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Quantitative risk assessment of human salmonellosis linked to the consumption of ground beef

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Abstract

This work reports a quantitative risk assessment of human salmonellosis linked to the consumption of ground beef patties in France. The risk assessment was based on data on the frequency, concentration and inactivation of *Salmonella* in ground beef. Different distributions were assumed for parameters of the model and Monte Carlo simulations were used to model the process and to quantify the resulting risk for public health. The probability of ground beef batches contamination was estimated to be 100% after 2000 iterations with an expected percentage of ground beef batch with patties contamination less than 1, 6, 12, and 18 percent were 22.5%, 52%, 69% and 95%, respectively. About 93% of ground beef patties (55.7 million out of 60 million patties) were expected not to be contaminated. The simulated concentration of *Salmonella* in a typical ground beef patty serving of 100 g before cooking ranged from 0 to 1.4 x 10^6 *Salmonella* cells with a median of 0 cells. The expected percentage of ground beef patties with contamination greater than 5, 10 and 100 *Salmonella* cells were 29%, 17% and 0.02%, respectively. For 10 million servings of 100 g, the expected number of cases of salmonellosis predicted by the model is in average 7 and 8 for fat content 7% and 24% respectively. The risk of salmonellosis per 100 g serving ranged from 0 to 2.33E-06 dependent on the type of cooking and the fat content. The risk of salmonellosis was closed to zero when the 100 g serving ground beef patties were consumed well done. The relative risk of getting salmonellosis from the consumption of rare ground beef patties is 312, 61 times higher for fat content 7% and 24% respectively comparing to the consumption of well done patties. There are 35 batches with at least one case out of 2000 batches (1.8%). 15 of them have 2 cases or more (0.75%).

Keywords: Risk assessment; *Salmonella*; Ground beef; Monte Carlo simulation.
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تقدير الخطر الكمي لمرض السالمونيلا في الانسان المرتبط باستهلاك اللحم البقري

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الخلاصة

يقرر هذا العمل تقدير الخطر الكمي لمرض السالمونيلا في الانسان المرتبط باستهلاك شطائر اللحم البقري في فرنسا. تقدير الخطر كَانَ مستند على البياناتِ كتكرار وتركيز ومدى اعاقة نمو السالمونيلا في لحم البقر. وقد افترضت توزيعات مختلفة لقياسات نموذج ومحاكاةِ مونتي كارلو كَانتْ تُستعملُ لتشكيل العمليةِ ولقياس كمية الخطر الناتج للصحة العامةِ. قدرت احتمالية تلوث وجبات اللحم البقري بانها تصل الى 100% بعد 2000 مكرر بنسبة مؤية لوجبة اللحم البقري مع تلويث توقع للشطائر وصل الى اقل من 1 ،6 ، 12 و 18 % لتكون 22.5 % و 52 % 69 % و95 % على التوالي. ويتوقع حوالي 93 % من شطائر اللحم البقري (55.7 مليون من 60 مليون شطيرة) كان متوقعا بانها غير ملوثة. التراكيز الظاهرية للسالمونيلا في شطائر اللحم بوزن 100 غم قبل الطهي تراوحت بين صفر – 1.4 × 10 من خلايا السالمونيلا وبمتوسط صفر خلية. النسبة المتوقعة في شطائر اللحم الملوثة التي تزيد عن

5 ، 10 ، 100 خلية لجرثومة السالمونيلا كانت 29 % ، 17 % و 0.02 % على التوالي ، عدد الحالات المتوقعة للتنبؤ بوجود السالمونيلا لعشرة ملايين شطيرة بوزن 100 غم بواسطة النموذج هو بمعدل 7 و 8 لمحتوى دهن 7 % و 24 % على التوالي. ان خطر مرض السالمونيلا لكل 100 غم تراوح بين صفر – 2.33 حسب نوع الطهي والمحتوى الدهني. وان خطر الاصابة بالسالمونيلا انحدر الى الصفر عندما كانت شطيرة اللحم البقري مطهية بشكل جيد. الخطر النسبي للاصابة بمرض السالمونيلا من استهلاك شطائر اللحم البقري غير المطهية بشكل جيد هو 312، أي بـــ 61 مرة اعلى في القطع ذات المحتوى الدهني 7% و 24 % على التوالي مقارنة مع استهلاك الشطائر المطهية جيدا. هناك 35 وجبة فيها على الاقل حالة واحدة من مجموع 2000 وجبة (1.8 %) و 15 منها لها حالتان أو أكثر (0.75 %).

Introduction

Foodborne illness due to *Salmonella* is a major public health problem. Bacterial pathogens contribute to ~60% of foodborne illnesses that lead to hospitalization and account for nearly two-thirds of the estimated number of foodborne pathogen-related deaths. (1) estimated that *Salmonella* spp. caused ~26% and >30% of foodborne illness-related hospitalizations and foodborne deaths respectively. Several food items, including ground beef, have been implicated in a great number of *Salmonella* disease outbreaks in the U.S., Canada, and Europe. The presence of *Salmonella* in ground beef is a known health hazard and outbreaks of salmonellosis linked to the consumption of ground beef have been reported. In the USA, in 1994, an outbreak of *Salmonella* serotype Typhimurium gastrointestinal illness in Wisconsin associated with eating contaminated raw ground beef during winter holiday season (2). In this outbreak, 107 confirmed cases. Between January and April 2002, multidrug-resistant *Salmonella* Newport emerged as a cause of salmonellosis in five states due to exposure to raw or undercooked ground beef (3*). Salmonella* Newport was isolated from 47 persons in five states: New York (34 cases), Michigan (five), Pennsylvania (four), Ohio (two), and Connecticut (two). In 2003-2004, the first multistate outbreak of multidrug resistant *Salmonella* Typhimurium DT104 associated with consumption of ground beef occurred in the northeastern United States (4). In the same year (August 11-October 2, 2004), multistate outbreak *Salmonella* Typhimurium infections associated with eating ground beef occurred In Canada, an outbreak of multidrug resistant *Salmonella* Typhimurium occurred in 2003 among at least 47 persons attending a school potluck (5). In Norway, October-November 2005, an outbreak of *Salmonella* Typhimurium DT 104 occurred that was linked to imported minced beef (6). In France, from 1990 to 2000, four outbreaks of salmonellosis occurred (7) that had been detected by CNR surveillance system and investigated. The results of the epidemiological, veterinary and laboratory investigations indicated that the *Salmonella* serotypes responsible for these four episodes were different (Meleagridis, Paratyphi B, Typhimurium, and Coeln respectively). The number of the cases is relatively low (8-58 with medium 32). In 1996, a study done in France to evaluate the risk factors for the occurrence of sporadic *Salmonella enterica* serotype Typhimurium infections in children less than 15 years old (8) gave the evidence that the consumption of raw or uncooked ground beef was the main risk factor of salmonellosis (OR= 5, IC 95= 1.7-8.4) and the population attributed risk for the children less than 15 years old was 35% (IC 95= 12-58). This study considered only *Salmonella enterica* serotype Typhimurium. Therefore, risk assessment for public health linked to the consumption of ground beef contaminated by *Salmonella* provides useful information for the management of the risk.

In general, relatively few papers dealing with quantitative risk assessment models for salmonellosis of food origin have been published in the scientific literature. Risk assessment models of the consumption of ground beef patties products have previously been developed for *E. coli* O157:H7 (9). To our knowledge, a quantitative risk assessment of salmonellosis linked to the consumption of ground beef has not been carried out. The present work therefore reports a first risk assessment model of salmonellosis linked to the consumption of ground beef using, a Monte Carlo simulation by SAS software.

Materials and methods

Risk assessment is a science-based process in which questions that have been formulated during the risk evaluation step of the risk management process are addressed to develop an understanding of the problem and to come up with risk estimates and risk mitigation options. In our study, the hazard is *Salmonella* and the risk qualifies the probability of human salmonellosis associated with the consumption of 100 g serving of ground beef patty.

Hazard identification

Salmonella is the most frequently reported cause of foodborne illness in the world. It is the major cause of childhood mortality in developing countries and constitutes a permanent threat in industrialized countries. However, the

disease can spread systemically and degenerate into a chronic condition such as reactive arthritis, osteomyelitis, cardiac inflammation or neural disorders. Groups at higher risk of severe illness and death from *Salmonella* infection are infants, elderly persons, and persons with an impaired immune system.

Previously reported surveys on *Salmonella* in Europe, Australia, United States and Canada have shown large variations in the prevalence of *Salmonella* in faecal, hide, and carcasses ranging from 2% to 50% These reported findings clearly suggest that *Salmonella* can be carried by healthy cattle at slaughter and can therefore serve as a reservoir and source of contamination of carcases during processing and may pose a health hazard (10).

Exposure assessment

In order to develop a risk assessment model of human salmonellosis associated with the consumption of ground beef, we attempted to estimate the potential exposure to *Salmonella* in a single serving. The exposure was characterized by the probability distribution of *Salmonella* colony-forming units (CFUs) in 100-g servings of meat at the time of consumption. A list of variables was identified and distribution was assumed for each variable. An accurate exposure assessment needs information such as the frequency and level of contamination of the selected foods and the growth and inactivation of the pathogen during the preparation steps. This information will be discussed below. Monte Carlo simulations were done using SAS software v.9.2 (SAS Institute, Inc.).

The microbial risk assessment (MRA) will be developed using a slaughter-to consumption framework. The use of this framework's exposure pathway is split into 4 modules, these are animals at slaughter house, slaughter and processing, distribution & storage and finally preparation and consumption. Each module of the pathway includes appropriate mathematical descriptions of the changes in the prevalence and levels of organism.

Collection of data in bovine faeces contaminated by *Salmonella* at slaughterhouse (Module 1)

Contamination of bovine faeces by *Salmonella*

To estimate the prevalence and concentration of *Salmonella* in bovine faeces, we used our data from a study conducted in 2006 at slaughterhouse located in Meaux, France. A total of 296 fecal samples were aseptically collected weekly in February and March 2006 (an average of 40 samples per visit) and the presence of *Salmonella* in bovine faeces was investigated using the real-time PCR. The results of this study indicated that 9.12% (27/296) of fecal samples were *Salmonella* positive (11).

Level of *Salmonella* contamination in bovine faeces

In general, literature data on the contamination of bovine faeces by *Salmonella* are qualitative and presented as presence or absence of *Salmonella* in samples analyzed. The absence of quantitative data could be due to the difficulty of applying enumeration methods to quantify low levels of contamination. In a previous work, we developed a method for the quantification of *Salmonella* in artificially contaminated faeces based on the real-time PCR assay combined with MPN (11). This developed MPN-real-time PCR assay was used to provide quantitative data by estimating the level of contamination of positive-*Salmonella* fecal samples. The MPN real-time PCR assay enabled the enumeration of *Salmonella* in fecal *Salmonella*-positive samples that ranged from <1.8 to 1609 CFU/g. Using tobit model (12), the estimates of mean and standard deviation of the log10 concentration of *Salmonella* in positive bovine fecal samples were respectively 0.6189 MPN/g and 2.7112.

Slaughter and Processing (Module 2)

Slaughter plant process

To identify the most important steps in the slaughter process, from a risk prospective, a flow diagram was constructed for live cattle entering the slaughter plant and going through typical France commercial butchering procedures.

Modelling the food pathway

In consecutive steps the transmission of *Salmonella* spp. is described by modelling the change in number of micro-organisms per unit N. A run of the Monte Carlo model simulates the production of ground beef batch and consecutive production and consumption of the ground beef from that ground beef batch. At each step, the number of micro-organisms at the end of the process (N') is given as a function of the number in the previous step (N).

Step 1: Contamination of carcasses

In order to assess carcasses contamination by *Salmonella*, we used a parameter a (gram of faeces/carcass) indicating the amount of faeces contaminating a single carcass. By multiplying this parameter by the concentration of faeces by *Salmonella* we are able to estimate the total amount of *Salmonella* contaminating each carcass. The total quantity of faeces on carcass i (in g) $a_i \sim a_{max}$ x Beta(α, β) with a_{max}, α and β model parameters expressing the level of faeces contamination and its variability per carcass. (13) estimated the parameters a_{max} = 10.1 g, α = 0.395, and β = 2.473 by using the result of the expert panel to assess carcass contamination.

We assume that trimming from n carcasses are contributing to ground beef batch of W_{gb} kg. Next, we suppose that faeces from m_i animals ($i = 1 \ldots n$) contribute to the contamination of carcass i. Parameters n and m_i will be

variable per ground beef batch produced. This variability is implemented as:

n ~ 1+Poisson(n_{mean} - 1)
m_i ~ 1+Poisson(m_{mean} - 1)

Both parameters n and m_i have a discrete value with a minimum of 1 and a probability density function characterised by one parameter only. The mean number of carcasses used for a ground beef batch (n_{mean}), and the mean number of animals from which the faeces contaminate a single carcass (m_{mean}) were assessed by the expert panel for the slaughter house (m_{mean} = 2.98 and n_{mean} =50.33) (13)

The prevalence of contaminated animals entering the slaughter house is P_f. P_f is assumed to be constant throughout this model (9.1%). The concentration of *Salmonella* spp. in faeces in animal j contaminating carcass i (c_{ij}) is assumed to be distributed as lognormal with parameter estimates.

The total number of cfu on a carcass is a function of:

The fraction of the faeces of animal *j* that contributes to the contamination of carcass *i*. The relative contribution of each animals to the total amount of contaminating faeces is described by a Beta distribution.

f_{ij} ~ Beta(b_1, b_1 (m_i – 1)), with 'beta factor' b_1 (13).

The concentration of *Salmonella* spp. in the faeces of animal *j*, contaminating carcass *i*: C_{ij}(cfu/g).

The expected number of cfu on carcass *i* is derived using the formula:

$$n_i = Poisson\left(a_i \sum_{j=1}^{m_i} f_{ij} c_{ij} \right).$$

Step 2: Partitioning to half carcasses

The carcass is split into two. The weight of the clean carcass is W_{carc} and the weight of the half carcass is 0.5 W_{carc} and $N = N$i, so Ni' ~ Binomial (Ni, Beta (b_2, b_2)), with b_2 a parameter describing the clustering at carcass halving. The mean estimated value of b_2 is 2.7 with 67% of cells on the half carcass with most cells (13). The mean carcass weight at slaughter in our study was 400 kg. This is thus incorporated in the model as a fixed number: W_{carc} = 400.

Step 3: Partitioning to trimmings

Trimmings are cut from carcasses. To assess the number of *Salmonella* spp. on the trimmings from one half carcass *i* used in the ground beef batch, we used the Beta-binomial distribution function:

n_i' ~ Binomial (n_i, Beta(b_4, 0.5 W_{carc} b_4/ W_{tri} - 1)),

with b_4 a parameter describing the clustering effect of cells on the carcass when trimmings are cut off. We therefore assume that the probability of finding *Salmonella* on meat destined for ground beef is equal to the probability of finding it at a random place on the carcass. As clustering is incorporated in the model, the cells are not assumed to be spread equally over the carcass. The mean estimated value of b_4 is 0.73 assessed by the expert panel (13), whereas the weight of these trimmings from this half carcass (W_{tri}) is determined in the mixing process in step 4 below (note that W_{tri} is not the weight of one trimming, but the total weight of all trimmings from carcass *i*).

Step 4: Mixing: the ground beef batch

When the ground beef batch is formed, it contains meat of n animals. The total weight of all the trimmings from one (half) carcass used for the ground beef batch depends on the number of carcasses used for the batch (n), and the weight of the batch (W_{gb}). The carcasses need not contribute equally to ground beef batch. Then the weight of trimmings of a random half carcass *i* that contribute to ground beef batch is:

W_{tri} ~ Beta (b_5, b_5 (n-1)) W_{gb}, with 'beta factor' b_5

The b_5 as a measure of the relative contribution of (trimming of the) carcasses to the ground beef batch, is set as b_5=1.

Implementing the distribution of W_{tri} in equation step 3, we calculate the total number of *Salmonella* per batch.

$N' = \sum_n n'_i$

Step 5: Partitioning to 100 g ground beef patties

Ground beef patties are produced from the ground beef batch. This is a typical partitioning process. Therefore the number of *Salmonella* on 100 g ground beef *j* is:

N_j' ~ Poisson (N, Beta (b_6, W_{gb} b_6/ $W_{gbp,j}$-1))

The beta factor, b_6, is a parameter describing the clustering effect of cells in the ground beef batch for 100 g ground beef patty formation. As the clustering effect may be rather large due to the fact that large batches are not easily well mixed, as default it is assumed that b_6 = 0.15.

Distribution & Storage (Module 3)
Module pathway

For each contaminated product, the distribution & storage module describes how the level of contamination for that product is affected by the following stages.
Transport (1): Processing plant to retail outlet.
Storage (1): Storage at retail outlet.
Transport (2): Retail outlet to storage facility at home/other.
Storage (2): Storage facility.

Even though, the level of contamination at each stage may increase due to temperature abuse; however, it is assumed that the number of *Salmonella* will stay the same, without any growth, within the Distribution & Storage module due to the lack of information on temperature and

time associated with transport (to and from retail) and storage of chilled and frozen ground beef patties in France. Therefore, the effect of the possible growth on the risk model result will be studied in the scenario analysis.

Preparation and Consumption (Module 4)
Module pathway

This module is directly linked to Distribution and Storage module and thus continues to track the progress of a random chilled or frozen product. For each contaminated product, the Preparation & Consumption module describes how the level of contamination on that product and the subsequent dose that an individual is exposed to through eating inadequately cooked meat, is affected by the following stages:

Cooking.

Consumption.

Modelling the Preparation and Consumption pathway
Cooking (inactivation during preparation of patties)

Thermal inactivation of bacteria is commonly modelled with the Bigelow model, using D- and z-values of Salmonella spp. in ground beef (13). According to (14) inactivation depends on fat content (higher fat content gives higher D-value, i.e. less inactivation). The Food and Drug Administration (FDA) and US Department of Agriculture (USDA) stated that 54.4°C endpoint temperature is typical for rare hamburgers, 62.7°C is typical for medium and 68.3°C is typical for "well done" we consider in this risk assessment that there are three ways for cooking ground beef: rare, medium, and well done.

For the present exposure assessment, traditional log-linear-death kinetic model will be considered. Many investigators do not show the inactivation data for their studies and merely quote D-values (i.e. time for a 90% reduction in the numbers of bacteria at a given temperature). Linear model was used to model the $\log_{10}$ bacteria numbers vs heating time. We fitted 121 curves with different temperature (55, 57.5, 60, 62.5, 65, 70°C) and fat contents (7, 12, 18, 24%) from published papers (15) and all the data related to inactivation of Salmonella in ground beef in ComBase. A set of D- values are calculated for each temperature at various fat contents.

$$\log_{10}\frac{N(t)}{N(0)} = \alpha \times time$$

The equation of the regression line was used to calculate a D-value over 1 log cycle reduction in the numbers of bacteria.

$$T_{temperature}\,(\text{for the first log reduction}) = -\frac{1}{\alpha}$$

When D-values are calculated for a number of different temperatures, a relationship between the D-value and the temperature were calculated. Data expressed as the reciprocal of the log10 D-value vs temperature of the D-value was analysed by linear regression to give a straight-line equation. This equation was used to calculate the z-value, which is the temperature change required to bring about 90% change in D-value.

$$LogD = \alpha + \beta * temperature$$

$$\text{D-value} = 10^{(-\beta T)+\alpha}$$

$$z = -\frac{1}{\beta}$$

The Food and Drug Administration (FDA) and US Department of Agriculture (USDA) have recommended a minimum temperature of 68.3°C at the lowest heating point at 16 s holding time to enhance safety (16). Since inactivation of the bacteria in ground beef patties is a function of temperature and time, and also the temperature varies during cooking time, it is important to use a dynamic model to predict the change of the temperature in the centre of the ground beef patties with time to adjust the D-value for each time interval at given temperature. The thermodynamics during cooking was assessed by using mathematical heat and mass (moisture and fat) transfer models described by Ou and Mittal (20). The temperature at the lowest microbial inactivation point (in the centre of ground beef patties) was predicted using this model. Using this result, we could estimate the efficiency (E), that is the number of 10-fold divisions or decimal reductions of bacteria caused by the operations (E_c: efficiency of cooking), by dividing the cooking (t_c) by the corresponding decimal reduction time D calculated above (D_c: cooking). The following integral was used, incremented by blocks of time of 15 seconds:

$$E_c = \int_0^{t_c} \frac{1}{D_c(T(t))}\,d(t)$$

If the heat treatment caused E decimal reductions, and the initial Salmonella number in one serving was C, the consumer would ingest a dose of Salmonella equal to: $d = C/10^{Ec}$ per serving.

Dose-response model

A dose-response model gives the probability of the studied effect according to the amount of ingested pathogenic microorganisms. Among *d*-ingested microorganisms, some might survive human barriers and later initiate infection and cause illness. Probability of the effect was defined as the probability of achieving this sequence of events.

Several dose-response models have been published and used for *Salmonella*, based on different types of data (feeding trials, outbreaks), outcomes (infection or illness) and assumptions on the dose-response relationship:

exponential (17), Beta-Poisson and Gompertz Actually, the exponential and the Beta-Poisson models are the most commonly used.

The probability of infection was described by the following equation:

$$PI = 1-(1-r)^{n}$$

The parameter r is the probability of one *Salmonella* cell to survive to the different host immune barriers and induce infection. Where n is the number of consumed microorganisms and r a parameter with a value r~Beta(α, β), α and β were equal to 0.3126 and 1.9^5, respectively (18). We consider here the principle of single hit model.

For each consumed 100 g serving we simulated the number of bacteria per serving (n), sampled from the beta distribution the parameter r (which represents the consumer susceptibility) and applied the formula $1-(1-r)^n$ to assess the probability of infection per serving of (rare, medium, or well done) frozen or chilled ground beef patties.

Illness was defined as the occurrence of gastroenteritis (abdominal cramps, diarrhoea, nausea, vomiting). According to feeding studies on human volunteers, the average probability of gastroenteritis among infected naïve subjects was varying between 0 and 75% with a mean of 16%. To build a more realistic model, we decided to reduce the reported probability of illness to 10% (19).

The risk of salmonellosis from the consumption of a single ground beef patty was estimated using the result of the previous steps, for each type of cooking (rare, medium and well done).

Risk characterization

Risk characterization integrates the results of dose-response and exposure assessment into a risk statement that includes one or more quantitative estimates of risk. An essential prerequisite to risk characterization is the clear definition of output. Examples of possible outcomes are expected risk of infection to a typical person, expected number of illnesses or deaths in a community, upper confidence limit to expected number of illnesses, upper confidence limit for illness to a highly exposed person, or maximum number of illnesses in a community at any time. The choice between all possible outcomes has to be decided in relation to the needs of the decision maker.

We first assessed the distribution of the probability of illness per serving. This distribution encapsulates the variability and uncertainty inherent to the different model input parameters. Second, we calculated the arithmetic mean of the probability of illness per serving. This constitutes the "marginal risk" (MR) which is one of the central possible outcomes. It can be defined as the "expected risk of illness for one random individual after one intake of the considered food product". To predict the expected number of salmonellosis cases one could multiply the MR by the number of consumed servings for the considered period of time in the susceptible population.

A food outbreak is defined as an incident in which at least two grouped cases became ill, with similar symptoms, after the consumption of the same food. This risk of outbreak and the number of outbreaks were estimated.

Scenario analysis

In a scenario analysis the baseline risk model with default value of parameters can be compared with alternatives. In our sensitivity analysis, we looked at the effect of varying the inputs parameters on the output of the model (number of salmonellosis cases) to find out for which parameters precise estimation is (not) important, or at which parameters intervention should be aimed. For different parameters at different steps along the food pathway, the default value is adapted and then a new risk model is run (2000 iterations). This alternative scenario gives an alternative prediction of the number of salmonellosis cases per 10^7 ground beef patties servings. The choice for the alternative scenario per parameter depended on the parameter characteristics and the available information.

Results

Ground beef batch contamination

The probability of ground beef batch being contaminated was estimated to be 100% after 2000 iteration. The distribution of the level of contamination ground beef patties with *Salmonella* within the ground beef batch was obtained. The expected percentage of ground beef batches with percentage of contaminated patties less than 1, 6, 12, and 18 were 22.5%, 52.1%, 69.07% and 95.07%, respectively with maximum of 18% as shown in Fig 1.

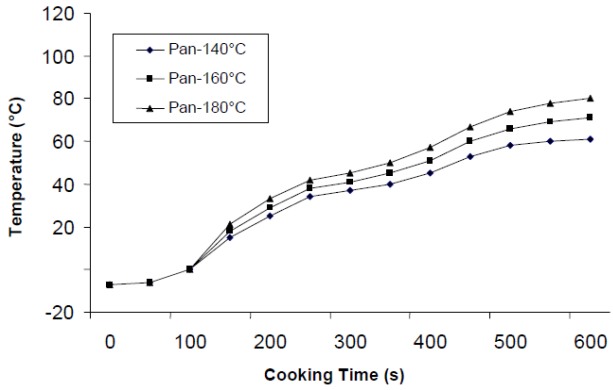

Figure 1. Comparison of simulated ground beef patty center temperatures at 140, 160, 180°C pan temperatures during

single-sided pan-frying of frozen patties with three flippings.

Ground beef patties contamination

The simulated concentration of *Salmonella* in a typical ground beef patty serving before cooking of 100 g ranged from 0 to 1.4 x 10^6 *Salmonella* cells with a median of 0 cells. About 92.9% of ground beef patties (55.74 million out of 60 million patties) were expected not to be contaminated. The expected percentage of ground beef patties with contamination greater than 5, 10 and 100 *Salmonella* cells were 29%, 17.1% and 0.02%, respectively.

Percentiles of the distribution of *Salmonella* in 100g serving of ground beef patty before cooking. The 99th percentile of *Salmonella* cell numbers in servings of 100 g of ground beef patty was 167 cells before cooking. The intra-class correlation (variance between batches/total variance) was equal to 87%. It means that 87% of *Salmonella* concentration per g of patty could be explained by batch-level factors.

Preparation and cooking practices

Cooking temperature

In our model ground beef patties could be consumed rare, medium, or well done. Dependent of the duration of cooking, the types of cooked ground beef patties are presented in Fig. 1 shows a comparison of the ground beef patty center temperatures at 140, 160, 180°C pan temperatures with three flippings. The cooking time was set up to 10 min. During the frozen period, the center temperature rises slowly and the increase rates are not significantly different for various pan temperatures. A higher pan temperature results in a shorter heating time to overcome the latent heat of fusion. The cooking temperatures for the patty geometric center at 140, 160 and 180°C pan temperatures were 61, 71.5, 80.04°C at 600s, respectively. We excluded pan 140°C from further analysis due to low temperature in the center of ground beef patties.

***Salmonella* reduction during cooking**

121 survival curves were constructed for analyzing inactivation of *Salmonella* spp. in ground beef for different fat levels (7, 12, 18, and 24%). The survival curves were linear on a semi-logarithmic plot. A linear model provided a fair-to-good fit at all temperatures, with R-square values of 0.98 to 0.99. It was assumed that 20% of *Salmonella* cells were submitted to the heating treatment pertaining to the coldest spot in the ground beef patty (in center) (20) where temperature measurements were estimated above.

The regression analysis was run for each fat content (7, 12, 18, 24%) with temperature as the only variable of the model to determine the equation of the regression line and to estimate the slope and its standard error: (i.e. for fat content 7%, the other fat content shown in Table 1).

$\text{Log } D_7 = 9.929 - 0.155T$

The Z-value is the reciprocal of the slope and was equal to:

$$Z_7 = \frac{1}{0.155} = 6.448$$

We attributed to the slope a normal distribution (fat content 7% as example) with a mean of -0.1555 and a standard deviation of 0.0084, resulting from the regression analysis. So D(T(t) becomes:

$D_7(T(t)) = 10^{[9.929 + \text{Normal}(-0.155,\ 0.0084)T]}$

Table 1: D- and Z- values and regression parameters (α, β) obtained for four fat levels (7, 12, 18, 24%) at different temperatures for Salmonella spp. in ground beef.

Fat level (%)	Temperature (°C)	α(se)	β(se)	D-value	R-square	Z-value
7	58	9,929	-0,155	8,581	0,983	6,448
	60	(0.517)	0.008)	4,201		
	62			2,057		
	65			0,705		
	67			0,345		
	70			0,118		
	72			0,058		
12	58	10,022	-0,156	9,753	0,988	6,421
	60	(0.435)	(0.008)	4,761		
	62			2,324		
	65			0,793		
	67			0,387		
	70			0,132		
	72			0,064		
18	58	10,018	-0,155	11,059	0,990	6,463
	60	(0.382)	(0.006)	5,423		
	62			2,659		
	65			0,913		
	67			0,448		
	70			0,154		
	72			0,075		
24	58	10,014	-0,154	12,464	0,981	6,503
	60	(0.538)	(0.009)	6,139		
	62			3,024		
	65			1,045		
	67			0,515		
	70			0,178		
	72			0,088		

This equation was applied for each cooking operation and for each type of meat preparation. The time/temperature profiles estimated by using mathematical heat and mass (moisture and fat) transfer models (20) were randomly generated, incremented by 15 sec.

The number of decimal reductions (E_c) was dependent on the level of fat, type of meat (frozen or chilled), pan temperature and finally the time of cooking. As shown in Fig. 2, Fig. 3, the heat treatment submitted by the pan 180°C was more efficient than the pan 160°C since the cooking time was shorter with a higher log destruction of Salmonella in the center of ground beef patties. For example, after 6.5 min cooking the number of log reductions in the center of patties is ranged from 10.34 to 15.69, 0.59 to 0.88 for pan 180°C and 160°C, respectively, dependent on fat content.

Comparing the temperature in the center of ground beef patties with the given time using the temperature-time profile (fig. 1), we could estimate the ranged and more likely temperature in the center of the patty corresponding to the type of cooking (rare ranged from 53.5 to 55.5°C, medium ranged from 61.7 to 63.7°C and well done ranged from 67.3 to 69.3°C. In a recent work on *E. coli* O157:H7 (21), it was estimated that 16%, 52% and 32% of the ground beef patties are consumed rare, medium and well done, respectively.

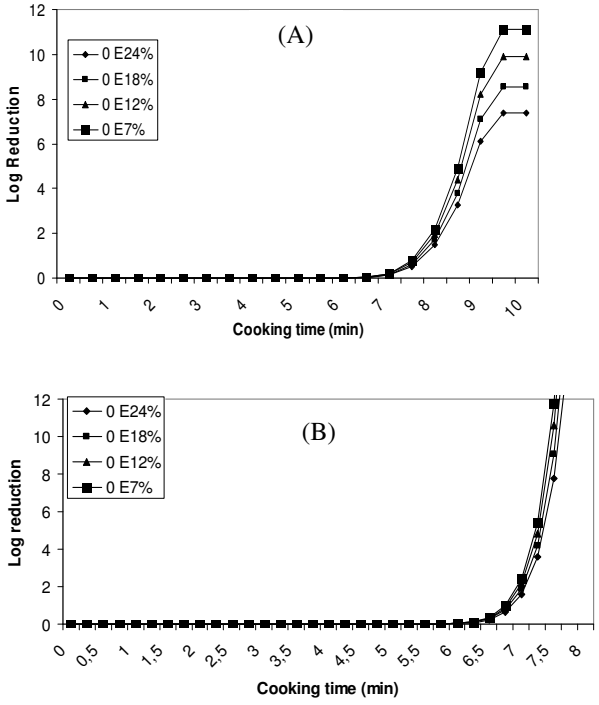

Figure 2: log reductions of Salmonella during time of cooking at various fat contents of frozen patties ($T_{initial}$ = -6°C) (A) and chilled patties ($T_{initial}$ = 4°C) (B) at pan temperature of 160°C with three flippings.

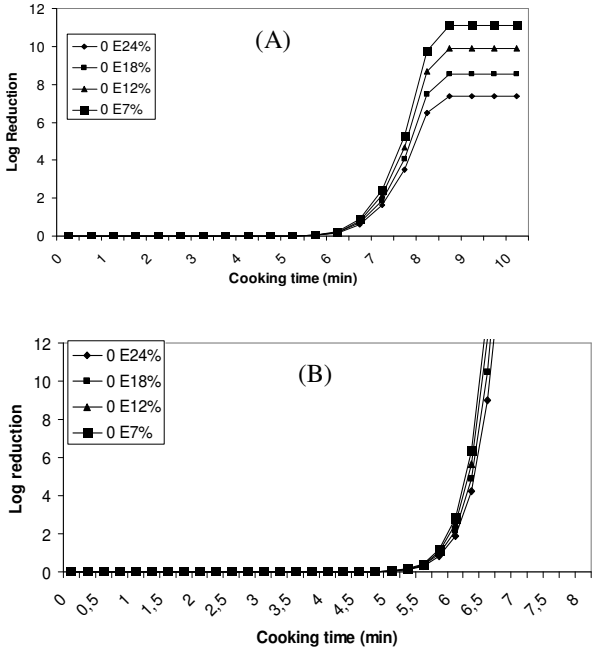

Figure 3: log reductions of Salmonella during time cooking at various fat contents of frozen patties ($T_{initial}$ = -6°C) (A) and chilled patties ($T_{initial}$ = 4°C) (B) at pan temperature of 180°C with three flippings.

The average number of log reductions (E_c) was equal to 0.005, 0.006, 0.006, and 0.007 for ground beef patties consumed rare with fat content 24, 18, 12, and 7% respectively and was 0.14, 0.16, 0.19, and 0.21 for ground beef patties consumed medium with fat content 24, 18, 12, and 7% respectively and was 1.87, 2.14, 2.48, and 2.78 for ground beef patties consumed well done with fat content 24, 18, 12, and 7% respectively as shown in Fig. 4.

Risk characterization

Risk of salmonellosis

The risk of salmonellosis was closed to zero when the 100 g serving ground beef patties were consumed well done, whatever the temperature (min, most likely, or max). The results obtained for the risk according to the different types of cooking and the expected numbers of cases per 10 million servings.

The risk of salmonellosis per 100 g serving ranged from 0 to 2.33E-06 dependent on the type of cooking and the fat content. The risk was equal to zero (no salmonellosis cases occurring) in 99% of the iterations when the meat consumed well done. For 10 million servings of 100 g of ground beef patty, the number of cases predicted by the model is in average 7, and 8 for fat content 7% and 24%

respectively when the data collected by (21) was used (rare 16%, medium 52%, and 32% well done).

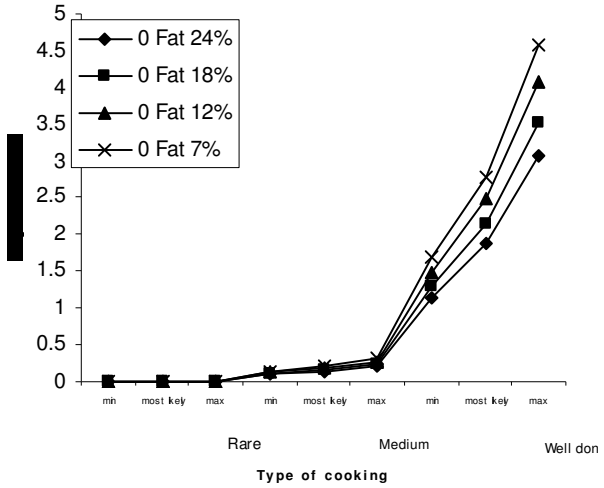

Figure 4: The expected number of log reductions estimated for each level of fat dependent on the type of cooking (rare, medium, and well done) in the center of ground beef patties.

The risk of getting salmonellosis from consumption of rare ground beef patties is more than 312, 60 times higher for fat content 7% and 24%, respectively, comparing to the consumption of well done patties, whereas consumption of medium ground beef patties would increase the risk by more than 188, 42 times comparing to the consumption of well done patties for fat content 7% and 24%, respectively.

Risk of outbreak

The risk of outbreaks was calculated as the probability of having at least 2 cases of salmonellosis from one ground beef batch for 2000 iterations (60 million patties). There are 35 batches with associated with at least one case of salmonellosis out of 2000 simulated batches (1.8%). Only 15 of them have 2 cases or more (0.75%, 15/2000).

Scenario analysis

An overview of the results of this scenario analysis is given in Fig. 5A and 5B. It shows that the largest increase in the number of salmonellosis cases is a twelve-fold increase which results from a tenfold increase of the prevalence of bovine faeces contamination by *Salmonella* at slaughter-house and the largest decrease is a fifteen-fold decrease which results also from a tenfold decrease of the prevalence of bovine faeces contamination by *Salmonella* at slaughter-house, whereas the other scenarios show little or no change in the number of salmonellosis cases.

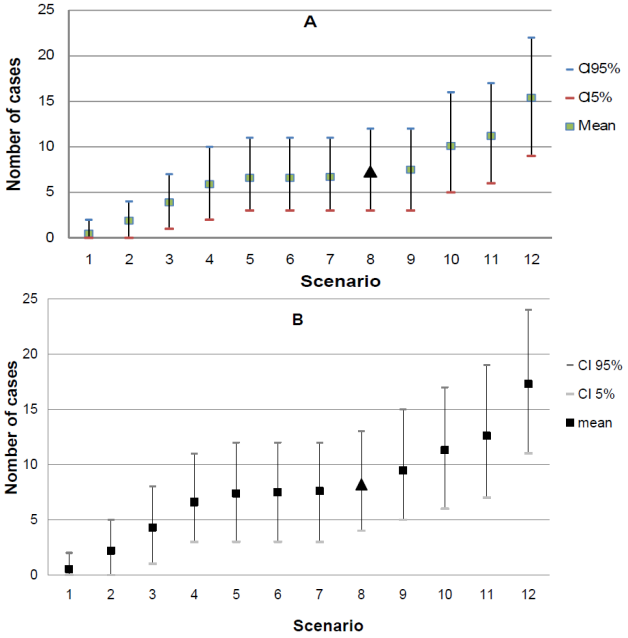

Figure 5: Comparison of the predicted number of salmonellosis cases per 10 million ground beef patties servings for different alternative scenarios with the results of the default model (scenario number 8 ▲) for fat level 7% (A) and 24% (B). Scenario number 13 (Pf =90%) was omitted from these graphs for clarity.

Discussion

Despite the amount of ground beef patties consumed daily in France, outbreaks of infection remain comparatively rare. *Salmonella* outbreaks due to the consumption of ground beef patties in France have been reported (7) Human cases of salmonellosis occur sporadically or as part of outbreaks. The role of ground beef patties in sporadic cases is not well assessed. Under-notification of sporadic salmonellosis cases and non systematic case investigation complicate the demonstration and quantification of ground beef productions role in sporadic cases.

Because of the origin of data and the assumptions made, the results of this risk assessment should be interpreted carefully. The simulations presented herein were based on our own data collected specifically to assess the risk of salmonellosis from consumption of ground beef patties. The model incorporated also data from published literature. This study showed a high frequency of ground beef batches contamination (100%) but mostly a low level of patties contamination. For the frequency of contamination, we observed a same prevalence rate of *Salmonella* in fecal

samples, 9.12%, close to the previously reported prevalence of 9.5% (22) and 8.1% (23). For the enumeration, we used the MPN-real-time PCR assay to enumerate *Salmonella* in fecal samples. The assay enabled the enumeration of *Salmonella* in fecal samples that ranged from <1.8 to 1609 MPN of *Salmonella* per g. We believe that these estimates overestimated the original concentration of the organism in faeces. It has been previously reported (24) that assays based on MPN-PCR tended to give higher estimates than traditional enumeration methods. These results clearly indicated that these higher estimates are due to the detection of DNA from dead and stressed cells, which are not able to form colonies.

The risk assessment model predicts about 8.1% of ground beef patties (4.86 million out of 60 million patties) to be contaminated. A large fraction of patties has only one CFU per 100g of *Salmonella* (70%), where the detection limit in the microbiological analysis is probably much higher than 1 cfu (no less than 1 CFU /25 gr). This implies that one would expect that the prevalence predicted by the model would be higher than the prevalence found in a surveillance study. We did not find any published data related to the prevalence of *Salmonella* in ground beef in France. The prevalence of *Salmonella* in ground beef meat in the EU is ranged from 0 to 2.1 and 0 to 1.7 in year 2005 and 2006 respectively dependent on the number of samples and the size of sample (25).

The minimum growth temperature associated with *Salmonella* on ground beef ranged from 6 to 10°C, with a very slow growth at that temperature (11). A model was developed for growth of *Salmonella* on ground beef from published data. Using this growth model, time and temperature governed the amount of growth during the product's transportation from the retail outlet to the home and it's storage in the refrigerator or freezer, prior to food preparation for both chilled and frozen patties (data not shown). The model predicts low and slow increase during this module. For example, at least 37 h needed for the *Salmonella* to increase by one log at 10° C. We assume this is unlikely to occur because the products become spoiled and unfit for human consumption. We excluded the transportation module from our model.

To estimate the number of decimal reductions and evolution of the microbial population before consumption, we did use literature data to develop inactivation model. By calculating D- and Z-values and using them with the temperature/time profile model, we were able to get the estimation of the number of log reductions at given temperatures in the centre of the ground beef patties.

Because of the lack of data on the infective dose of *Salmonella,* we chose to use the dose-response model published by (18) Haas which was fitted to the naïve human data from *Salmonella* feeding trials and outbreaks investigations. We did not use the dose response model developed for the WHO/FAO risk assessment of *Salmonella* in eggs and broilers (26). This latter model was developed using various outbreak data where the exposure doses estimate are judged not enough accurate and that led to high uncertainty on risk estimate.

This study indicated that the risk of salmonellosis after a well done cooking was closed to zero whatever the fat content in the product as cooking times were sufficient to reach the recommended temperature (68.3°C) in the centre of the patties. Yet the risk could be multiplied by just 312 if the patty was cooked during a short time (rare). Consumers, especially the most susceptible ones (immunocompromised, elderly, young children, and pregnant women), should continue to be made aware of the risks associated with eating raw or uncooked ground beef, tasting ground beef during food preparation, and cross-contamination from raw meat to ready-to-eat-food, as well as the importance of hand washing after handling raw ground beef.

Salmonellosis outbreaks associated with ground beef continue, despite Hazard Analysis and Critical Control Point System (HACCP), enhanced adherence to good manufacturing practices and education of food processors, preparers, and servers at all levels in the food industry and at home. Targeting interventions at various steps, from beef production through consumption, might help to reduce the risk of salmonellosis. The scenario analysis has been set up to study the effect of quantified uncertainties and some model assumptions on the baseline model results. It was found that by multiplying or dividing animal prevalence by 10 at slaughter had large impact on the result. The scenario analysis identifies important and less important gaps of knowledge, and allows a provisional insight in the comparison of intervention strategies for risk management. Based on the present knowledge and expertise the model suggests that the number of salmonellosis cases due to ground beef patties consumption may be lowered mostly by lowering the prevalence of *Salmonella* in cattle at the point of slaughter. It could be suggested that potential *Salmonella* excretors be slaughtered at the end of the day or better be removed from the production of ground beef. Product control (and monitoring) at retail level seems rather useless, due to the predicted low prevalence and low concentration. Growth of *Salmonella* spp. during storage is unlikely. An information campaign for consumers promoting better cooking of the steak may be helpful.

The model simulation did not take into account the possible cross-contaminations occurring during ground beef patty production, transport and distribution or in the consumer's fridge. Yet, provided basic hygiene rules are followed along the whole chain, the model remains a practical value and fairly low numbers of expected cases tend to confirm that ground beef patties are low risk foods as far as salmonellosis is concerned if they are consumed well done.

Despite the limitations that we underscored, the present work is the first attempt to model the risk of *Salmonella* infection linked to the consumption of ground beef patties which tended to show that the risk of salmonellosis could be considered relatively low and is manageable at the farm and processing levels. The model could be used to assess different mitigations options such as the effect of more strict hygienic procedures during slaughtering, meat mixing, patties formation and the way of cooking the ground beef patty. Efforts for risk mitigation should be focused on reducing the risk estimated, even if this represents a relative, rather than absolute value.

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