTEC	$P(D>0)=P_{FGB}\times(1-e^{-10\ Cckh\times m_{hm}})$
P_{dr}	Beta-Poisson model	$1-[1+D/\beta]^{-\alpha}$
α	Beta-Poisson parameter	0.0571
β	Beta-Poisson parameter	2.2183
P_i	Probability of illness	$P_{dr}\times P_e$
$P_{HUS\|i}$	Probability of HUS given illness	Uniform(0.03,0.09)
$P_{mort\|HUS}$	Probability of mortality given HUS	Child: Uniform(0.022,0.048) Adult: 0.12
P_{HUS}	Probability of HUS	$P_{HUS\|I}\times P_i$
P_{mort}	Probability of mortality	$P_{mort\|HUS}\times P_{HUS}$

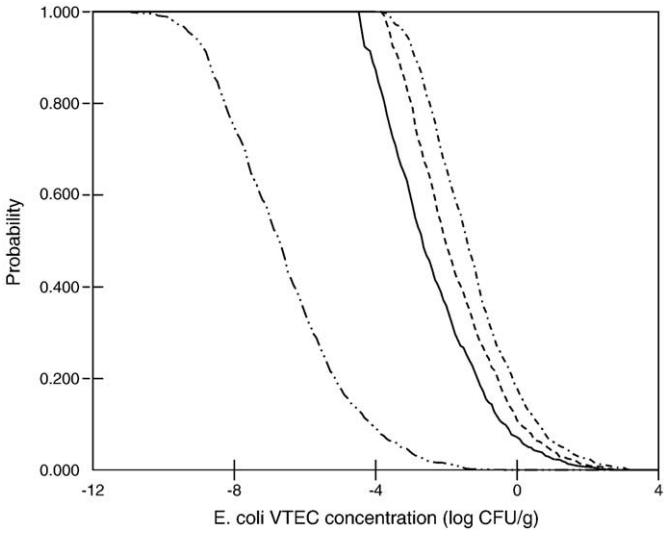

Fig. 3. Predicted *E. coli* VTEC concentration (log CFU/g) throughout the food chain. *E. coli* VTEC concentration (log CFU/g) in fresh ground meat, - - - - during retail storage, -·-·-·- during home storage, and —··—·· after cooking.

2.4. Risk characterisation

Risk characterisation is the estimation of the probability of occurrence and severity of adverse health effects in a given population based on hazard characterisation and exposure assessment. The number of *E. coli* VTEC in a meal was estimated using the predictions of the exposure assessment, and it was the input for the dose–response model. Thus, the probability of illness was the product of the probability of non-zero exposure and the probability of illness from the output of the Beta-Poisson dose–response model.

3. Results

The amount of *E. coli* VTEC that a consumer was exposed to in a single serving of hamburger was a function of the original number of *E. coli* VTEC in the cattle carcass and the subsequent effects of storage, handling and cooking on the growth or decline in the pathogen number (Fig. 3). In fresh ground meat, the average concentration of the pathogen per kg of meat was 3.89 CFU. The number of bacteria increased during distribution and home storage, reaching a microbial concentration of 51.28 CFU/kg hamburger prior to cooking. Cooking resulted in a dramatic reduction of microbial load with an average reduction of 3.75 log CFU (95%CI 1.54–4.24 log CFU). The average pathogen doses ingested by adults and children were 30.96 CFU/kg hamburgers and 81.30 CFU/kg hamburgers, respectively. The data above were for hamburgers containing *E. coli* VTEC. The model predicted the prevalence of hamburgers contaminated with the pathogen to be 1.82% (95% CI; 2.55×10^{-8}% to 14.9%).

Each iteration predicted a probability of illness for a single hamburger meal (Fig. 4). The range of this probability extended from 10^{-9} to 2.29×10^{-3} for adults and from 5.75×10^{-10} to 9.54×10^{-4} for children (95% confidence interval). The distribution depicts the central tendency of the distribution at risks of 8.12×10^{-7} and 3.23×10^{-7} for adults and children, respectively. The log probability of illness was chosen as a convenient representation for the probability of risk, because it was so concentrated near zero that it was not useful to display on a linear scale. The simulated risk for children under 5 years of age was the result of a lower exposure to the pathogen, as they consume fewer burgers (mass) compared to adults. The conditional probability of *E. coli* VTEC infection resulting in HUS was 4.6×10^{-8} (95% CI; 7.41×10^{-11}–1.58×10^{-4}) in adults and 1.8×10^{-8} (95%CI 3.23×10^{-11}–5.24×10^{-5}) in children. Although the illness probability

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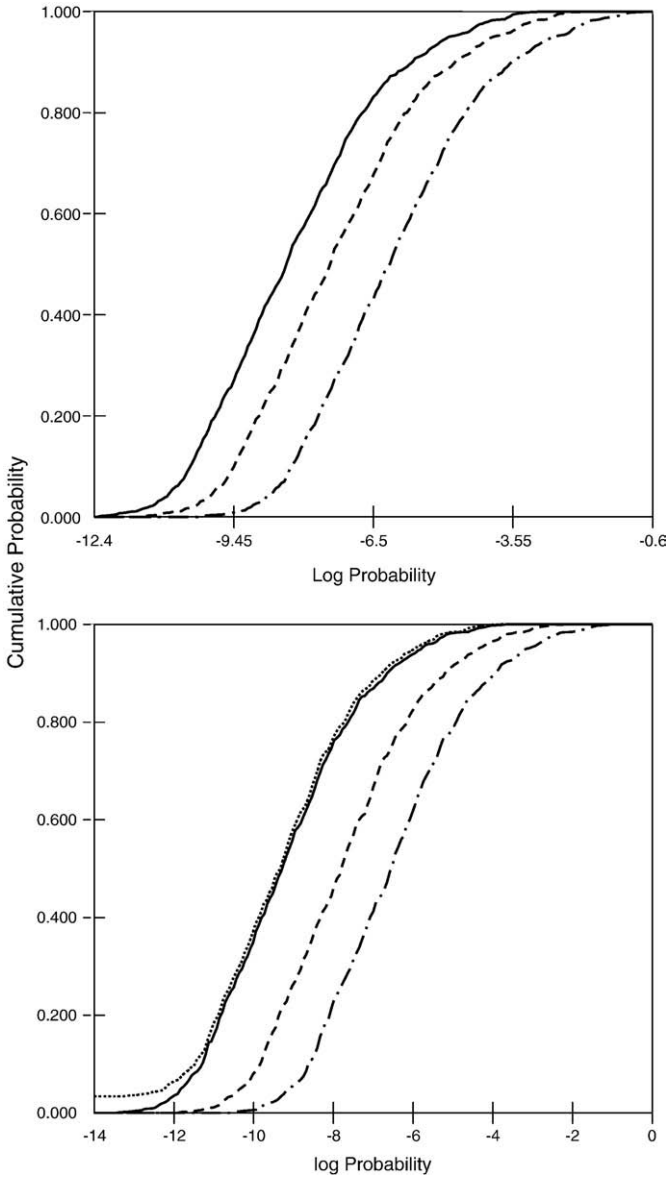

Fig. 4. Cumulative probability distribution for the probabilities of illness, HUS and mortality from a single hamburger meal. -·-·-·- Log Probability of illness, - - - - - HUS, –––– mortality, and ..... chronic renal failure.

values were extremely small, the risk was not insignificant and should be viewed in light of the magnitude of exposure to fully understand the public health impact. Using the conditional probability of HUS-induced mortality, the mean probabilities of mortality for the elderly and children under age 5 were 5×10^{-9} (95% CI; 8.91×10^{-12}–1.9×10^{-5}) and 6.31×10^{-10} (95% CI; 8.91×10^{-13}–2.29×10^{-6}), respectively. Chronic renal failure is another potential outcome of *E. coli* VTEC infection in children; its mean probability was estimated at 9.33×10^{-10} (95% CI; 1.58×10^{-12}–2.63×10^{-6}).

The risk of *E. coli* VTEC infection and its subsequent outcomes was sensitive to the type of meat storage at home ($r=-0.416$), slaughterhouse cooling conditions ($r=0.240$), the bacterial concentration in the cattle hide ($r=0.239$), the origin of the hamburger ($r=-0.116$), retail storage conditions ($r=-0.110$) and increases in the pathogen concentration on the carcass during the cutting and deboning process ($r=0.109$) (Fig. 5).

The storage conditions throughout the agri-food chain were important factors affecting pathogen survival and multiplication and therefore the likelihood of infection. The average probability for a child to acquire HUS as a result of hamburger consumption was 8.31×10^{-8} for refrigerated meat storage at home and 8.00×10^{-9} for frozen meat storage at home. Therefore, refrigerated meat storage increases the risk of HUS in a child ten-fold over frozen meat storage. The lessened risk for HUS can be explained in two ways: frozen meat storage can prevent *E. coli* VTEC growth and it has the potential to reduce the bacterial load, decreasing the ingested dose by approximately two logs. Similarly, the risk of HUS in a child is significantly affected by the cooling of carcasses in the slaughterhouses, which can cause the level of risk to vary between 2.00×10^{-9} and 1.69×10^{-7} (95% CI) (Fig. 6).

This risk assessment is distinctive, because approximately half of the target population eats home-prepared hamburgers. Children were at a 2.75-fold greater risk for developing HUS after consuming home-prepared burgers (3.3×10^{-8}) than commercially-prepared burgers (1.2×10^{-8}) (95%CI 1.37–5.62; $p<0.0017$). To identify confounding factors present among the variables that were sensitive to the likelihood of developing HUS, 1,000 additional iterations were performed in two simulations, keeping only the home meat storage conditions (i.e., refrigeration, freezing) fixed. When refrigerated meat storage was modelled, the risk of developing HUS that was derived from the consumption of home-prepared hamburgers was 5.55×10^{-5} (IC 95%; 5.9×10^{-8}–1.6×10^{-4}), and the risk derived from the consumption for commercially-prepared hamburgers was 4.77×10^{-5} (IC 95%; 5.9×10^{-8}–1.6×10^{-4}); no statistically significant differences were found ($p<0.42$). When frozen meat storage was modelled, the risk of developing HUS from home-prepared or commercially-prepared hamburgers was 2.23×10^{-6} (IC 95%; 9.62×10^{-11}–1.16×10^{-6}) or 1.24×10^{-6} (IC 95%; 3.34×10^{-10}–2.77×10^{-6}), respectively; no statistically significant differences were found ($p<0.56$). Therefore, the greatest risk of becoming ill by consuming home-prepared hamburgers derived from the storage conditions during production, distribution, and in the home and not from any intrinsic characteristic of the product.

The pathogen level in the cattle hide was another important factor that influenced the risk of *E. coli* VTEC infection. The cattle hide served as the source of the microorganisms on the carcass and then in the fresh ground beef and hamburger. When the *E. coli* VTEC concentration in the hide was −2.4 log CFU/100 cm^2 (5th percentile), the probability of developing HUS was 9.33×10^{-10} (95%CI 1.44×10^{-11} – 1.02×10^{-7}), but if the pathogen concentration was increased to 2.61 log CFU/100 cm^2 (95th percentile), the probability of developing HUS was 2.23×10^{-7} (95%CI 4.26×10^{-10} – 2.04×10^{-4}) (Fig. 6).

Pathogen growth during the cutting and deboning process had a strong influence on the likelihood of developing HUS. At this stage of the process, the risk for a child to develop HUS ranged from

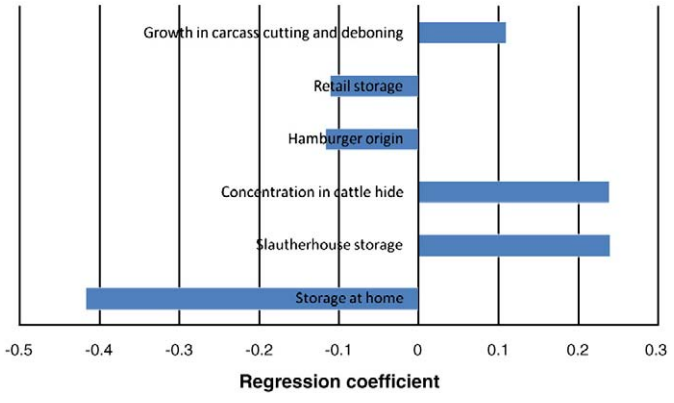

Fig. 5. Regression coefficient between the estimated probability of children developing HUS and the most important predictive factors.

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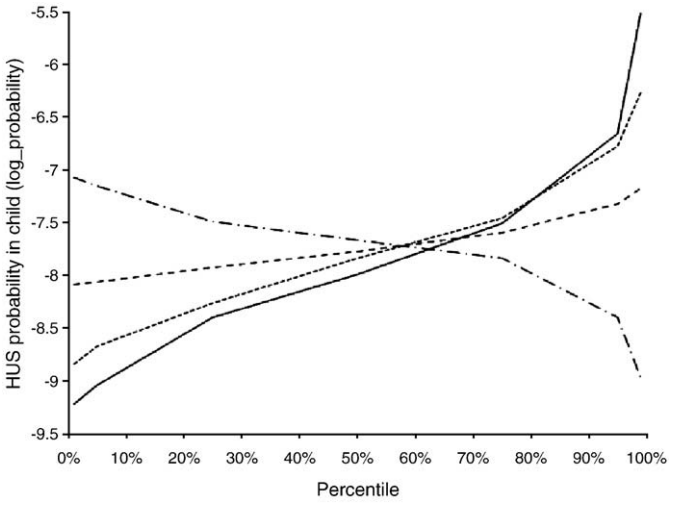

Fig. 6. Spider diagram: relationship between the change in the probability of children developing HUS and changes in the most important predictive factors. –––– *E. coli* VTEC concentration in cattle hide. ..... Slaughterhouse storage temperature. - - - - - Growth in carcass cutting and deboning. —··—·· Effect of freezing storage at home.

8.00×10^{-9} to 4.7×10^{-8} between the 5th and 95th percentiles of the microbial rate growth distribution (Fig. 6).

4. Discussion

The quantitative risk assessments for *E. coli* VTEC infection following the consumption of hamburgers that have been published to date have taken into account storage conditions, distribution and consumption patterns from developed countries like Canada (Cassin et al., 1998), Australia (Lammerding et al., 1999), the Netherlands (Nauta et al., 2001), the United States (USDA-FSIS, 2001) and the Republic of Ireland (Duffy et al., 2006). All of these models used the same approach, and differed only in the pathogen prevalence in cattle and the dose–response model. The probabilities for *E. coli* VTEC infection, HUS and mortality following hamburger consumption were estimated by these models at 6.0×10^{-7}–4.6×10^{-4}, 4.2×10^{-9}–4.6×10^{-5} and 5.9×10^{-10}–2.3×10^{-6}, respectively.

Our model is the first in Latin America. We considered hamburger manufacturing, sale, distribution, consumption patterns in Argentina, and the prevalence of *E. coli* VTEC in local cattle. One distinctive characteristic of this population was the habit of eating hamburgers prepared at home. This has important implications for the risk of infection, because the supply chain of commercially-prepared hamburgers involves transport and retail storage at temperatures lower than −10 °C, which both prevents microbial growth and reduces the pathogen concentration. Conversely, when burgers are prepared at home, they are made from ground beef that has been chilled in a butcher's shop. Chilling does not prevent microbial growth and results in an increase in the pathogen content of hamburger. This was apparent in the sensitivity analysis, in which one of the most important factors was home storage, and the risk of illness was estimated to be higher when burgers were refrigerated than when they were frozen.

In agreement with previously published risk assessments, the risk of illness was highly correlated with the initial concentration of the pathogen in cattle. Therefore, risk management should focus on reducing the microbial load in the raw material. Previous studies have demonstrated that *E. coli* O157:H7 is prevalent in cattle faeces and varies from 0.1% to 53% (Duffy et al., 2006). In the present study, the average prevalence of pathogenic strains of *E. coli* VTEC was 21%.

In our study, the risk of infection and the subsequent development of HUS was not sensitive to the internal temperature of cooked hamburgers, as has previously been reported (Cassin et al., 1998; Duffy et al., 2006; Lammerding et al., 1999). However, this does not mean that the microbial load and the risk of illness are not reduced at this stage; instead, it reflects the large percentage of the target population that prefers to eat hamburgers well done. For that reason, the process presented a low variability and did not alter the final estimate of risk.

The absence of local information on the *E. coli* VTEC counts on bovine hides and carcasses was recognised as one of the most important limitations of this model. Similar practices and equipment are used for cattle slaughtering in Argentina as in Europe and the United States of America. Argentina is an important beef-exporting country and it must meet the health requirements imposed by importing countries to introduce its products in the international markets. Some authors (Lasta and Gimeno, 1993) have reported that the microbiological meat quality produced by Argentine slaughterhouses was equal to or lower than those reported in other countries including Australia, Europe and the United States of America. Additionally, the distribution for *E. coli* VTEC counts estimated in this model (mean = 2.3 log CFU/cm^2, 95% CI; 0–4.32 log CFU/cm^2) was similar to the contamination levels reported by the USDA (range = 0–3 log CFU/cm^2) in beef carcasses produced in U.S. slaughterhouses (USDA-FSIS, 2001).

In our model, the probability of HUS in adults was higher than in children. This is in disagreement with the fact that young children have an increased probability of severe outcomes such as HUS and death following infection. In Argentina, it was reported that 95% of the HUS cases were in children under 5 years old (Rivas, 2006). A possible explanation for this discrepancy is because, following the Cassin et al. (1998) approach, the susceptible population was assumed to have a similar vulnerability to illness following ingestion of *E. coli* VTEC given the lack of data to separate the susceptible groups. It would therefore be appropriate to focus only on the probability of HUS reported for children.

Although the results seem reasonable, there is still room for improving the model: a) incorporate data from historical records of storage temperatures during different stages of the process to incorporate a more accurate representation of the *E. coli* VTEC concentration in the hide and carcass into the microbial growth model, b) improve the assessment of cross-contamination sources, c) we assumed that there was a homogenous distribution of faeces on the carcass, but an assumption of some clustering may yield different results, d) incorporate other variations in the prevalence of the disease with consideration of seasonal variations and different feeding systems and e) incorporate information on the initial levels of bacteria on animal hides in Argentina.

5. Conclusions

The pathogen concentration in the hides of cattle destined to slaughter was one of the most important factors associated with the risks for *E. coli* VTEC infection and HUS. In addition, the temperature during meat processing and thermal abuse during storage and food preparation were determinant in the risk for infection and its subsequent outcomes. These parameters differed depending on the type of hamburger consumed (commercially- or home-prepared) and resulted in different risks for illness. This model will enable risk managers to adopt risk management measures for the appropriate steps that we have identified in this sensitive analysis with the aim of reducing the risk for *E. coli* VTEC infection, especially in those populations that frequently consume home-prepared hamburgers.

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References

Ansay, S.E., Darling, K.A., Kaspar, C.W., 1999. Survival of *Escherichia coli* O157:H7 in ground-beef patties during storage at 2, −2, 15 and then −2 degrees C, and −20 degrees C. Journal of Food Protection 62, 1243–1247.

Bacon, R.T., Belk, K.E., Sofos, J.N., Smith, G.C., 2000. Incidence of *Escherichia coli* O157:H7 on hide, carcass and beef trimmings samples collected from United States packing plants. Presented at FSIS public meeting on *E. coli* O157:H7, February 29, 2000, Arlington, VA.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology 23, 277–294.

Buchanan, R.L., Bagi, L.K., 1994. Expansion of response surface models for the growth of *Escherichia coli* O157:H7 to include sodium nitrite as a variable. International Journal of Food Microbiology 27, 317–332.

Buchanan, R.L., Bagi, L.K., 1997. Effect of water activity and humectants identity on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiology 14, 413–423.

Buchanan, R.L., Klawitter, L.A., 1992. The effect of incubation temperature, initial pH and sodium chloride on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiology 9, 185–196.

Buchanan, R.L., Bagi, L.K., Goins, R.V., Phillips, J.R., 1993. Response surface models for the growth kinetics of *Escherichia coli* O157:H7. Food Microbiology 10, 303–315.

Byrne, C.M., Bolton, D.J., Sheridan, J.J., McDowell, D.A., Blair, I.S., 2000. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. Letters in Applied Microbiology 30, 142–145.

Cassin, M.H., Lammerding, A.M., Todd, E.C.D., Ross, W., McColl, R.S., 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. International Journal of Food Microbiology 41, 21–44.

Coll Cárdenas, F., Giannuzzi, L., Zaritzky, N.E., 2008. Mathematical modelling of microbial growth in ground beef from Argentina. Effect of lactic acid addition, temperature and packaging film. Meat Science 79, 509–520.

Cornell Collaborative Project, 1998. Prevalence of *E. coli* O157:H7 pre-slaughter/*E. coli* O157:H7 Associated risk factors. New York State Cull Cow Sub-Project. Final Report.

Cummins, E., Nally, P., Butler, F., Duffy, G., O'Brien, S., 2008. Development and validation of a probabilistic second-order exposure assessment model for *Escherichia coli* O157:H7 contamination of beef trimmings from Irish meat plant. Meat Science 79, 139–154.

Dorsa, W., 1997. Review: new and established carcass decontamination procedures commonly used in the beef-processing industry. Journal of Food Protection 60, 1146–1151.

Duffy, G., Cummins, E., Nally, P., O'Brien, P., Carney, S., Butler, F., 2006. *E. coli* O157:H7 in beef burgers produced in the Republic of Ireland: a quantitative microbial risk assessment. Report published by Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland.

Elder, R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmaraie, M., Laegreid, W.W., 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in faeces, hides, and carcasses of beef cattle during processing. Proceeding of the National Academy of Sciences of the United States of America 97, 2999–3003.

FAO-WHO, 2006. Food safety risk analysis. A guide for national food safety authorities. FAO Food and Nutrition Paper N° 87. Food and Agriculture Organization of the United Nations, Rome. ISBN: 978-92-5-105604-2, p. 121.

Gill, C.O., 1999. HACCP by guesswork or by the numbers? Food Quality 6, 28–32.

Gill, C.O., Bryant, J., 1997. Assessment of the hygienic performances of two beef carcass cooling processes from product temperature history data or enumeration of bacteria on carcass surfaces. Food Microbiology 14, 593–602.

Gill, C.O., Landers, C., 2003. Microbiological effects of carcass decontaminating treatments at four beef packing plants. Meat Science 65, 1005–1011.

Gill, C.O., McGinnis, J.C., Badoni, M., 1996. Use of total *Escherichia coli* counts to assess the hygienic characteristics of a beef carcass during processing. International Journal of Food Microbiology 31, 181–196.

Jackson, T.C., Hardin, M.D., Acuff, G.R., 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. Journal of Food Protection 59, 230–237.

Juneja, V.K., Snyder, O.P., Williams, A.C., Marmer, B.S., 1997. Thermal destruction of *Escherichia coli* O157:H7 in hamburger. Journal of Food Protection 60, 1163–1166.

Lammerding, A.M., Fazil, A., Paoli, G., Desmarchelier, P., Vabderkubde, P., 1999. Shiga toxin-producing *E. coli* in ground beef manufactured from Australian beef: process improvement. Food Science Australia, Brisbane Laboratory.

Lasta, J.A., Gimeno, E.J., 1993. Hygiene conditions of beef carcasses and cuts in Argentine meat packing plants. Fleischwirtsch 73, 169–171.

Lebert, I., Robles-Olvera, V., Lebert, A., 2000. Growth of mixed cultures of *Listeria* and *Pseudomonas* spp. in beef meat and their prediction using polynomial models. 46th ICoMST, pp. 700–701.

Marks, H.M., Coleman, M.E., Lin, C.T.J., Roberts, T., 1998. Topics in microbial risk assessment: dynamic flow tree process. Risk Analysis 18, 309–328.

Ministerio de Salud y Ambiente de la Nación, 2005. Diarrea aguda en Argentina. Precauciones especiales durante el período de verano. Epi-Noticias, Año IV, Número 527, p. 3.

Meichtri, L., Miliwebsky, E., Gioffré, A., Chinen, I., Baschkier, A., Chillemi, G., Guth, B.E.C., Masana, M.O., Cataldi, A., Rodríguez, H.R., Rivas, M., 2004. Shiga toxin-producing *Escherichia coli* in healthy young beef steers from Argentina: prevalence and virulence properties. International Journal of Food Microbiology 96, 189–198.

McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Thomson-Carter, F.M., Garvey, P., McGuire, L., Blair, I.S., McDowell, D.A., 2003. The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. Journal of Applied Microbiology 95, 256–266.

Nauta, M., Evers, E., Takumi, K., Havelaar, A., 2001. Risk assessment of shiga-like producing *Escherichia coli* O157 in steak tartar in the Netherlands. Report 257851003/2001. RIVM, Bilthoven, The Netherlands, p. 169.

Noris, M., Remuzzi, G., 2005. Hemolytic uremic syndrome. Journal of the American Society of Nephrology 16, 1035–1050.

Notario, R., Fain, J.C., Prado, V., Ríos, M., Borda, N., Gambande, T., 2000. Prevalence of enterohemorrhagic *Escherichia coli* in a cattle area of Argentina. Genotypic characterization of the strains of animal origin. Revista Médica de Chile 128, 1335–1341.

O'Brien, S.B., Duffy, G., Daly, D., Sheridan, J.J., Blair, I.S., McDowell, D.A., 2004. Detection limit and recovery rates achieved using direct plate and enrichment/immunomagnetic separation methods for *Escherichia coli* O157:H7 in minced beef and bovine hide. Letters in Applied Microbiology 41, 88–93.

O'Brien, S.B., Duffy, G., Carney, G., Sheridan, J.J., McDowell, D.A., Blair, I.S., 2005. Prevalence and numbers of *Escherichia coli* O157 on bovine hide at a beef slaughter plant. Journal of Food Protection 68, 660–665.

Omisakin, F., MacRae, M., Ogden, I.D., Strachan, N.J.C., 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle faeces at slaughter. Applied and Environmental Microbiology 69, 2444–2447.

Oteiza, J.M., Chinen, I., Miliwebsky, E., Rivas, M., 2006. Isolation and characterization of shiga toxin-producing *Escherichia coli* from precooked sausages (morcillas). Food Microbiology 23, 283–288.

Padola, N.L., Sanz, M.E., Lucchesi, P.M., Blanco, J.E., Blanco, J., Blanco, M., Etcheverría, A., Arroyo, G.H., Parma, A.E., 2002. First isolation of the enterohaemorrhagic *Escherichia coli* O145:H- from cattle in feedlot in Argentina. BMC Microbiology 2, 6–10.

Padola, N.L., Sanz, M.E., Blanco, J.E., Blanco, M., Blanco, J., Etcheverria, A.I., Arroyo, G.H., Usera, M.A., Parma, A.E., 2004. Serotypes and virulence genes of bovine shigatoxigenic *Escherichia coli* (STEC) isolated from a feedlot in Argentina. Veterinary Microbiology 100, 3–9.

Powell, M., Schlosser, W., Ebel, E., 2004. Considering the complexity of microbial community dynamics in food safety risk assessment. International Journal of Food Microbiology 90, 171–179.

Quinteros, C., Marín, V., Tarabla, H., Signorini, M., 2008. Consumption patterns of hamburger in Santa Fe (Argentina) Province. XVII Technical Scientific Meeting of the Argentine Association of Diagnostics Laboratory Veterinary, Santa Fe, October 28th to 31st. Abstract E9.

Rearte, D., 2007. Situación de la ganadería Argentina en el contexto mundial. Proyecto Carne—Instituto Nacional de Tecnología Agropecuaria. Available at http://www.inta.gov.ar/balcarce/Carnes/sitgan.htm.

Rice, D.H., Ebel, E.D., Hancock, D.D., Herriott, D.E., Carpenter, L.V., 1997. *Escherichia coli* O157 in cull dairy cows on farm, and at slaughter. Journal of Food Protection 60, 1–2.

Rivas, M., 2006. The epidemiology of hemolytic uremic syndrome in Argentina. Diagnosis of the etiologic agent, reservoirs and routes of transmission. Medicina 66, 27–32.

Roberts, T., Malcolm, S.A., Narrod, C., 1999. Probabilistic risk assessment and slaughterhouse practices: modelling contamination process control in beef destined for hamburger. Proceedings of the probabilistic safety assessment specialty meetings, pp. 809–815. Washington, DC, August 1999.

Rosmini, M.R., 2006. Métodos de insensibilización y matanza. In: Limusa (Ed.), Ciencia y Tecnología de Carnes. ISBN: 968-18-6549-9, pp. 43–85. México D.F.

Sage, J.R., Ingham, S.C., 1998. Survival of *Escherichia coli* O157:H7 after freezing and thawing in ground beef patties. Journal of Food Protection 61, 1181–1183.

Sanz, M.E., Vinas, M.R., Parma, A.E., 1998. Prevalence of bovine verotoxin-producing *Escherichia coli* in Argentina. European Journal of Epidemiology 14, 399–403.

Sheridan, J.J., 2000. The effectiveness of commercial decontamination treatments on fresh meat carcasses. International livestock conference, Beef Program 2000. Houston, TX (USA).

Strachan, N.J.C., Doyle, M.P., Kasuga, F., Rotariu, O., Ogden, I.D., 2005. Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks. International Journal of Food Microbiology 103, 35–47.

Tamplin, M.L., Paolib, G., Marmera, B.S., Pillips, J., 2005. Models of the behavior of *Escherichia coli* O157:H7 in raw sterile ground beef stored at 5 to 46 °C. International Journal of Food Microbiology 100, 335–344.

USDA-FSIS, 2001. Draft risk assessment of the public health impact of *Escherichia coli* O157:H7 in ground beef. Available from http://www.fsis.usda.gov, last accessed: April 2008.

Vose, D., 1996. Quantitative Risk Analysis: A Guide to Monte Carlo Simulation Modelling. John Wiley and Sons, Chichester, England.

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