Quantitative risk assessment of human salmonellosis from the consumption of a turkey product in collective catering establishments

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Abstract

The quantitative risk assessment (QRA) approach recommended by the Codex Alimentarius Commission was used to assess the risk of human salmonellosis from the consumption of ‘cordon bleu’, a specific turkey product, in collective catering establishments (CCEs) of a French department. The complete process was modeled and simulated, from the initial storage in the CCE freezer to the consumption, using a Monte Carlo simulation software. Data concerning the prevalence of contaminated ‘cordon bleu’, the level of contamination of Salmonella, the cooking and storage process were collected from 21 CCEs and 8 retailers of ‘cordon bleu’ in the selected department. Thermal inactivation kinetics for Salmonella were established to estimate the effect of heat treatment on the concentration in the product and to calculate the dose that could be ingested by the consumer. The Beta-Poisson dose-response model of Rose and Gerba [Water Science and Technology 24 (1991) 29] with the specific parameters for Salmonella was used to estimate the probability of infection related to the ingestion of a particular dose and a factor was applied to estimate the probability of illness from ingestion. The individual risk of salmonellosis, the risk of outbreak and the number of cases were calculated using Monte Carlo simulation method. The risk of salmonellosis was close to zero when the ‘cordons bleus’ were cooked in the oven. Therefore, the risk was calculated for the fryer cooking since the insufficient cooking time observed was, sometimes, at the origin of low temperatures (37–89 °C). The influence of both the initial concentration of Salmonella in the product and the heat storage before consumption on the final risk was studied. For a high initial concentration of Salmonella in the product, when the ‘cordons bleus’ are fryer cooked, the average risk of salmonellosis was equal to $3.95 \times 10^{-3}$ without storage before consumption and $2.8 \times 10^{-4}$ if the product is consumed after storage. This paper presents the results of the QRA and discusses risk management options to minimize the risk of salmonellosis.

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Keywords: Salmonella; Microbial risk assessment; Turkey product; Dose-response; Thermal inactivation; Monte Carlo simulation

1. Introduction

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. This pathogen is at the origin of a foodborne illness called salmonellosis. Fever, nausea, sometimes vomiting, abdominal cramps and diarrhea characterize human salmonellosis. Dehydration can be particularly serious in infants, old people and persons with impaired immune system.

Salmonellosis is reported to be responsible for 87% of the cases of reported foodborne illness in France (World Health Organization, 1997). In 1997, Salmonella caused 76% of the outbreaks (Pierre et al., 1996; Haeghebaert et al., 1998).

In the USA, the Centers for Disease Control and Prevention (CDC) record the cases of salmonellosis since 1943: between 1973 and 1987, 59% of the foodborne outbreaks were attributed to Salmonella, more often from animal origin (Dubbert, 1998). Improperly handled chicken and turkey products were the most common sources (Tietjen and Fung, 1995).

The last outbreak of salmonellosis associated to turkey meat has been at the origin of 32 cases (2 deaths) in August 1999 in Sweden (National Food Administration, 1999).

This pathogen is widely found in the environment and animals, particularly poultry (chicken, turkey, etc.) which represents its main source.

Turkey and chicken carcasses, giblets and skin (particularly neck skin) are frequently contaminated with Salmonella. Contamination occurs because of the deposition of fecal material on feet and feathers which reach the carcasses during slaughtering, defeathering and evisceration, and cross-contamination during the processing from the utensils or the hand of workers frequently occurs (Uyttendaele et al., 1998).

If the poultry carcass is consumed as such, the cooking temperature allows for the destruction of the surface contamination. On the other hand, when several pieces of different carcasses are minced and mixed, cross-contamination occurs.

‘Cordon bleu’ is a meat turnover coated with breadcrumbs made of a slice of reconstituted turkey meat (mixing of any part of boned minced meat and skin) on which a slice of turkey ham and a slice of processed cheese are placed. The consumption of this type of products could present a risk when good hygienic practices are not followed in the industrial plants and/or the actual cooking practices do not allow for the inactivation of the Salmonella cells that could be present in the product.

Owing to the recent increase of ‘cordon bleu’ consumption, mostly for catering, the question was raised of a potential increase of exposition to Salmonella, it was then decided to realize an assessment of the risk of human salmonellosis outbreaks arising from ‘cordon bleu’ consumption in collective catering establishments (CCEs) of a French department. A quantitative risk assessment (QRA) was done on the basis of the recommendations of the Codex Alimentarius Commission (Anonymous, 1994, 1995, 1999). The first step of risk assessment is hazard identification. This was done above.

For the purpose of this study, four specific steps were defined: (i) estimation of the frequency and the level of contamination in turkey ‘cordons bleus’ used in CCEs of the selected department; (ii) description of cooking and storage practices; (iii) description of the evolution of the concentration of Salmonella in the product until consumption; and finally, (iv) the quantitative risk assessment linked to the consumption of turkey ‘cordons bleus’ by combining the results of the three previous steps.

2. Materials and methods

2.1. Estimation of the frequency and the level of contamination

2.1.1. Sampling method

To determine the sample size necessary to estimate the frequency of ‘cordons bleus’ contamination with Salmonella, we used as a probable frequency the result obtained in a preliminary study conducted by the French Agency for Food Sanitary Safety (AFSSA) that estimated the contamination rate of the ‘cordons bleus’ at 13%.

According to the desired level of relative precision, namely 25% or 30%, the sample size should be respectively 300 or 400 (Toma et al., 2000).

A sample size equal to 300 or 400 units and an expected frequency of contamination equal to 13%, corresponds to, respectively, at least 95% or 99% of chance to observe more than 30 contaminated ‘cordons bleus’ in the sample which allowed to estimate the parameters of concentration distribution.
Thus, the ‘cordons bleus’ were sampled both from the visited CCEs (75 ‘cordons bleus’ from 15 different batches), and from the 8 retailers of ‘cordons bleus’ in the studied region (250 ‘cordons bleus’ from 50 different batches) so that a total of 325 ‘cordons bleus’ from 65 different batches were collected. Sampling period lasted for 10 months from April 1998 to January 1999, including all seasons.

2.1.2. Salmonella detection and enumeration

Each frozen ‘cordon bleu’ sample was divided in two equal portions. One of them was immediately refrozen at \(-24\,^\circ\text{C}\) and the other one was thawed at room temperature and then homogenized in a Stomacher\textsuperscript{TM} bag. *Salmonella* detection was realized according to the French Standard NF V08-052 on 25 g of the homogenized sample. As soon as the presence of *Salmonella* was detected, the enumeration using a most probable number (MPN) method was immediately done on the remaining part of the ‘cordon bleu’. After thawing, 25 g of this portion was homogenized and diluted in 100 ml Buffered Peptone Water (BPW—AES Laboratories, Combourg, France) in a Stomacher bag with filter.

2.1.2.1. Preenrichment. Twenty-five milliliters of the liquid blended sample was collected and serially diluted (1/5 dilutions) in BPW to obtain five dilutions noted d1–d5. Two milliliters of the initial suspension was distributed with a multipipette in each of the 8 wells of the first row of a 96-well polypropylene plate of 2 ml (Poly Labo, Strasbourg, France). The same was repeated in the third to the ninth row with the other dilutions. Alternative rows of the microplate remained empty to limit cross-contamination. The plate was incubated at \(37\,^\circ\text{C}\) for 16–20 h.

2.1.2.2. Selective enrichment. A second polypropylene plate of 2 ml was filled up with 1 ml of Rappaport Vassiliadis broth medium (Biokar Diagnostics, Beauvais, France) used as a selective enrichment medium and inoculated with 10 µl of the preenrichment plate in the corresponding wells. The plate was incubated at \(42\,^\circ\text{C}\) for 18–24 h.

2.1.2.3. Postenrichment. Thirty microliters from the selective enrichment broth was inoculated into the corresponding wells of a polyethylene microplate which had been previously dispensed with 300 µl of Bacto M Broth (Difco, Fischer Scientific, Elancourt, France) and incubated at \(41\,^\circ\text{C}\) for \(12\pm1\) h.

2.1.2.4. Enrichment serology. After this period of incubation, Bacto M broth cultures were tested individually using a miniaturized enrichment serology technique (Humbert et al., 1997). Rows of eight wells (Nunc-Immuno module, Poly Labo) were studied individually because of the better visual control of each well. Fifteen microliters of a formaldehyde saline solution (Sigma, St. Quentin Fallavier, France), 250 µl of Bacto M Broth and 30 µl of a mixture of nine polyclonal antisera (Difco, Fischer Scientific) were added to each microwell. The modules were incubated at \(41\,^\circ\text{C}\) for 20–30 min. A presumptive positive reaction gave a flocculent agglutination in the well. The number of positive wells for each dilution was enumerated and translated into MPN of *Salmonella* per gram of sample.

2.1.3. Serotyping

The detection of the somatic «O» antigen and the flagellar «H» antigen has been realized from a pure colony of *Salmonella* known to be nonautoagglutinating. The research has been done by slide agglutination technique with monovalent and polyvalent antisera (Difco, Fischer Scientific). The determination of these antigenic factors allows the recognition of the serovar via the Kauffmann-White scheme.

2.1.4. Thermal resistance

To study the thermal resistance of *Salmonella* in turkey ‘cordons bleus’ and determine the \(D\)-value (the time in minutes required to reduce the microbial population by a factor of 10) and \(z\)-value (numbers of degrees Celsius required to bring about a 10-fold change in decimal reduction time \(D\)), *Salmonella hadar* and *S. typhimurium* isolated from ‘cordons bleus’ were used to contaminate the samples. This was deemed more prudent than using literature data. A suspension of \(10^7\) cells/ml (0.2 ml) was mixed with 5 g of a mixture of brined meat and turkey ham, and blended. The blending was done in sterile polyethylene bags (Whirl-Pack, Poly Labo). The watertight bags were sealed, and stored at \(3\,^\circ\text{C}\) for \(2\) h to ensure a homogeneous temperature within samples. They were then heated in a water bath at 55, 57, 60 and
62 °C. After heating, samples were diluted in TSB (AES Laboratories, Combourgire, France) and Bril-
liant Bile Agar (Oxoid, Dardilly, France) was used for
the enumeration of survivors.

D-values were analyzed with SAS software (GLM
procedure). We used a regression analysis weighted
by the determination coefficient ($R^2$) with the loga-
rithm of the D-value as the dependent variable and the
temperature, the serovar and the interaction between
them as the independent variables.

2.2. Preparation and cooking practices

The study on the preparation practices of the
‘cordons bleus’ in the CCEs was realized in two steps.
Firstly, a phone inquiry was done next to 200 CCEs
randomly drawn among the 1174 CCEs of the studied
department. One hundred forty-five of them used
turkey ‘cordons bleus’. The cooking was usually done
in an oven, yet frying in pans or a combination of
oven heating and frying is used in some CCEs.

We selected the CCEs that used frozen raw and
frozen precooked ‘cordons bleus’ and visited 21 of
them between April 1998 and January 1999. Time,
environmental temperature, as well as ‘cordons bleus’
temperature were measured at each step of the proc-
ess.

The size of the establishment (number of meals/
day, number of sittings/day, etc.) and information
about ‘cordons bleus’ (preparation frequency/month,
storage time before use, use-by-date, brand or distrib-
utor, characteristics of the product—raw or pre-
cooked, instruction for use) were recorded.

During cooking, thermometers were placed in the
oven or directly in the frying oil and cooking temper-
ature and time were monitored. At the end of cooking,
the temperatures of five ‘cordons bleus’ were meas-
ured. The ‘cordons bleus’ were then placed in a stove
until consumption. The same measurements were
done during this step.

2.3. Exposure assessment model

This step of the QRA aims at the estimation of the
ingested concentration of Salmonella relative to the
consumption of a single serving of ‘cordon bleu’. An
accurate exposure assessment needs information such
as the frequency of contamination of the selected
food, its level of contamination and the growth of
the pathogen during preparation steps.

The proposed exposure assessment model is pre-
sented in Fig. 1.

Monte Carlo simulation was done using @Risk
software (version 3.5d, Palisade, Newfield, NY, USA)
to analyze different scenarios and suggest risk man-
agement strategies. During each iteration, a single
value of each variable of the model was selected
according to the attributed distribution. Finally, the
complete simulation provided a distribution of the
final output.

Using the collected data, the percentage of con-
taminated batches (PL) was modeled as a Beta dis-
tribution with parameters $a$ $(k + 1)$ and $b$ $(N - k + 1)$,
where $N$ is the total number of batches and $k$ the
number of contaminated batches (Vose, 1996). At
each iteration, the batch contamination status ($S$)
was randomly generated according to the distribution
described above. If the batch was contaminated, the
probability of finding a contaminated ‘cordon bleu’
($F$) was generated, assuming a Beta distribution with
the corresponding parameters.

The enumeration provided data on the concentra-
tion of Salmonella in the product. The Poisson dis-
tribution seemed to be the most appropriate
distribution.

To estimate the number of microorganisms to
which consumers were exposed, the weight ($W$) of
the servings had to be known. In this study, the weight
of sampled portions was 100 or 125 g. It was assumed
to follow a discrete distribution with two possible
values: so that the probability of occurrence of 100 g
was $1/4$ and the probability of 125 g was $3/4$. Each
parameter, divided by the sum $(1 + 3 = 4)$, corresponds
to the observed percentages.

To estimate the dose of Salmonella that could be
ingested by the consumer, the reduction of concen-
tration caused by cooking and storage before con-
sumption was estimated, based on the estimated D-
and z-values. The influence of unpacking and storage
before cooking was not taken into account since the
product was frozen and the duration of these steps was
not sufficient to induce a significant increase of the
product temperature.

Using the results provided by the regression anal-
ysis, we determined the equation of the regression line
and estimated the corresponding parameters (mean)
and their standard deviation. These values were then used as the parameters of the normal distribution (mean, standard deviation) attributed to the significant variables of the model.

The thermal effect of cooking and heat storage was assessed by using the time/temperature profiles measured in the study. At each iteration, a situation was randomly generated according to the observed values. 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; \( E_s: \) efficiency of storage) by dividing the cooking \( (t_c) \) or storage time \( (t_s) \) by the corresponding decimal reduction time \( D \) calculated above \( (D_c: \) cooking; \( D_s: \) storage). The following integral was used, incremented by blocks of 1 min:

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

\[ (1) \]
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: 

$$d_1 = \frac{C}{10^E_c}$$

if the product was consumed without storage) or 

$$d_2 = \frac{C}{10^E_c + E_s}$$

if the product was consumed after storage per serving of 'cordon bleu'.

Details on distributions and variables of the model are summarized in Table 1.

### 2.4. Risk characterization

The risk characterization step allowed for the estimation of the probability of an adverse effect to human health, such as infection or illness, from the ingestion of a particular dose of the pathogen.

#### 2.4.1. Dose–response relationship

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 (Rose et al., 1996), Beta-Poisson (Rose and Gerba, 1991; Fazil, 1996; USDA-FSIS, 1998), Gompertz (Coleman and Marks, 1998) and the Health Canada reparameterized Weibull model (Paoli, unpublished; Ross, unpublished).

Actually, the exponential and the Beta-Poisson models are the most commonly used.

The probability of infection was described by the following equation:

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

where $n$ is the number of consumed microorganisms and $r$ is the probability of infection caused by a single cell, assuming that each microorganism has a same probability of causing infection.

On the other hand, if we assume that the number of microorganisms was Poisson distributed with a mean $d$, we obtain the exponential model:

$$PI = 1 - \exp(-rd)$$

### Table 1

**Distribution of the variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Unit</th>
<th>Distribution/Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>Percentage of contaminated batches</td>
<td>–</td>
<td>Beta (20, 47)</td>
</tr>
<tr>
<td>$S$</td>
<td>Batch contamination status</td>
<td>–</td>
<td>Uniform (0, 1) (if &lt; PL, 1, 0)</td>
</tr>
<tr>
<td>$F$</td>
<td>Percentage of contaminated CB$^*$</td>
<td>–</td>
<td>Beta (37, 60)</td>
</tr>
<tr>
<td>$C_s$</td>
<td>Concentration of Salmonella per gram of cordon bleu</td>
<td>CFU/g</td>
<td>Poisson (0.5)</td>
</tr>
<tr>
<td>$W$</td>
<td>Weight of 'cordon bleus'</td>
<td>g</td>
<td>Poisson (2)</td>
</tr>
<tr>
<td>$C$</td>
<td>Concentration of Salmonella per serving</td>
<td>CFU/CB$^*$</td>
<td>Poisson (10)</td>
</tr>
<tr>
<td>$(T_c, t_c)$</td>
<td>Temperature–duration profiles of cooking</td>
<td>–</td>
<td>Observed values</td>
</tr>
<tr>
<td>$(T_s, t_s)$</td>
<td>Temperature–duration profiles of storage</td>
<td>–</td>
<td>Observed values</td>
</tr>
<tr>
<td>$D_c(T(t))$</td>
<td>Decimal reduction time after cooking</td>
<td>min</td>
<td>$10^{\left(\text{Normal}(-0.213, 0.01)\times T_c + 12.91\right)}$</td>
</tr>
<tr>
<td>$D_s(T(t))$</td>
<td>Decimal reduction time after storage</td>
<td>min</td>
<td>$10^{\left(\text{Normal}(-0.213, 0.01)\times T_s + 12.91\right)}$</td>
</tr>
<tr>
<td>$E_c$</td>
<td>Number of log-reductions caused by the whole operation of cooking</td>
<td>–</td>
<td>$E_c = \int_0^{t_c} \frac{1}{D_c(T(t))}d(t)$</td>
</tr>
<tr>
<td>$E_s$</td>
<td>Number of log-reductions caused by the whole operation of storage</td>
<td>–</td>
<td>$E_s = \int_0^{t_s} \frac{1}{D_s(T(t))}d(t)$</td>
</tr>
<tr>
<td>$E_c + E_s$</td>
<td>Number of log-reductions caused by the whole process</td>
<td>–</td>
<td>$E_c + E_s$</td>
</tr>
<tr>
<td>$d_1$</td>
<td>Concentration of Salmonella in the product if consumed without storage</td>
<td>CFU/CB$^*$</td>
<td>$C/10^{E_c}$</td>
</tr>
<tr>
<td>$d_2$</td>
<td>Concentration of Salmonella in the product if consumed with storage</td>
<td>CFU/CB$^*$</td>
<td>$C/10^{E_c} + E_s$</td>
</tr>
</tbody>
</table>

$^*$ CB: Cordon bleu.
On the other hand, if we assume that the probability of infection from a single cell varies among hosts and attributes to $r$ a Beta distribution with the parameters $\alpha$ and $\beta$, one obtains, by a mathematical approximation, the following equation called the Beta-Poisson model (Furumoto and Mickey, 1967):

$$PI = \frac{1}{C0} \left( 1 + \frac{d}{139.9} \right)^{-1}$$  \hspace{1cm} (4)

For the purpose of this study, the Beta-Poisson model was used with the specific parameters for *Salmonella* proposed by Rose and Gerba (1991). This model predicts the percentage of the population that becomes infected in function of the ingested dose of *Salmonella*.

$PI$ was the probability of infection related to the ingestion of $d$ *Salmonella*, $\alpha$ and $\beta$ were equal to 0.33 and 139.9, respectively (Rose and Gerba, 1991).

### 2.4.2. Probability of illness

Illness was defined as the occurrence of gastroenteritis (abdominal cramps, diarrhea, nausea, vomiting).

According to feeding studies on human volunteers (McCullough and Eisele, 1951a,b), the average probability of illness among infected naive subjects was varying between 0 and 75% with a mean of 16%. Because of the high doses administered in the study, it seemed difficult to extrapolate this probability to the general population. So, to build a more realistic model, we decided to reduce the reported probability of illness to 10%.

Distribution of variables and models for the risk of human salmonellosis are presented in Table 2.

The risk of salmonellosis from the consumption of a single turkey ‘cordon bleu’ was estimated using the results of the previous steps, for each type of cooking, without storage ($RI_1 = PI_1 \times 10\%$) or with storage before consumption ($RI_2 = PI_2 \times 10\%$).

#### 2.4.3. Risk of outbreak

A foodborne outbreak is defined as an incident in which at least two grouped cases became ill, with similar symptoms, after the consumption of a same food.

The risk of outbreak and the number of cases ($N_1$ without storage; $N_2$ with storage) were estimated for 5000 sittings. For each sitting, a number of meals was simulated using a triangular distribution (100, 500, 1000) to represent all the interrogated CCEs (phone questionnaire and observation study).

The maximum number of cases ($N_{1\text{max}}$ without storage; $N_{2\text{max}}$ with storage) and the expected probability of outbreak with a number of cases $z$ ($RO_{1z}$ without storage; $RO_{2z}$ with storage) and $z_5$ ($RO_{1z_5}$ without storage; $RO_{2z_5}$ with storage) were estimated. The probability of outbreak with at least five cases was calculated because outbreaks causing fewer cases could remain unreported.

Table 2
Description and distribution of variables and models for the risk assessment of human salmonellosis from the consumption of turkey ‘cordons bleus’

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Distribution/Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$PI_1$</td>
<td>Probability of infection if the product is consumed without storage</td>
<td>$1 - \left(1 + d/(139.9)\right)^{-0.33}$</td>
</tr>
<tr>
<td>$PI_2$</td>
<td>Probability of infection if the product is consumed after storage</td>
<td>$1 - \left(1 + d/(139.9)\right)^{-0.33}$</td>
</tr>
<tr>
<td>$PM$</td>
<td>Probability of illness</td>
<td>0.10</td>
</tr>
<tr>
<td>$RI_1$</td>
<td>Individual risk of salmonellosis/serving if the product is consumed without storage</td>
<td>$0, \text{ if } S=0; F \times PI_1 \times PM, \text{ if } S=1$</td>
</tr>
<tr>
<td>$RI_2$</td>
<td>Individual risk of salmonellosis/serving if the product is consumed after storage</td>
<td>$0, \text{ if } S=0; F \times PI_2 \times PM, \text{ if } S=1$</td>
</tr>
<tr>
<td>$NR$</td>
<td>Number of meals/sitting</td>
<td>Triangular (100, 500, 1000)</td>
</tr>
<tr>
<td>$N_1$</td>
<td>Number of cases if the product is consumed without storage</td>
<td>Binomial (NR, $RI_1$)</td>
</tr>
<tr>
<td>$N_2$</td>
<td>Number of cases if the product is consumed after storage</td>
<td>Binomial (NR, $RI_2$)</td>
</tr>
<tr>
<td>$N_{1\text{max}}$</td>
<td>Maximum number of cases for the 5000 iterations if the product is consumed without storage</td>
<td>Max ($N_1$ for the 5000 iterations)</td>
</tr>
<tr>
<td>$N_{2\text{max}}$</td>
<td>Maximum number of cases for the 5000 iterations if the product is consumed after storage</td>
<td>Max ($N_2$ for the 5000 iterations)</td>
</tr>
<tr>
<td>$RO_{1z}$</td>
<td>Risk of outbreak with at least two cases if the product is consumed without storage</td>
<td>Pr($N_1 \geq 2$) for the 5000 iterations</td>
</tr>
<tr>
<td>$RO_{1z_5}$</td>
<td>Risk of outbreak with at least five cases if the product is consumed without storage</td>
<td>Pr($N_1 \geq 5$) for the 5000 iterations</td>
</tr>
<tr>
<td>$RO_{2z}$</td>
<td>Risk of outbreak with at least two cases if the product is consumed after storage</td>
<td>Pr($N_2 \geq 2$) for the 5000 iterations</td>
</tr>
<tr>
<td>$RO_{2z_5}$</td>
<td>Risk of outbreak with at least five cases if the product is consumed after storage</td>
<td>Pr($N_2 \geq 5$) for the 5000 iterations</td>
</tr>
</tbody>
</table>
3. Results

3.1. Frequency of contamination, level of contamination and serotypes

Among the 65 collected batches, 19 batches contained at least one contaminated ‘cordon bleu’ (29.2%). Among the 95 ‘cordons bleus’ from the 19 contaminated batches, 36 were positive (37.9%).

The enumeration showed that only two ‘cordons bleus’ out of 36 contained 2 *Salmonella*/g. The concentration in the 34 others was lower than the detection threshold, that is, 2 CFU/g.

Serotypes found in ‘cordons bleus’ were distributed as presented in Fig. 2. *S. hadar* represented 30% of occurrences.

3.2. Preparation and cooking practices

3.2.1. Phone inquiry

Among the 200 questioned collective restaurants, 163 used poultry ‘cordons bleus’: 89.2% of them prepared turkey ‘cordons bleus’. Frozen ‘cordons bleus’ were most frequently used (72.8%) than refrigerated ones. The ‘cordons bleus’ were purchased more often as cooked (43.2%) than precooked (40.8%) or raw (16%).

The majority of CCEs preparation was done in oven or fryer.

The frequency of preparation was one time/month in 58.1% of the CCEs.

3.2.2. Observation study

The results provided by the observation study were close to those from the inquiry. The product was prepared once a month in 66.6% of cases. ‘Cordons bleus’ were usually cooked without previous thawing in oven or fryer (33% and 29%, respectively). We then decided to include these two types of cooking in the risk assessment model.

3.2.2.1. Cooking temperatures. ‘Cordons bleus’ temperatures ranged from 74 to 97 °C with a mean of 83 °C after cooking in oven, and from 37 to 89 °C with a mean of 57 °C when cooked in the fryer. The insufficient cooking time was at the origin of the low temperatures observed when the ‘cordons bleus’ were fried. Indeed, when the product was cooked in the fryer, time varied from 3 to 7 min with a mean of 5 min (Table 3).

The temperature of the oven ranged from 133 to >200 °C and the frying oil one from 135 to >200 °C.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Results for cooking and storage practices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cooking temperature (°C)</td>
</tr>
<tr>
<td>Oven</td>
<td>Minimum 74</td>
</tr>
<tr>
<td></td>
<td>Maximum 97</td>
</tr>
<tr>
<td></td>
<td>Mean 83</td>
</tr>
<tr>
<td></td>
<td>S.D. 6.3</td>
</tr>
<tr>
<td>Fryer</td>
<td>Minimum 37</td>
</tr>
<tr>
<td></td>
<td>Maximum 89</td>
</tr>
<tr>
<td></td>
<td>Mean 57</td>
</tr>
<tr>
<td></td>
<td>S.D. 12.3</td>
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</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>D- and z-values and regression parameters obtained for <em>S. hadar</em> and <em>S. typhimurium</em> at different temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T</em>&lt;sub&gt;ref&lt;/sub&gt; (°C)</td>
</tr>
<tr>
<td><em>S. hadar</em></td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>57</td>
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<td>57</td>
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<td></td>
<td>60</td>
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<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>62</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>55</td>
</tr>
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</tr>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>62</td>
</tr>
</tbody>
</table>

Fig. 2. Serotypes of *Salmonella.*
Salmonella and for different concentrations of 0.5 CFU/g RI 1 5.59
Poisson distribution parameters Risk Mean Maximum 99th Percentile 99.9th Percentile
(a) Oven
\( E_\text{c} \)
7.16 \( \times 10^4 \) 1.06 \( \times 10^5 \) 1.58 \( \times 10^{12} \) 1.22 \( \times 10^3 \) 7.41 \( \times 10^5 \)
\( E_\text{c} + E_s \)
5.62 \( \times 10^2 \) 6.46 \( \times 10^5 \) 6.66 \( \times 10^{11} \) 8.3 \( \times 10^5 \) 3.04 \( \times 10^6 \)
(b) Fryer
\( E_\text{c} \)
1.8 \( \times 10^{-7} \) 1.35 \( \times 10^4 \) 9.04 \( \times 10^6 \) 1.55 \( \times 10^{-6} \) 3.31 \( \times 10^{-2} \)
\( E_\text{c} + E_s \)
1.04 \( \times 10^{-2} \) 3.75 \( \times 10^7 \) 1.36 \( \times 10^{10} \) 6.34 \( \times 10^{-2} \) 4.35 \( \times 10^4 \)

3.2.2.2. Storage temperatures. All the CCEs stored the ‘cordons bleus’ before consumption in a stove where the temperature varied from 54 to 81 °C with a mean of 68.6 °C for those cooked in the oven, and from 51 to 88 °C with a mean of 69 °C for the ‘cordons bleus’ cooked in the fryer as shown in Table 3. Sometimes, this storage allowed a temperature increase of the ‘cordons bleus’, especially with the fryer.

3.3. Exposure assessment model

The data obtained for the estimation of the decimal reduction time \( D \) are presented in Table 4. Survival curves were linear on a semi-logarithmic plot. Since no tailing off was observed over six decimal reductions, a logarithmic decline was assumed also for greater numbers of decimal reductions. It was assumed that Salmonella cells were submitted to the heating treatment pertaining to the coldest spot in the ‘cordon bleu’ where temperature measurements were performed.

The regression analysis showed that the serovar and the interaction between serovar and temperature were not significant \((p>0.05)\). Another regression analysis was then run with the 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:

\[
\log D = 12.91 - 0.213T
\]  \( (5) \)

The \( z \)-value is the reciprocal of the slope and was equal to:

\[
z = \frac{1}{0.213} = 4.69
\]  \( (6) \)

We attributed to the slope a normal distribution with a mean of \(-0.213\) and a standard deviation of 0.01, resulting from the regression analysis. So, Eq. (5) becomes:

\[
D(T(t)) = 10^{12.91+\text{Normal}(-0.213, 0.01)T}
\]  \( (7) \)

This equation was applied for each operation (cooking, storage) and for each type of cooking (oven, fryer). The time/temperatures profiles observed in the study were randomly generated, incremented by 1 min.

For products cooked and consumed without storage, the average number of decimal reductions \( (E_c) \) was equal to \( 1.06 \times 10^5 \) for oven and \( 1.35 \times 10^4 \) for fryer.

Table 6

<table>
<thead>
<tr>
<th>Poisson distribution parameters</th>
<th>Risk</th>
<th>Mean</th>
<th>Maximum</th>
<th>99th Percentile</th>
<th>99.9th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 CFU/g</td>
<td>RI1</td>
<td>5.59 ( \times 10^{-4} )</td>
<td>1.49 ( \times 10^{-2} )</td>
<td>1.07 ( \times 10^{-2} )</td>
<td>1.43 ( \times 10^{-2} )</td>
</tr>
<tr>
<td></td>
<td>RI2</td>
<td>3.55 ( \times 10^{-5} )</td>
<td>1.11 ( \times 10^{-2} )</td>
<td>1.43 ( \times 10^{-2} )</td>
<td>7.05 ( \times 10^{-3} )</td>
</tr>
<tr>
<td>2 CFU/g</td>
<td>RI1</td>
<td>1.88 ( \times 10^{-3} )</td>
<td>2.37 ( \times 10^{-2} )</td>
<td>1.60 ( \times 10^{-2} )</td>
<td>1.90 ( \times 10^{-2} )</td>
</tr>
<tr>
<td></td>
<td>RI2</td>
<td>9.99 ( \times 10^{-5} )</td>
<td>1.70 ( \times 10^{-2} )</td>
<td>3.63 ( \times 10^{-3} )</td>
<td>1.25 ( \times 10^{-2} )</td>
</tr>
<tr>
<td>10 CFU/g</td>
<td>RI1</td>
<td>3.95 ( \times 10^{-3} )</td>
<td>3.05 ( \times 10^{-2} )</td>
<td>2.47 ( \times 10^{-2} )</td>
<td>2.80 ( \times 10^{-2} )</td>
</tr>
<tr>
<td></td>
<td>RI2</td>
<td>2.80 ( \times 10^{-4} )</td>
<td>2.30 ( \times 10^{-2} )</td>
<td>1.09 ( \times 10^{-2} )</td>
<td>2.06 ( \times 10^{-2} )</td>
</tr>
</tbody>
</table>
for fryer cooking. For products consumed after a heat storage, this number \((E_s + E_c)\) was equal to \(1.71 \times 10^9\) for the oven and \(3.75 \times 10^7\) for the fryer.

As shown in Table 5a and b, the heat treatment submitted by the oven was most efficient than the fryer since the cooking time was longer and allows a higher destruction of initial number of bacteria.

### 3.4. Risk characterization

#### 3.4.1. Risk of salmonellosis

The risk of salmonellosis was close to zero when the ‘cordons bleus’ were cooked in oven, whatever the initial distribution of *Salmonella*. The results obtained for the fryer are summarized in Tables 6 and 7. Risk distribution for each concentration, with or without storage, is presented in Fig. 3.

When cooked in fryer and stored before consumption, the risk of illness \((RI_2)\) varied from 0 to \(1.11 \times 10^{-2}\) with a mean of \(3.55 \times 10^{-5}\) for a low initial concentration \((0.5 \text{ CFU/g})\) and from 0 to

![Poisson (0.5)](image)

![Poisson (10)](image)

![Poisson (2)](image)

Fig. 3. Risk of illness associated to the consumption of a single serving of ‘cordon bleu’ cooked in the fryer: 5000 iterations \((RI_1): \text{Individual risk of illness if the product is consumed without storage}; RI_2: \text{Individual risk of illness if the product is consumed after storage})

<table>
<thead>
<tr>
<th>Poisson distribution parameters</th>
<th>Number of cases</th>
<th>Mean (E_s + E_c)</th>
<th>99th Percentile</th>
<th>99.9th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 CFU/g</td>
<td>(N_1^a)</td>
<td>0.33</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>(N_2^b)</td>
<td>0.02</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>2 CFU/g</td>
<td>(N_1)</td>
<td>0.93</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>(N_2)</td>
<td>0.04</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>10 CFU/g</td>
<td>(N_1)</td>
<td>2.28</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>(N_2)</td>
<td>0.20</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>

* Number of cases if the product is consumed without storage.

* Number of cases if the product is consumed after storage.
2.3 \times 10^{-2} \text{ with a mean of } 2.8 \times 10^{-4} \text{ for a high initial concentration (10 CFU/g). The risk was equal to zero (no salmonellosis cases occurring) in 98.4% and 95.4% of iterations, respectively.}

When the ‘cordons bleus’ were consumed without storage, the risk (RI₁) varied from 0 to 1.49 \times 10^{-2} with a mean of 5.59 \times 10^{-4} for the lower concentration and from 0 to 3.05 \times 10^{-2} with a mean of 3.95 \times 10^{-3} for the higher concentration. RI₁ was equal to zero in 90.7% and 75.1% of iterations, respectively.

### 3.4.2. Risk of outbreak

The detailed results are summarized in Table 8. The risk of outbreak was calculated for the three distributions of concentrations of Salmonella. The presented results are expressed in terms of probability of an outbreak for 5000 sittings.

When the initial concentration was low (Poisson distribution with a mean equal to 0.5 Salmonella/g) and the product consumed immediately after cooking, the median probability of outbreak with at least two or five cases was respectively 6.68 \times 10^{-2} and 2.90 \times 10^{-2}. For the same concentration, when the product was consumed after storage, the median probability of outbreak with at least two or five cases was respectively 3.80 \times 10^{-3} and 1.00 \times 10^{-3}. For an initial concentration of 10 Salmonella/g, the same probabilities are 2.10 \times 10^{-1} and 1.78 \times 10^{-1} without storage and 1.66 \times 10^{-2} and 1.02 \times 10^{-2} with storage.

### 4. Discussion

As far as we could know, no case of salmonellosis associated with the consumption of ‘cordons bleus’ was reported during the study period.

This study showed a high frequency of contamination but mostly a low level (<2 CFU/g). The high concentrations used in the simulations, which were not observed in our study, were used in this risk assessment as an example of what could happen if the initial concentration is high and an accident occurred during the preparation of the product. The Poisson distribution seemed to be appropriate for this.
type of data but more information could allow to find a more accurate distribution as the lognormal (Brown et al., 1998) or the negative binomial distributions also associated with microbial counts in food.

For the enumeration, we used the MPN method. The latter is used for the enumeration of low number of microorganisms in foods. It is based upon two assumptions. The first one is that the organism is randomly distributed in the food sample. The second one is that each dilution will exhibit growth if one or more organisms are present.

To estimate the number of decimal reductions and the evolution of the microbial population before consumption, we did not use literature data, but instead used our own data on decimal reduction times that we measured for two serotypes of Salmonella isolated from the ‘cordon bleu’ (S. hadar because it was the most frequently found in this study and S. typhimurium because it is the most virulent serovar).

Because of the lack of data on the infective dose of Salmonella, we chose to use the Beta-Poisson model proposed by Rose and Gerba (1991). In the establishment of this relation, several factors that could influence the severity of the illness had to be taken into account. Three kinds of factors related to the pathogen (ingested dose, virulence of the strain, serovar, ability to grow and survive in the food vehicle or the host organism), the host (age, gender, immune status) and the environment (stomach contents, food vehicle composition, microorganisms competitiveness in the food) can affect the infective dose of the pathogen (Blaser and Newman, 1982; Coleman and Marks, 1998). Yet the estimation of the infective dose is difficult because of the lack of epidemiological data since the pathogen is rarely enumerated in food vehicles in outbreaks.

The probability of illness was assumed by reducing the reported attack rate in feeding studies on human volunteers (McCullough and Eisele, 1951a,b), but the serotypes used in the latter (S. meleagridis, S. anatum, S. bareilly, S. newport, S. derby), were different from those found in our study (S. virchow, S. heidelberg, S. bredeney, S. indiana, S. kottbus, S. typhimurium, S. newport, S. st paul, S. hadar) except for S. newport.

We did not have details about the virulence and the pathogenicity of these strains.

Furthermore, in the study of McCullough and Eisele (1951a,b), the volunteers ingested high doses of pathogen. The lower dose tested was higher than $1.2 \times 10^4$ Salmonella while in the majority of outbreaks, when the pathogen was enumerated, the higher dose was lower than $1.2 \times 10^4$ cells (Armstrong et al., 1970; Boring et al., 1971; Craven et al., 1975; D’Aoust et al., 1975; George, 1976; Lipson, 1976; Fontaine et al., 1978, 1980; Hennessy et al., 1996; Levy et al., 1996; Vought and Tatini (1998). Finally, the volunteers were healthy adult males. The extrapolation to the general population seemed to be difficult while salmonellosis is particularly serious in children and the elderly. Therefore, our assumption on the probability of illness was most probably overestimated, and therefore was conservative.

This study indicated that the risk of salmonellosis after an oven cooking was close to zero whatever the initial number of bacteria in the product as cooking times were sufficient to reach the recommended medium temperature of 63 °C. Yet the risk could be important if the product is fryer cooked during a short time and the initial concentration of bacteria is high.

Also, we noted that there was a confusion between cooked and precooked ‘cordon bleus’ since the labeling rarely specified the heat process submitted by the product and the recommended cooking practices.

Labels should therefore provide recommendations to the consumers and CCEs about the risk of illness linked to the consumption of an insufficiently cooked meat. At the end of the study, a report was presented to the industrials. The ‘cordon bleus’ makers decided to recommend to no longer produce precooked ‘cordon bleus’ and to improve the labeling of the packages to prevent every confusion.

The main objective of this study was to pinpoint an atypical or a risk behavior that could be the origin of an incident if the product was highly contaminated. It could now be possible to extrapolate the approach and the results to products that necessitate the same type of manufacturing process, to propose risk management strategies to the industry.

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References


