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Use of quantitative microbial risk assessment when investigating foodborne illness outbreaks: The example of a monophasic *Salmonella* Typhimurium 4,5,12:i:— outbreak implicating beef burgers

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ABSTRACT

A major community outbreak of salmonellosis occurred in France in October 2010. Classical epidemiological investigations led to the identification of beef burgers as the cause of the outbreak and the presence of the emerging monophasic *Salmonella* Typhimurium 4,5,12:i:—.

The objective of this study was to understand the events that led to this large outbreak, that is to say, what are the contributing factors associated with consumer exposure to *Salmonella*. To this end, intensive microbiological investigations on several beef burgers were conducted and a risk assessment model was built.

The microbiological results confirm the presence of *Salmonella* in all analysed frozen burgers at high levels of contamination above 1000 MPN/g. These results in frozen burgers combined with a model of thermal destruction were used to estimate the dose ingested by the exposed persons. Most people that consumed cooked beef burgers were exposed from 1.6 to 3.1 $\log_{10}$ (MPN). The number of sick people predicted with a dose–response relationship for *Salmonella* is consistent with the observed number of salmonellosis cases.

The very high initial contamination level in frozen beef burgers is the primary cause of this large outbreak rather than bad cooking practices. Intensive investigations, modelling of the initial contamination and quantitative exposure and risk assessments are complementary to epidemiological investigation. They can be valuable elements for the assessment of missing information or the identification of the primary causes of outbreaks.

1. Introduction

Salmonella is a leading cause of bacterial foodborne disease in France (Delmas et al., 2006) and Europe (EFSA, 2010), for which foodstuffs of animal origin are most often implicated. The relative share of human cases associated with beef products is lower than those associated with products derived from poultry and pork meat or with eggs (David, 2009).

Nevertheless, two recent studies prioritising microbial hazards associated with beef, identified *Salmonella* as the most significant hazard, surpassing Shiga toxin-producing *Escherichia coli* [STEC] (Fosse et al., 2008; Greig and Ravel, 2009).

Over the last two decades, several salmonellosis outbreaks associated with consumption of minced beef have been detected by the French National Reference Center (CNR) for *Salmonella* and investigated by the French Institute for Public Health Surveillance [InVS] (Guillois-Bécel et al., 2009; Haeghebaert et al., 2001). A case–control study also showed that minced beef consumed without thorough cooking was a risk factor for the onset of sporadic infections in children under the age of 15 (Delarocque-Astagneau et al., 2000).

In October 2010, an outbreak of salmonellosis was triggered in over 500 people, mainly located in the city of Poitiers in Vienne department western France, after eating beef burgers served in school canteens (Raguenaud et al., 2012). Epidemiological investigations conducted by the veterinary services identified a production batch of frozen beef burgers. This batch identification led to the rapid withdrawal and recall of the burgers. This recall alert was published at the European level in the Rapid Alert System for Food and Feed by the French authorities in November 2010 (Anonymous, 2010).

The microbiological risk of STEC associated with the consumption of beef burgers prompted the Ministry of Agriculture to recommend conducting a visual inspection for the absence of pink meat when beef burgers are prepared by institutional caterers and checking that a core temperature of at least 65 °C was reached (DGAL, 2007). According to thermal inactivation parameters of *Salmonella* (van

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Asselt and Zwietering, 2006), inactivation at 65 °C is important and consequently, these control measures can also be thought as efficient to control *Salmonella* risk.

The magnitude of the community outbreak in Poitiers raises questions about its causes insofar as the local health department did not detect any improper practices by the centralised kitchen facility that prepared the burgers. Several hypotheses were possible, alone or in combinations: the contamination level was high, the *Salmonella* strain implicated was highly virulent, or the existing control measures were inadequate.

A retrospective study of the food in question, through the production and distribution chain, can sometimes identify the most likely scenario leading to contamination (Tauxe et al., 2010). Intensive investigations of outbreaks may help in preventing future illnesses by providing information needed to take new control or prevention measures (Jones et al., 2004; Schoder et al., 2012).

Modelling may then be invaluable for analysing all available information. The use of modelling, particularly of the dose–response relationship, was recently recommended in the investigation of community outbreaks when attempting to assess missing information such as quantity consumed or population exposed (Teunis et al., 2010). It was also recently shown that combinations of several modelling approaches including quantitative microbial risk assessment, and spatial epidemiology can help to improve the understanding of how humans become infected by a pathogen (Rotariu et al., 2012).

The purpose of this study was to understand which events led to this large-scale community outbreak. To achieve this goal, it was necessary to (i) characterise the concentration and distribution of *Salmonella* in burgers from the same batch incriminated in the community outbreak and, (ii) to aggregate all available information in a risk assessment model to estimate the doses to which consumers were exposed.

2. Materials and methods

2.1. Characterisation of contamination in the implicated batch of minced beef

2.1.1. Samples

Two series of beef burgers from the batch incriminated in this foodborne outbreak were analysed. The first series consisted of five burgers (referenced hereafter as burgers I to V) sent by the routine laboratory which analysed samples taken by the caterer. This laboratory thawed the stored frozen burgers at 4 °C and confirmed the presence of *Salmonella* (in 25 g). The unanalysed part of burgers were refrozen and sent to our laboratory.

The second series consisted of frozen beef burgers from the withdrawal and recall by the burger supplier. Two packets of beef burgers were sent to our laboratory. Eighteen burgers, nine per box, (referenced hereafter as burgers 1 to 18) were randomly selected for analysis.

2.1.2. Quantification and biomolecular identification of Salmonella

Salmonella serotyping was performed following the Kauffman–White Scheme (Grimont and Weill, 2007).

Quantification of *Salmonella enterica* subsp. enterica serovar 4,5,12:i:—, strain isolated in the frozen burgers incriminated, was carried out using the mini-MSRV (Oxoid, Dardilly, France) MPN method (Fravalo et al., 2003) and by the surface plating method on XLD agar (AES Chemunex, Bruz, France). Both enumeration methods were used for the first series of burgers. Only the MPN method was used for the second series.

The confirmation step was performed on presumptive colonies by real-time PCR, targeting the *ttrC* gene (Bugarel et al., 2011). Negative PCR results were tested by multiplex PCR targeting *rpoB* (all *Enterobacteriaceae*) and *invA* (*Salmonella*-specific) genes (Malorny et al., 2004).

2.1.3. Quantification of other microflora

For the second series of thawed beef burgers, the determination of *E. coli* counts, aerobic colony count and *Enterobacteriaceae* counts were made for ten beef burgers using the following validated standardised methods: the NF ISO 16649-2 standard method using tryptone bile X-glucuronide agar (AES Chemunex) with an incubation period of 24 h at 44 °C, the NF ISO 4833 standard method using plate count agar (Oxoid) at 30 °C and incubation for 72 h, and the NF V08-054 method using violet red bile glucose agar (AES Chemunex) at 30 °C for 24 h, respectively. Buffered peptone water (Oxoid) or 0.1% peptone solution (AES Chemunex) was used for initial suspension and for the 1:10 dilutions of the samples.

2.2. Assessment and validation of consumer exposure

This section describes how the consumer exposures to *Salmonella* were estimated and how this result was validated. The epidemiological investigation pointed out that 1559 students and employees ($n_{exposed}$) attending lunch in one of the four schools of the city of Poitiers were exposed to the incriminated batch of frozen beef burgers. Each cooked burger was assumed to be entirely consumed. The burgers weight was 120 g (w). Consumer exposures were calculated from the initial contamination in frozen burgers (N_0) and the inactivation during cooking of beef burgers (R). Both the uncertainty and variability of exposures to *Salmonella* ($dose_{ij}$) were estimated. To validate these results of exposure, we used different available dose–response models by comparing predicted number of salmonellosis (N_{ill}) to the observed number (554 out of 1559 exposed persons). Dependence between the variables of the model to calculate exposures is shown in Fig. 1 and Table 1. The

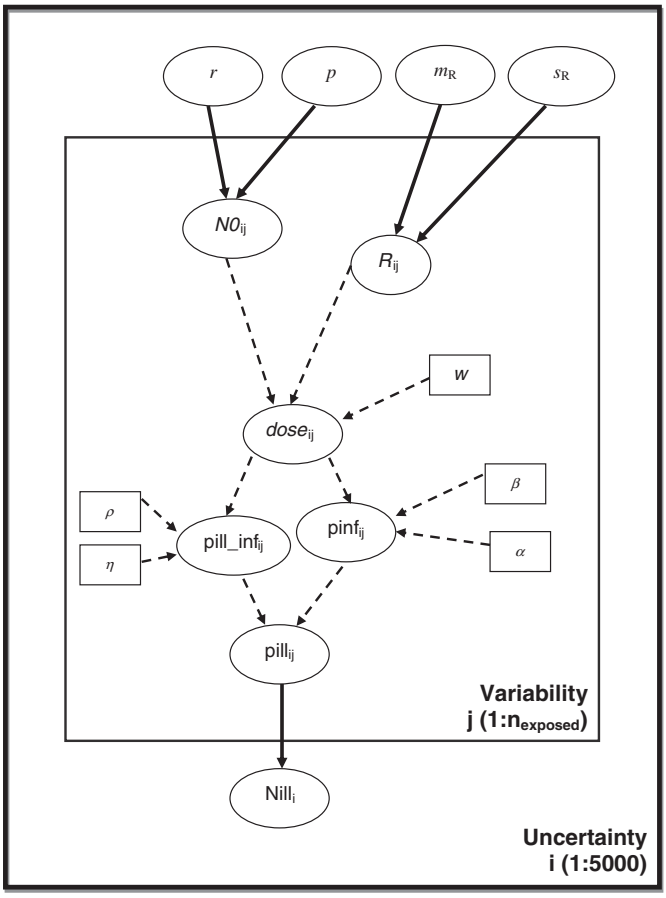

Fig. 1. Directed acyclic graph of model #2. Deterministic links between nodes are represented by dotted arrows. Stochastic links are represented by plain arrows. Links are reported in Table 2.

link between exposure to *Salmonella* and illness is detailed in Fig. 1 and Table 2.

2.2.1. Initial contamination of beef burgers before cooking

Two statistical distributions, Poisson and negative binomial (also called Poisson–Gamma) distributions were considered for describing the microbial concentration of *Salmonella* in the samples of beef burgers before cooking (N_0). The R (R version 2.14.1: A Language and Environment for Statistical Computing, 2011) *fitdistrplus* package (Delignette-Muller et al., 2010; Pouillot and Delignette-Muller, 2010) was used to fit and compare both distributions. Skewness-kurtosis plot, the Akaike information criterion (AIC) and Schwarz Bayesian criterion (BIC) were used to choose the most appropriate distribution to model N_0. This package includes a set of functions dedicated to facilitating the entire process of fitting parametric distributions for different types of data, including censored data. Bootstrap resampling was used to characterise uncertainty on parameters of the fitted distributions.

2.2.2. Modelling the destruction of Salmonella during cooking

Information collected during the investigation highlighted that cooking practices were the same in the different schools. Unthawed burgers were fried in a sauté pan and then stored in a preheated hot cabinet until serving. Storage durations in hot cabinets were about 1 h. It should be noted that three of the four school facilities measured the temperature at the centre of the burgers and performed a visual inspection after cooking to confirm the absence of any pink colour. One facility measured temperature three times in beef burgers. The reported measured temperatures were above 65 °C.

To assess the inactivation corresponding to these cooking practices (R), results of a study on the impact of cooking practices of beef burgers on microbiological contamination (Bergis et al., 2009) were used. The authors measured the actual *Salmonella* inactivation of inoculated beef burgers according to different factors: for unthawed or thawed out burgers cooked in an oven or an electric contact grill and for different levels of cooking, i.e. rare, medium and well done. All the observed $\log_{10}$ reductions were modelled with a generalised linear model according to the three input variables, i.e. the initial state of beef burgers, the method of cooking and the final doneness. According to the model of Bergis et al. (2009), the average inactivation for sautéing (m_R) was 2.85 $\log_{10}$ of *Salmonella* with confidence limits at 95% for this average, ranging between 2.6 and 3.1 $\log_{10}$ for a medium level of cooking under these conditions. The results of this study also showed that the between-burger variability of reduction is independent of the cooking method and of the cooking level. Standard deviations of decimal reductions were used to model the uncertainty of the standard deviation of the log reductions (s_R). A gamma distribution was fitted to these data.

2.2.3. Dose–response relationships for Salmonella

The two dose–response relationships used were those proposed by the FAO/WHO (2002) and by Teunis et al. (2010). Both equations are presented in Table 2. The Teunis et al. relationship takes into account the risk of infection (p_{inf}) and the probability of becoming ill when infected (p_{illinf}) to calculate the probability of illness ($p_{ill} = p_{inf} \cdot p_{illinf}$) as a function of the ingested dose. FAO/WHO relationship directly calculates the probability of illness (p_{ill}) as a function of the ingested dose. The models that predict number of salmonellosis are indicated hereafter as models #1 and #2, for the FAO/WHO or the Teunis et al. models respectively.

For each model, two situations were considered: taking into account (models #1u, #2u) or not taking into account (models #1 and #2) the uncertainty of the parameters of the dose–response model. A set of 5000 values of the four parameters (α, β, η and ρ) of the Teunis et al. model (Teunis et al., 2010) was provided by the authors. The parameters selected for the dose–response relationship of Teunis et al. correspond to the parameters obtained for *Salmonella* Typhimurium and for those people who are believed to be non-susceptible.

The uncertainty of α and β of the FAO/WHO model was simulated using triangular laws with parameters that comply with the minimum, maximum, and most likely values given in the report (FAO/WHO, 2002).

2.2.4. Two dimensional Monte Carlo simulations

Two-dimensional (or second-order) Monte-Carlo simulations, in which the estimation of variability and uncertainty in the exposure/risk estimates is separated (Pouillot et al., 2010), were performed as follows:

> For one set of uncertain parameters, i.e. (i) parameters of negative binomial distribution (r and p) describing the number of *Salmonella* cells in frozen burgers, (ii) parameters on the normal distribution describing reduction corresponding to the cooking step, and eventually (iii) parameters of used dose–response relation (Table 2), the contamination with *Salmonella* (N_0) and a reduction during cooking of burgers (R) were randomly selected from their estimated distributions (Table 1). The number of cells ingested (dose) was then calculated and the number of salmonellosis cases (N_{ill}) out of the number of people exposed ($n_{exposed}$) was estimated.

These simulations were performed using 5000 sets of uncertain parameters, leading to a credibility distribution of the number of cases, summarised by its median and its 2.5th and 97.5th percentiles. The model was implemented using Matlab 2012 software (MathWorks Inc., Natick, Mass.).

3. Results

3.1. Quantification of indicator microflora

For the ten beef burgers, aerobic colony counts (ACC) were between 4.4 and 6.4 $\log_{10}$ (CFU/g). Within these ten concentrations, one is above 5 $\log_{10}$, the M value of the process hygiene criteria of the European Regulation EC 2073/2005 for ACC in minced beef. *E. coli* counts (ECC) were between 2.8 and 3.1 $\log_{10}$ (CFU/g), all above the M value fixed for ECC in the same regulation. The ten *Enterobacteriaceae* counts were comprised between 3.4 and 3.9 $\log_{10}$ (CFU/g).

3.2. Biomolecular identification of Salmonella

Isolates from burgers were collected and serotyped by the *Salmonella* Network (Anses, Maisons-Alfort, France, http://www.afssapro.fr/reseausalmonella/). They were identified as monophasic variants of *S.* Typhimurium with the antigenic formula 1, 4,5,12:i:—. The same identification was determined by the CNR-Salm (Pasteur Institute, Paris, France, http://www.pasteur.fr/ip/easysite/pasteur/fr/sante/centres-nationaux-de-reference-et-centres-collaborateurs-de-l-oms/cnr-et-ccoms/cnr-des-salmonella/identite-et-coordonnees) for the patient's isolates.

All the isolates were further characterised by additional typing methods such as MLVA, PFGE, CRISPOL, or antibiogram. None of these characterization methods were capable of differentiating the isolates.

3.3. Quantification, and modelling of Salmonella contamination

AIC statistics associated to colony counts of *Salmonella* on XLD plate were 72 and 74 for series 1, 91 and 127 for series 2, for negative binomial and Poisson distribution respectively. BIC statistics indicated in the same way (data not shown) that the negative binomial distribution is suitable to fit the distribution of *Salmonella* contamination in raw beef burgers, even if this distribution has one more parameter than the Poisson distribution. As the negative binomial distribution better described the variability of contamination in raw beef burgers than the

Table 1
Relationship between the different nodes. 'F' for fixed, 'V' for variability, 'U' for uncertainty and 'VU' for variability and uncertainty.

Parameters		Dispersion	Abbreviation	Description	Unit
Parent nodes					
Parameters of negative binomial distribution (NB) describing N_0	Number of successes	U	r	5000 pairs of (r, p) from	–
	Probability of success in a single trial	U	p	bootstrap resampling	–
Parameters of the normal distribution describing R	Mean	U	m_R	Normal (2.85, 0.13)	$\log_{10}$ (MPN/g)
	Standard deviation	U	s_R	Gamma (0.779,0.239)	$\log_{10}$ (MPN/g)
Weight of burgers		F	w	120	g
Dose–response parameters and laws		See Table 2			
Child nodes					
Contamination in frozen beef burger		VU	$N0_{ij}$	NB (r, p)	(MPN/g)
Log reduction		VU	R_{ij}	Normal (m_R, s_R)	$\log_{10}$ (MPN/g)
Ingested dose		VU	$dose_{ij}$	$N0_{ij} \cdot 10^{R_{ij}} \cdot w$	MPN
Probability of infection		VU	$pinf_{ij}$	See Table 2	Probability
Probability of illness, given infection		VU	$pill\_inf_{ij}$		Probability
Probability of illness		VU	$pill_{ij}$		Probability
Number of people exposed		F	$n_{exposed}$	1559	People
Number of salmonellosis cases		U	$Nill_i$	$\sum_{j=1}^{n_{exposed}} \text{Binomial}(1, p_{ill}ij)$	People

Poisson distribution, it can be concluded that there is a substantial clustering of *Salmonella* contamination.

Individual results of the observed CFU/g or MPN/g of *Salmonella* for each series of burgers are shown in Table 3. Mean of each series for both methods of enumeration were also retrieved from parameters of the fitted negative binomial distribution. The beef burgers of series 2, those that did not undergo thawing out and refreezing, have a significantly higher mean, i.e. confidence intervals of means did not overlap, whatever the enumeration method was used. Even though the impact of thawing out for *Salmonella* in meat seems to be limited (Dykes and Moorhead, 2001; Lianou and Koutsoumanis, 2009), results from burgers I to V were not used for modelling initial contamination in thawed beef burgers.

The mean enumeration levels obtained with plating method on XLD are significantly lower than those obtained with the mini-MSRV MPN method (Table 3) for both series of beef burgers. The mean values for the MPN results are approximately 2 and 4 times higher than the CFU results, for series 1 and 2 respectively.

3.4. Assessment and validation of consumer exposure

Results on the variability in doses and of associated uncertainty are summarised in Fig. 2. Eighty percent of consumers were exposed to doses ranging between 43 MPN (with a credibility interval at 95% [8, 149]), and 1242 MPN (CI 95% [531, 4530]). The median exposure dose was 315 MPN (CI 95% [142, 685]).

In order to confirm the estimated exposure, the predicted number of salmonellosis giving the exposure for the four different dose–response relationships described above was used. Uncertainty distributions of predicted number of salmonellosis for each model are presented in Fig. 3. Using the FAO/WHO dose–response relationship, the observed number of salmonellosis, i.e. 554 ills, is >95th percentile of the uncertainty distribution of the number of salmonellosis cases predicted by models #1 and #1u (Fig. 3a). When using the dose–response relationship published by Teunis et al. (models #2 and #2u), the credibility interval of the number the predicted number of salmonellosis is consistent with the 554 patients identified in the InVS investigation (Fig. 3b). However, for model #2u, which incorporates uncertainty in the parameters of the dose–response law, the credibility interval of the number of patients predicted is large, ranging between 0 and 1534 (almost all of those exposed).

4. Discussion

All the information available in the context of foodborne illness was compiled in order to determine whether a risk assessment approach would be useful and valid as part of a foodborne outbreak investigation. Its purpose was to estimate the variability and uncertainty of doses to which people who consumed the burgers were exposed, by taking into account the burger contamination before cooking and the reduction of *Salmonella* concentration during cooking. Dose estimation was then checked using two dose–response relationships for *Salmonella* (FAO/WHO, 2002; Teunis et al., 2010). The predicted number of people becoming ill was compared with the results of the epidemiological investigations.

The microbiological results confirmed the presence of *Salmonella* at very high levels of contamination in each frozen beef burgers analysed. There is relatively little information on concentration levels of *Salmonella* in contaminated raw minced meat (Rhoades et al., 2009). The few studies which have investigated contamination levels showed contamination levels mostly < 1 MPN or CFU/g (Anonymous, 1996; Bosilevac et al., 2009; Brichta-Harhay et al., 2007; Wong et al., 2007) and the maximum observed concentration was 40 CFU/g (Bosilevac et al., 2009). In our study, *Salmonella* concentrations were almost as high as *Enterobacteriaceae* concentrations, 3.2 $\log_{10}$ MPN/g and 3.6 $\log_{10}$ CFU/g, respectively. This contamination level is hard to explain as concentrations in the potential contamination vectors, i.e. bovine faeces or rumen (Koohmaraie et al., 2012), are generally lower than 4 $\log_{10}$ MPN/g (Fegan et al., 2005; Rhoades et al., 2009). Another explanation could lie in a potential break in the cold chain, but ACC levels, although high, may not support this point.

On the same samples, we observed that MPN results were higher than CFU results. This is consistent with literature showing that stressed *Salmonella* cells are often difficult to recover by direct plating on selective medium (Gurtler, 2009), even for frozen and thawed cells (Janssen and Busta, 1973). As stressed *Salmonella* cells have the same, or potentially an enhanced virulence compared to non-stressed cells (Wesche et al., 2009), MPN results were used to model initial contamination.

Our results also show that contamination is slightly clustered, i.e. negative binomial distribution was preferred over the Poisson distribution. The clustered spatial distribution could also be confirmed with the analysis of the variance and mean of the logarithm of observed numbers of *Salmonella* per portion. Indeed, Jongenburger et al. (2012b) proposed that clustering can be quantified by the ratio variance/mean of the log numbers of observed microorganisms. Contamination is not "homogenous" when this ratio is above 6. Jongenburger et al. (2012b) recommended the use of a member of the family of generalised Poisson distributions (negative binomial or Poisson lognormal) to model microbial distributions in foods, especially to investigate spatial clustering. Negative binomial distribution was chosen to model contamination in beef burgers as this distribution "*may be easier to apply in practice and is comparatively as appropriate as the Poisson-lognormal*" (Jongenburger et al., 2012b).

Although there was substantial clustering, doses of *Salmonella* were not so dispersed, with 80% of them having contamination levels ranging

Table 2
Two dose–response models used to predict the number of salmonellosis and the associated parameters considering or not the uncertainty.

Equation	Used in risk model	α	β	η	ρ	Reference
$p_{ill\,ij} = 1-\left(1+\frac{dose_{ij}}{\beta}\right)^{-\alpha}$	#1	0.1324	51.45			FAO/WHO (2002)
	#1u	Triang (0.0763,0.1324,0.2274)	Triang (38.49,51.45,57.96)			
$p_{inf\,ij} = 1-\frac{\Gamma(\alpha+\beta)\cdot\Gamma(\beta+dose_{ij})}{\Gamma(\beta)\cdot\Gamma(\alpha+\beta+dose_{ij})}$	#2	7.75×10^{-3}	2.72×10^{-3}	1.92×10^{-6}	1.37×10^{3}	Teunis et al. (2010)
$pill_{infij} = 1 - (1 + \eta \cdot \mathrm{dose}_{ij})^{-\rho}$	#2u	5000 sets of $\{\alpha, \beta, \rho, \eta\}$ parameter				
$pill_{ij} = pinf_{ij} \cdot pill_{infij}$		values furnished by Teunis et al. (2010)				

from 1.6 to 3.1 $\log_{10}$ (MPN) as shown on Fig. 2. This point is important because in any given foodborne outbreak, not all people exposed present with symptoms. This can be explained by variation in the susceptibility of individuals. Another explanation lies in the variability of the dose consumed, either by the amount of food consumed varying between individuals or through a heterogeneous distribution of the contaminant in the food matrix (Jongenburger et al., 2012a). Significant variability of exposure could also be explained by variations in the cooking process (ILSI Europe, 2010; Jongenburger et al., 2012b). The results of exposure assessment of burgers can exclude the heterogeneity of contamination between portions as a key element within the context of the community outbreak under consideration because each beef burgers consumed contained at least 1 MPN.

Table 3
Results of *Salmonella* counts on raw frozen beef burgers from the batch implicated in the outbreak.

Series	Burgers	Samples[a]	Counts on XLD (CFU/g)	Mini-MSRV (MPN/g)
Series 1: frozen burgers thawed out for analysis by the routine lab and refrozen	I	I.1	1091	710
		I.2	200	>710
		I.3	700	>710
	II	II.1	200	220
		II.2	640	710
		II.3	700	>710
	III	III.1	400	>710
		III.2	<100	710
		III.3	100	>710
	IV	IV.1	300	>710
		IV.2	200	>710
		IV.3	500	710
	V	V.1	200	380
		V.2	400	400
		V.3	200	>710
		Mean[b]	**393**	**839**
		CI of mean[c]	**[261, 541]**	**[650, 1322]**
Series 2: frozen beef burgers withdrawn and recalled by supplier	1		–	2400
	2		–	18000
	3		–	1600
	4		–	5600
	5		–	1600
	6		–	1000
	7		–	1000
	8		–	1600
	9		491	1600
	10		536	790
	11		655	1000
	12		1345	9400
	13		655	790
	14		591	470
	15		682	1100
	16		673	320
	17		773	2800
	18		573	530
		Mean[b]	**697**	**2867**
		CI of mean[c]	**[581, 826]**	**[1681, 4402]**

– = Not performed.
[a] Test portions were of 25 g for series 1 and of 120 g (whole beef burger) for series 2.
[b] Means were estimated from the parameters of negative binomial distribution fitted to data.
[c] Credibility intervals of means were estimated with bootstrap uncertainty bounds of parameters.

For *Salmonella*, several dose–response relationships were established to link the level of exposure and the probability that an effect will occur. The adjustment of dose–response models to data obtained from human volunteers in the 1950s gave ID_{50} values (the dose causing the considered effect in 50% of individuals in the exposed population) greater than 10^6 CFU (Oscar, 2004; Teunis et al., 2010). These relationships are now being questioned because the strains used do not correspond to the primary serotypes encountered for actual outbreaks. It is also legitimate to question the representativeness of volunteers and the impact of storage at the laboratory on the strains (Teunis et al., 2010).

Three dose–response relationships were established for *Salmonella* from data from community outbreaks. The first relationship proposed by FAO/WHO (2002) is based on twenty community outbreaks. In this study, the same weight was given to each of the outbreaks and a Beta-Poisson distribution was used. The model focuses on the probability of illness but does not take into account the probability of infection. The ingested dose causing illness in 50% of cases was estimated at 9610 CFU. Based on the same data used by FAO/WHO (2002), Bollaerts et al. (2008) proposed another modelling approach. They used fractional polynomials to model the probability of illness by considering the variation between outbreaks. In the most recent publication by Teunis et al. (2010), the parameters were estimated based on data from 38 community outbreaks. According to recent risk assessments (Pouillot et al., 2012; VLA/DTU/RIVM, 2011) both FAO/WHO and Teunis et al.'s models seemed to be the most appropriate and were thus used in the current study.

The systematic data collection of *Salmonella* concentrations in foods involved in salmonellosis outbreaks, could enable the extension of the database of outbreaks which could be incorporated into dose–response assessments (Teunis et al., 2010). Although the real exposure is not accurately known due to uncertainty of cooking effect, the community outbreak that is reported here, could help in the establishment of an updated dose–response relation. The data collected here may be of particular interest because they concern the Typhimurium variant 4,5,12:i:−, which is emerging in Europe (Hauser et al., 2010; Hopkins et al., 2010; Mossong et al., 2007).

By the use of dose–response, the exposure of the 1559 persons attending the schools to *Salmonella* was validated as the predicted number of salmonellosis was in accordance with the overall illness rate of 36% with 554 cases identified by the epidemiological investigation. It is worth mentioning that the same model was used for assessing *Salmonella* inactivation during cooking for the four schools, as investigations revealed no significant differences of preparation between them. Yet, the illness rate of one school was significantly lower than the rates of the three other schools (Raguenaud et al., 2012).

In France, there is no defined performance criterion for cooking of beef burgers, e.g. a logarithmic reduction, but two control measures have been proposed for *E. coli* risk management. These control measures are visual inspection of colour and temperature monitoring. According to the first, i.e. inspection of visual colour, and data from Bergis et al. (2009), a mean of 2.85 $\log_{10}$ reduction was expected for *Salmonella*. Yet, according to the second control measure, i.e. internal temperature of beef burgers that should be above 65 °C, one could expect that the reduction would be higher than 2.85 $\log_{10}$. At 65 °C, a 7 $\log_{10}$ reduction is achieved in 5 min according to classical *D* values for *Salmonella*. The

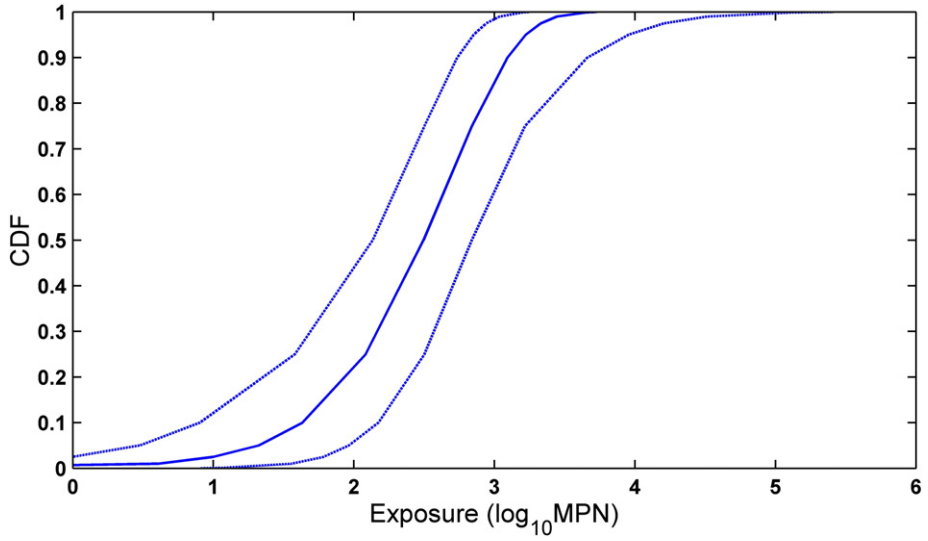

Fig. 2. Cumulative variability distribution of the *Salmonella* dose at which consumers of beef burgers were exposed (—) at 95% credibility interval (between the dotted lines).

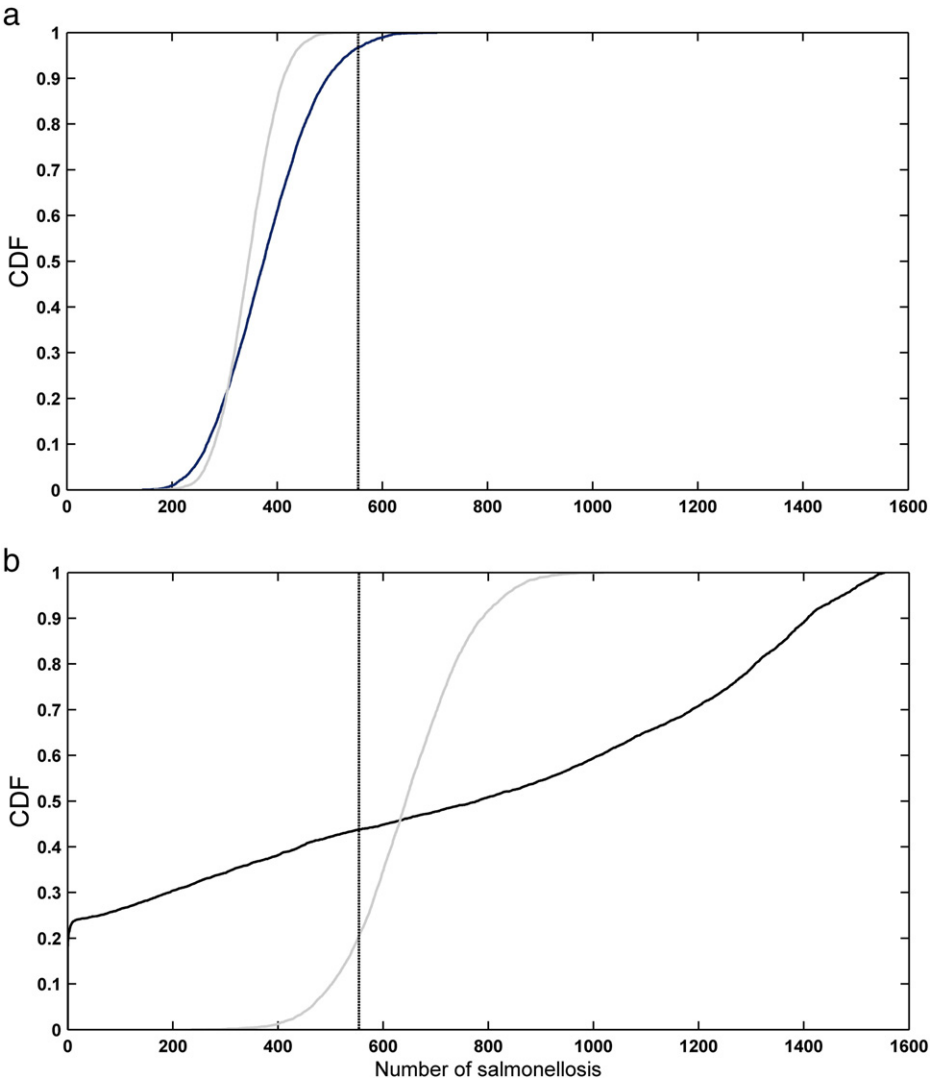

Fig. 3. Cumulative uncertainty distributions of the predicted number of patients, taking into account the uncertainty of the parameters (dark line) or not (light line) of the dose–response relationship for *Salmonella*. (a) FAO/WHO dose–response (b) Teunis et al., dose–response. (———) Observed number of salmonellosis cases, 554 cases, listed by the epidemiological investigation.

discrepancy between these two $\log_{10}$ reductions may be explained by the fact that temperatures measured in beef burgers are not representative of the actual temperatures. Indeed, temperature gradients occur during cooking of beef burgers (Huang, 2012; Ou and Mittal, 2007). Temperatures at the geometric centre of beef burgers significantly differ from temperatures at 0.5 cm of the centre (Huang, 2012). And the temperature monitored by food operators was probably not the temperature at the geometric centre.

Despite compliance with good practices currently proposed for cooking beef burgers, a major community outbreak occurred. It must be underlined that when a lower contamination, and probably more usual contamination level, is introduced in the model, e.g. with a mean concentration of 1 MPN/g, the number of predicted cases of salmonellosis is zero. The number of predicted cases of salmonellosis is also zero for a high contamination, as described in the present study, and a cooking step for which a 6.5 $\log_{10}$ reduction would be achieved. These results indicated that current control measures are efficient only for a low contamination level. Current official recommendations in terms of cooking can reduce the risk of exposure, but they are complementary to other measures in place on the food chain. Cooking recommendations as currently proposed, alone, cannot be considered an adequate response to the exceptional events of extreme foodborne bacterial pathogen contamination. This outbreak appears to show failure control measures in the food chain. Current cooking recommendations could be changed in order to achieve a higher reduction of pathogens and thus ensure safety even when high levels of contamination are present. These modified cooking recommendations, e.g. new temperature to reach, need to be defined.

5. Conclusion

It is now well known that information gathered in epidemiological studies can provide useful data to microbial risk assessment (Delignette-Muller and Cornu, 2008; Miliotis et al., 2008). Through this example, it is illustrated that quantitative risk assessment can also be a complementary approach to epidemiological investigations especially for the assessment of missing information or the identification of the primary causes of outbreaks.

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