Collaboration
Risk assessment of the consumption of fresh meat derivatives by certain population groups with respect to the modification of Royal Decree 1376/2003, establishing the health requirements for the production, storage and marketing of fresh meat and derived products in retail establishments

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Abstract
Royal Decree 728/2011 (Real Decreto, 2011), of 20 May, amends article 4 of Royal Decree 1376/2003 (Real Decreto, 2003), dated 7 November, establishing the sanitary conditions for the production, storage and marketing of fresh meats and derived products at retail establishments; an exception is established in this article concerning the supply of fresh meat preparations to authorised catering establishments.

This paper assesses the risk derived from these fresh meat preparations to the consuming population visiting bars and restaurants, and those people staying in geriatric nursing homes, hospitals, schools and nurseries to which the supply of fresh meat preparations is restricted.

The results obtained revealed the existence of differences in the health response of the subpopulations studied to specific doses of pathogenic microorganisms. The estimated number of ill people is greater in the case of groups from geriatric nursing homes, nurseries, schools and hospitals in comparison with the groups from bars and restaurants, for both Salmonella and L. monocytogenes.

Key words
Fresh meat derivatives, retail sale, Salmonella, Listeria monocytogenes, at-risk groups.
Introduction


Royal Decree 1376/2003 (Real Decreto, 2003) establishes an exception in the supply of meat derivatives to authorised catering establishments; this exception, which is subsequently modified by Royal Decree 728/2011, applies to fresh meat preparations. Catering establishments include nursing homes, geriatric nursing homes, hospitals, nurseries and schools, in which the populations groups at most risk stay; these groups include the elderly, the sick and children.

The amendment quoted is listed in a unique article in Royal Decree 728/2011, and affects the second paragraph of article 4 of Royal Decree 1376/2003 which is replaced with the following 1:

"Authorised establishments, pursuant to the above, may supply the products listed in this Royal Decree to authorised catering establishments provided that:

1. The supplying establishment has appropriate equipment and facilities for hygienic preparation in proportion to their volume of production.
2. This is limited to the types of meat defined in section 1 of article 2 and the meat-derived products defined in section 3 of article 2, with the exception of fresh meat preparations defined in paragraph a).1. of article 3. And, in this case, supply to bars and restaurants is limited to the meats defined in section 1 of article 2 and to the meat-derived products defined in section 3 of article 2.
3. They must not supply to establishments subjected to inscription in the General Register of Food Hygiene.
4. Distribution shall be within the scope of the area in which the establishment is located or within the local health unit, health zone or territory with similar characteristics and purpose defined by the corresponding competent authority”.

This modification allows supply of meats and meat-derived products to bars and restaurants, while maintaining the restriction (no supply of fresh meat preparations) to the rest of catering establishments such as in the case of nursing homes, geriatric nursing homes, hospitals, nurseries and schools, based on the fact that the most susceptible population groups from a food safety point of view are found in these establishments.

This paper will assess the magnitude of the risk which may be derived from the consumption of the fresh meat-derived products described in paragraph a.1 of section 3 of article 2 of Royal Decree 1376/2003, among the consuming population visiting bars and restaurants and the population of geriatric nursing homes, hospitals, schools and nurseries to which the supply is restricted from retail establishments.

1The new text is in italics.
Application of the Risk Assessment methodology to the proposed situation

The main purpose of a Risk Assessment (RA) is to support/substantiate decisions. The RA process must start by identifying the problem to be dealt with or the decision to be made by the risk assessor. The RA must provide the information required.

1. Approach to the issue

In the case in question, the amendment to article 4 of Royal Decree 1376/2003, introduces the liberalisation of the supply of fresh meat preparations (described in paragraph a.1o. of section 3 of article 2) from retail establishments, to bars and restaurants and maintains the restriction of said supply to geriatric nursing homes, hospitals, schools and nurseries, without being sustained by an objective Risk Analysis methodology.

To provide technical support to this liberalisation and avoid the arbitrariness which may arise with the conclusion that this liberalisation is rational in its application to bars and restaurants but not to other catering establishments, any approach which is adopted must hinge on the assessment of the health response of the two groups of consumers involved to the same microbiological hazards that may be conveyed in these meat-derived products.

This report provides the scientific basis that permits a differentiation to be made in the magnitude of risk derived from the consumption of fresh meat preparations among the consumers in bars and restaurants and those found in geriatric nursing homes, nurseries, schools and hospitals, who are considered to be higher risk groups. In short, the paper studies whether or not the response produced in the different groups is different and, where applicable, if this response should be considered to be of higher or lower risk for any of the groups mentioned. For this purpose, this technical report is based on the RA methodology in the framework of the Risk Analysis described by the FAO/WHO (1995).

To address a RA study, the data must be sufficient and of adequate quality. The collection of data for the development of a RA is probably the most time-consuming aspect of all tasks involved. To prepare this report, diverse epidemiological information related to outbreaks by fresh meat preparations consumption is required; data on prevalence and concentration of pathogenic microorganisms and hygiene indicator microorganisms in these products; consumption data in the sectors concerned (bars, restaurants, geriatric nursing homes, school dining rooms, hospitals, etc.); information on the degree of compliance to the hygiene management systems by food business operators, etc.

However, this information is often dispersed which does not make it possible to have access to these data quickly. This results in the search for and use of data from scientific bibliography, that implies approximations and/or assumptions to substantiate this document. According to CAC (1999), when such limitations exist, it is essential, for the sake of transparency, that these are formally described in the report, including their repercussions on the RA.

Irrespective of the above, it must be highlighted that, to find microbiological hazards in fresh meat preparations at risk levels, there must be some deviation from the compliance of the sanitary requirements for production, storage and distribution of these products, as established in Royal Decree 1376/2003. In addition, ineffective heat treatment during cooking, post-processing cross-
contamination and/or inadequate conditions of the cooked product prior to consumption, might allow
the presence and subsequent growth of pathogenic microorganisms. We consider that only
inadequate handling during preparation and/or maintenance prior to consumption would lead to the
survival and growth of pathogenic microorganisms in fresh meat preparations, as the application of
a code of Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) would effectively
eliminate or reduce this possibility.

This report does not intend to assess the differences in the sanitary conditions for the production,
storage and distribution of fresh meats and their derivatives between the different types of suppliers,
thus considering a similar degree of compliance. This report evaluates whether the health response in
the consumer groups concerned, given the same type and level of microbiological hazard conveyed
in fresh meat preparations, is or not significantly different and, where applicable, if this response
should be considered as a higher or lower risk for any of the above-mentioned groups. For this
purpose, the RA methodology adopted follows the approaches of different studies (Buchanan et al.,
2000) (FAO/WHO, 2004) (Teunis et al., 2010), which apply mechanistically-based and empirical dose-
response models, allowing differentiation, where applicable, of the magnitude of the response in the
different groups.

2. Stages in the Quantitative Risk Assessment of the proposed situation
Hazard identification
The identification of the hazard is defined as “the identification of biological, chemical, and physical
agents capable of causing adverse health effects and which may be present in a particular food or
group of foods” (CAC, 1999). In the case in question, we have only assessed those microbiological
hazards of a bacterial nature which may be of interest in fresh meat preparations, and which are
representative for the object of this report (Salmonella and Listeria monocytogenes). We have used
scientific bibliography for their selection.

Salmonella
Salmonellosis is one of the most frequent food-borne pathogens in spite of the progress made in respect
to food hygiene, both at industrial and primary production levels. The common reservoir of Salmonella
is the intestinal tract of a wide range of domestic and wild animals, resulting in a multitude of foods of
animal and plant origin as sources of infection. In general, in the European Union (EU), Salmonella
Enteritidis and Salmonella Typhimurium are the serotypes most frequently associated with the disease
in humans; specifically, S. Enteritidis is often associated with the consumption of contaminated eggs and
poultry, whereas the cases by S. Typhimurium are usually attributed to consumption of contaminated
pork, poultry and beef.

Salmonella is often transmitted when the pathogen is introduced in food preparation areas and
when storage conditions permit its survival and growth in foods, such as inadequate storage
temperatures, insufficient cooking or cross-contamination of ready-to-eat (RTE) foods. In addition, the
pathogen may be transmitted through direct contact with infected animals or humans or faeces-
contaminated environments.
Since 2004, a gradual reduction has been observed in the EU in the number of notifications of salmonellosis from 195,947 cases in 2004 to 109,844 cases in 2009, being the notification rate 23.7 cases/100,000 population in 2009 (EFSA/ECDC, 2011). S. Typhimurium and S. Enteritidis were the serotypes most frequently associated with toxinfections in humans.

The distribution of Salmonella cases by age in 2009 is very similar to that observed in previous years (Figure 1). Age data were available in 93.3% of the 108,614 cases confirmed. The highest notification rate is in the 0-4 year-old age range, with 112.4 cases/100,000 population. Although a reduction has been observed in this rate in comparison to 2008 (118.8 cases/100,000 population), it is still three times higher than the rate observed in the 5-14 year-old age group, and between six and nine times higher than in the +15 year-old age group.

**Figure 1.** Distribution of salmonellosis cases by age in the European Union. 2009.

In Spain the number of cases of salmonellosis reported has decreased significantly from 2004 (7,109 cases) to 2009 (4,304 cases), with a notification rate in 2009 of 37.6 cases/100,000 population.

**Listeria monocytogenes**

*Listeria monocytogenes* is a pathogenic microorganism causing listeriosis, which has a high mortality rate ranging between 20 and 50% (Rocourt and Bille, 1997) (Vázquez-Boland et al., 2001). Nevertheless, its impact is not always recognised on public health, probably due the sporadic nature of the disease in comparison with other common foodborne diseases of bacterial origin such as salmonellosis. Experts on listeriosis agree in the underestimation of listeriosis, probably due to the fact that notification of this disease is not compulsory in many countries including Spain, and the lack of adequate surveillance programmes for this disease.

The genus *Listeria* currently includes six species, and the cases of listeriosis in humans are almost exclusively caused by the species *Listeria monocytogenes*. *Listeria* spp. is a ubiquitous microorganism widely distributed in the environment, especially in plant matter and soils. The principal reservoirs of *Listeria* spp. are soil, forage and water, although domestic and wild animals may also act as reservoirs.
The main transmission route to humans and animals is assumed to be the consumption of contaminated food or feed. Nevertheless, the infection may also be transmitted directly from infected animals to humans, and between humans. *Listeria* is capable of multiplying at temperatures as low as 2-4 ºC, making its presence in RTE foods with a relatively long shelf-life or in inadequately cooked foods, particularly hazardous.

In 2009, the rate of listeriosis was 0.36 cases/100,000 population in the EU (EFSA/ECDC, 2011), a 14% greater than in 2008, with a notification rate of 0.30 cases/100,000 population. The distribution by age of listeriosis cases in 2009 is similar to that observed in previous years (Figure 2). The notification rate is higher among people aged over 65 (1.1 cases per 100,000 population), being the age group most affected, with 58.5% of the cases. It should be noted that the majority of cases in the 0-4 year-old age group (88.5%) correspond to newborn babies and infants (<1 year old).

![Figure 2. Distribution of listeriosis cases by age in the European Union. 2008.](image)

In 2008, data on transmission routes are available only for 34 out of the 1,381 cases notified at the European level. From these 34 cases, 32 were infected by *L. monocytogenes* through food, and two cases were associated with pregnancy. The result of the disease is known in almost half of the cases (653 cases). Out of these reported cases, 134 individuals died, 87 being over 65 years old.

In Spain, the number of cases confirmed in 2009 was 121, with a notification rate of 1.06/100,000 population. Although the incident rate of listeriosis is relatively low in comparison with other food toxifications, as mentioned above, the mortality rate is particularly high (Rodríguez Ferri, 1992). This, combined with its prevalence in fresh meat, makes this pathogen of special interest for inclusion in this RA.

**Exposure Assessment**

The Exposure Assessment is the “qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food, as well as exposures from other sources if relevant” (CAC, 1999). There are four areas requiring analysis in order to carry out a quantitative exposure
assessment: (i) exposed population; (ii) frequency of consumption of food in question; (iii) frequency of exposure of consumers to hazard in the food; and (iv) contamination level of the food at the time of consumption.

Obviously, as the value of any of the above four variables rises, the probability of exposure to the hazard increases. Therefore, a collection of accurate quantitative data would help to quantify this probability and contrast it with other hazards and/or foods. Nevertheless, the variability and uncertainty of the four variables is large, and in many cases, data are not available. Therefore a baseline scenario has been defined that will serve to illustrate different theoretical cases. This represents the worst case scenario of product contamination. In other words, it assumes the existence of a batch which is 100% contaminated (by the microorganisms identified above) which is supplied to different groups: bars and restaurants (BR); geriatric nursing homes, nurseries, schools and hospitals (GNSH). Note that in an outbreak event, the vehicle involved is normally a contaminated food lot. The scenario is based on the following assumptions:

(i) With respect to the population exposed, two populations are defined as the object of study: BR and GNSH, both consisting of 1,000 people. The age profile of the population exposed in BR is considered to be representative of the Spanish population (National Institute of Statistics, INE, 2011). Current data for the Spanish population distributed by age groups is shown in Table 1. In the same Table, it can be seen the percentage associated with each age group and the number of people in each group that randomly visit BR with an average of 1,000 customers per day.

(ii) Instead of considering a particular frequency for the consumption of fresh meat preparations, a single intake of this food per person is assumed in both populations.

(iii) The exposure of the consumer to the potential hazard in the food depends, on the one hand, on the serving size (the larger the serving size, the greater the likelihood of ingesting the potential pathogen), and on the other hand, on the prevalence of the pathogen in the food. In the baseline

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>Spanish population (x1000)</th>
<th>Percentage</th>
<th>Population 1000 people</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 0 to 1</td>
<td>497</td>
<td>1.1 %</td>
<td>11</td>
</tr>
<tr>
<td>From 0 to 4</td>
<td>1,988</td>
<td>4.3 %</td>
<td>43</td>
</tr>
<tr>
<td>From 5 to 11</td>
<td>3,216</td>
<td>7.0 %</td>
<td>70</td>
</tr>
<tr>
<td>From 12 to 64</td>
<td>32,574</td>
<td>70.5 %</td>
<td>705</td>
</tr>
<tr>
<td>≥65</td>
<td>7,878</td>
<td>17.1 %</td>
<td>171</td>
</tr>
<tr>
<td>Total</td>
<td>46,153</td>
<td>100.0 %</td>
<td>1,000</td>
</tr>
</tbody>
</table>
scenario, data concerning the serving size was not considered in this report as the need to know the serving size arises from the need to calculate the dose, and as different dose levels were set, serving size was not taken into account. Regarding the prevalence of the pathogen, as mentioned above, it was assumed that 100% of the product was contaminated.

(iv) The contamination level of the food at the time of consumption, together with the serving size, is directly related to the dose of pathogen ingested. The most recent available Spanish data were taken from the report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks for 2009 (AESAN, 2009). These data refer to the prevalence (percentage of positive samples), whereas the concentration data of Salmonella and L. monocytogenes were absent (Salmonella) or had a qualitative nature (Listeria monocytogenes). This lack of data makes it more difficult to quantify the concentration level before consumption. Therefore, various dose levels were assumed, ranging from 1 cfu to $10^8$ cfu/serving (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Dose levels of Salmonella and L. monocytogenes (cfu/serving) considered in this study</th>
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</table>

Hazard characterisation

Hazard characterisation involves the "qualitative or quantitative evaluation of the nature of the adverse health effects associated with the hazard. For the purpose of Microbiological Risk Assessment the concerns relate to microorganisms and/or their toxins" (CAC, 1999). Any dose-response relationship must consider the virulence factors associated with the different foodborne bacterial pathogens. Without knowledge about how a pathogen causes disease, it is difficult to assess and interpret the effect of the virulence, as well as the characteristics of the host and the food itself on the degree and severity of the disease.

There are three types of response to be measured: infection, morbidity and mortality. Infection refers to the colonisation of the intestinal tract by the pathogenic bacteria. Morbidity and mortality mean respectively the proportion of individuals infected and the individuals who die as a result of the symptoms caused by the disease.

The capacity of a microorganism to cause disease is associated with the presence of one or more virulence factors (toxin synthesis, degree of adherence, resistance to host immune response, resistance to adverse conditions and to antimicrobial agents, etc.). These characteristics are often associated with different genes which can be found in bacterial species.

In addition to the specific virulence features associated with pathogenic strains, the number of ingested cells may significantly affect the frequency and extent of the adverse effects produced by the pathogen. An increase in the ingested dose in food generally leads to an increase in the percentage of population who may become infected and/or ill, leading to a reduction in the time required to overcome the host barriers and cause physiological damage. This relation is usually linear in a certain range of doses until reaching a stationary stage in which high dose levels do not result in a significant increase in the probability of infection. The relation between the initial dose and the severity of the
disease is not well defined. Therefore, whereas for *Campylobacter jejuni* the infection rate is related to the dose, this is not the case for the morbidity rate (Medema et al., 1996). In contrast, Coleman and Marks (1998) observed higher severity rate of *Salmonella* infection when the dose increased.

For Buchanan et al. (2000), both conclusions may be plausible, considering that the individual response to a pathogen is highly dependent on the physiological condition of the individual, such that it is possible that severe infections are only caused in susceptible individuals (immunocompromised).

*Salmonella* is a foodborne microorganism of great public health significance. The usual reservoir of *Salmonella* is the intestinal tract of various animals, and consequently it is present in a large variety of foods. *Salmonella* is the cause of 1.4 million infections per annum, 15,000 hospitalisations and 400 deaths (Voetsch et al., 2004). Human salmonellosis is usually characterised by the sudden onset of fever, abdominal pain, nausea and sporadic vomiting. The symptoms are usually mild and most symptoms last only a few days. Nevertheless, in some patients, the infection may be more severe and the associated dehydration may endanger the patient’s life (Gonzales-Barrón et al., 2010). In these cases and in those in which *Salmonella* causes an infection of the bloodstream, a treatment with antimicrobial agents is needed. Salmonellosis has also been associated with long-term and sometimes chronic consequences, for instance, reactive arthritis.

*Listeria monocytogenes* is a foodborne microorganism which causes disease (listeriosis), and is more likely in individuals with a compromised immune system. The various clinical manifestations associated with listeriosis can be grouped in two categories: invasive listeriosis and non-invasive listeriosis. Invasive listeriosis refers to those cases in which an initial infection of the intestinal tissue by *L. monocytogenes* results in the invasion of parts of the body which are usually sterile, such as the gravid uterus, the central nervous system or the blood, or combinations of these. Invasive listeriosis is characterised by a high death rate, from 20 to 30% (Mead et al., 1999) and the infections may have consequences (McLauchlin, 1997), although their incidence has not been systematically determined (Rocourt, 1996). The cases of non-invasive listeriosis (known as febrile gastroenteritis caused by listerias) have been observed in certain outbreaks in which the majority of cases have symptoms of gastroenteritis, such as diarrhoea, fever, headaches and myalgia, after a short period of incubation (Dalton et al., 1997) (Aureli et al., 2000). These outbreaks have normally been caused after the intake of high doses of *L. monocytogenes* by healthy individuals. Neither the incidence nor the factors that cause the appearance of this non-invasive form of the disease are known.

In humans, severe listeriosis occurs mainly in foetuses, children, the elderly and immunocompromised individuals. Symptoms range from mild flu and diarrhoea to lethal infections characterised by septicaemia and meningocencephalitis. In pregnant women, the infection may spread to the foetus, which may die in the uterus resulting in miscarriage or it may be born with a severe illness. Listeriosis is one of the most important causes of death from foodborne infections in developed countries. The death rate from listeriosis has also been studied by the FDA (2003), providing with different “disease/death” ratios, as can be seen in Table 3. The highest ratio corresponded to the neonates group.
Individual susceptibility to bacterial pathogens is identified as one of the host-associated factors. The response of the population to infectious agents is varied, and is reflected in genetic terms, health status, nutrition, age, immunological state, stress and prior exposure to these pathogens. In the case of certain biological hazards, it would appear that prior exposure to the same hazard provides individual resistance to subsequent exposures to the same pathogen. Nevertheless, for many other foodborne pathogens, immunity is of limited importance, either because the presence of the pathogen is restricted to the intestinal tract (E. coli) or due to the huge variety of serotypes with virulence genes (Salmonella).

In some population groups, the exposure to these food-borne pathogens may cause higher risk because these groups have a depressed immune capacity. In particular, children and the elderly are considered to be high-risk populations from foodborne toxinfections. In addition, certain medical treatments (immunodepressors) or pathological conditions (AIDS), which have a negative effect on the immune response or the general health of the patient, may affect the incidence or severity of these foodborne infections. It has been estimated that immunocompromised population, including children and the elderly, could represent the 20% of total population (CAST, 1994) (Gerba et al., 1996) (Smith, 1998). However, it is not clear whether a depression in the immune system makes individuals more susceptible to an initial infection, or whether the infection rates for immunocompromised and healthy individuals are similar while infected immunocompromised persons present higher probability of becoming ill.

The final aspect affecting the dose-response relationship is the food matrix. Although, traditionally, food characteristics have been considered to have relative importance in the dose-response relation, in recent years the impact of the matrix effect on the probability of becoming ill has drawn increasing attention. Many published studies to date have focused on the adaptation of the microorganisms to acid conditions, and subsequent enhanced capacity to overcome the gastric barrier of the host. This feature has been demonstrated in some enteric pathogens such as E. coli, Salmonella, Shigella or L. monocytogenes. In addition, in the case of L. monocytogenes, exposure to acid conditions has been confirmed to have an influence on the expression of certain virulence genes, and their properties of adhesion and invasion. It should be remarked that physical properties of foods have an effect on the dose-response relationship. Thus, foods containing a significant quantity of fat in their composition may have a protective effect for the microorganisms as fat acts as a barrier against gastric juices. On

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>Estimates of cases per year</th>
<th>Cases reported over 4-year period</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases of listeriosis</td>
<td>Deaths</td>
<td>Cases of listeriosis</td>
</tr>
<tr>
<td>Neonates</td>
<td>216</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Age-Intermediate</td>
<td>702</td>
<td>67</td>
<td>113</td>
</tr>
<tr>
<td>Age-Elderly</td>
<td>1,159</td>
<td>307</td>
<td>194</td>
</tr>
<tr>
<td>Total</td>
<td>2,078</td>
<td>390</td>
<td>345</td>
</tr>
</tbody>
</table>
the other hand, the consumption of food which increases the stomach pH decreases the gastric barrier of the host against certain pathogens. This effect may be increased with the consumption of antacids, or substances that produce achlorhydria (reduction in the production of acid in the gastric fluids). Lastly, the consumption of liquid or solid foods may also modulate the dose-response relationship.

To characterise the hazard it is necessary to evaluate the dose-response relation, defined as “the determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response)” (CAC, 1999). A quantitative assessment of the dose-response relationship requires the development of dose-response mathematical models. A brief description of the models selected for the hazards identified, i.e. *Salmonella Typhimurium*, *Salmonella Enteritidis* and *L. monocytogenes*, is given below.

**Dose-response models of *Salmonella***

The majority of the reported cases of salmonellosis are foodborne (meat products, egg products, dairy products, etc.). Although numerous outbreaks associated with *Salmonella* have been reported, the majority of the cases are sporadic (Voetsch et al., 2004). *Salmonella* has a high capacity for host colonisation; this high degree of infection has been scientifically described using dose-response models based on different types of data (humans or animals trials, data from outbreaks etc.), and types of responses (infection or disease). Dose-response relationships are commonly assumed to follow any of the following mathematical forms: exponential (Rose et al., 1995), Beta-Poisson (Fazil, 1996) (Teunis et al., 1999) (WHO, 2003), Gompertz (Coleman and Marks, 1998), and three-phase linear models (Oscar, 2004). The most widely used models are exponential-type and Beta-Poisson models, although other authors have developed more sophisticated models. For example, Latimer et al. (2001) considered different levels of virulence of the pathogen; Bollaerts et al. (2008) modelled the dose-disease relationship based on epidemiological data and the specificity between *Salmonella* serotype and foods.

The RA developed by the United States Department of Agriculture and Food Safety Inspection Service (FDA/FSIS, 1998) for *S. Enteritidis* in eggs included different Beta-Poisson-type dose-response models for immunocompetent and immunocompromised population groups. The parameters of these models were estimated through the use of epidemiological data and investigations on salmonellosis outbreaks.

Other authors (Buchanan et al., 2000) have proposed a compartmentalised diagram to describe the dose-response relationship in which three compartments are established in the development of bacterial enteric diseases: gastric barrier, capacity for adhesion/infection and morbidity/mortality. Table 4 lists the most relevant factors associated with each of these compartments.
More recent work states that many of the experiments concerning infections caused in humans by Salmonella spp. are considered unsuitable due to an inappropriate design of the experiment, the use of unrealistic infective doses, the method of inoculation and the use of Salmonella serotypes not related to toxinfections in humans. In this respect, alternative serotypes used in some studies require very high doses to produce disease (Coleman and Marks, 1998). For example, the values of Infective Dose 50 or ID 50 (dose required to cause a 50% probability of infection) of an alternative serotype may be higher than $10^4$ cfu/serving, which often overestimates the true dose. On the other hand, it should be noted that the outbreaks caused by S. Enteritidis and S. Typhimurium usually involve a high number of cases, whereas the toxinfections associated with other serotypes of Salmonella are sporadic (Teunis et al., 2010).

Therefore, the usual procedure is the construction of dose-response models using the data from outbreaks of salmonellosis which relate the dose ingested and the infection rate (number of infected population/number of exposed population) (Bollaerts et al., 2008). Teunis et al. (2010) recently developed a dose-response model for Salmonella which allowed the estimation of the probability of infection and the probability of disease separately. The model also considered the heterogeneity of the dose ingested and the pathogen-host interaction. The model was fitted to data collected from outbreaks of salmonellosis in different countries. The variables used in this model serotype (S. Enteritidis and S. Typhimurium) and degree of susceptibility (immunocompetent and immunocompromised population). In the baseline scenario, the dose-response model proposed by Teunis et al. (2010) has been used.

The mathematical development of the model is shown below, together with the results obtained for the different dose levels and their application to both population groups (BR and GNSH).

In the model proposed by Teunis et al. (2010), the probability of exposure ($P_{\text{exp}}$) to a pathogen is given by the following equation:

$$P_{\text{exp}} (CV_{\text{ing}}) = 1 - e^{-CV_{\text{ing}}} \quad (1)$$

where $C$ is the concentration of the pathogen (cfu/g) and $V_{\text{ing}}$ is the quantity of food consumed (g).

When the infection arises irrespective of the number of cells ingested and these survive inside the body with a certain probability ($p_m$), the probability of infection is given by:

$$P_{\text{inf}} (CV_{\text{ing}} | p_m) = 1 - e^{-CV_{\text{ing}}p_m} \quad (2)$$

### Table 4. Factors associated with each of the three compartments of the mechanistic model proposed by Buchanan et al. (2000)

<table>
<thead>
<tr>
<th>Gastric barrier</th>
<th>Adhesion/Infection</th>
<th>Morbidity/Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adhesion capacity</td>
<td>Capacity to produce virulent genes</td>
</tr>
<tr>
<td>Stomach pH</td>
<td>Contact time between pathogen and epithelium</td>
<td></td>
</tr>
<tr>
<td>Time of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance to acid conditions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This model expresses the probability of infection \( P_{\text{inf}} \) as a consequence of the intake of a specific dose. According to this methodology, we consider \( P_{\text{inf}} \) as the individual probability of infection (individual risk), whereas the probability of becoming ill \( P_{\text{ill}} \) is calculated for a specific population group (population risk).

Heterogeneity of the dose
The dose-response model assumes that the microorganisms are not uniformly distributed in the food, but they form groups or clusters. In the case of solid foods, such as meat products, this type of distribution is usual. In this case, \( P_{\text{exp}} \) is given by:

\[
CV_{\text{ing}} P_{\text{exp}}(r) = 1 - \left(1 + \frac{CV_{\text{ing}}}{r}\right)^{-r}
\]  

where \( r \) is a dispersion parameter of the model related to the heterogeneity of the distribution of the dose. The value of the parameter is directly proportional to the homogeneity in the distribution of microorganisms in the food. \( P_{\text{inf}} \) is therefore established as:

\[
P_{\text{inf}}(CV_{\text{ing}} | r, p_m) = 1 - \left(1 + p_m \cdot \frac{CV_{\text{ing}}}{r}\right)^{-r}
\]

Heterogeneity of the pathogen-host interaction
The heterogeneity of parameter \( p_m \) (probability of survival of the pathogen in the host) is established by a Beta-type distribution \( g(p_m | \alpha, \beta) \). The final relation between \( P_{\text{inf}} \) and the dose consumed, considering the assumptions defined above, is determined as follows:

\[
P_{\text{inf}}(CV_{\text{ing}} | r, \alpha + \beta, -CV_{\text{ing}} / r) = \text{2F1}(\alpha, \alpha + \beta, r, r)
\]

where the probability of infection is described by a hypergeometric function \( \text{2F1} \) with parameters \( \alpha \) and \( \beta \) (Teunis et al., 2008).

\( P_{\text{inf}} \) calculation was based on a set of 5,000 data referring to parameters \( \alpha \) and \( \beta \) of the model (Eq. 5), obtained by Bayesian networks, and kindly provided by the authors of the model. From different dose levels \( 1-10^8 \text{ cfu/serving} \), the 50th percentile (median) of \( P_{\text{inf}} \) was calculated (Table 5).
From Table 5 the high infective capacity of Salmonella can be observed, as low doses (<10^2 cfu/serving) produce in all cases a $P_{inf}$ above 0.741. The infection rate is higher for S. Enteritidis, and also, there are differences between immunocompromised and immunocompetent individuals.

**Dose-response models of L. monocytogenes**

Data gaps are detected with regards to dose-response studies based on volunteers exposed to L. monocytogenes or by using surrogate pathogens. Consequently, dose-response relationships have been developed and assessed based on expert opinions, epidemiological data, animal trials, or a combination of all this information. Dose-response models available are based on the assumption that each microbial cell acts independently and that one bacterial cell is enough to cause the disease. The model used in this study is based on the RA for L. monocytogenes developed by FAO/WHO (2004). The model introduces a parameter, $r$, which is the probability that a single cell causes invasive listeriosis. This parameter is estimated by relating consumption patterns (exposure) of the population with epidemiological data from numerous cases of invasive listeriosis in the population. The estimated value of the parameter $r$ is used to calculate, using the exponential model, specific risks according to the number of cells of L. monocytogenes ingested through the consumption of contaminated fresh meat preparations. This type of model has been satisfactorily applied in numerous RA studies (Buchanan et al., 1997) (Notermans et al., 1998) (Lindqvist and Westöö, 2000) (FDA, 2003). The model applied in this report considers a probability of disease by L. monocytogenes cell of 5.85x10^{-14} and 5.34x10^{-12} for the inmunocompetent and inmunocompromised populations, respectively.

The general form of the exponential model is shown below:

$$P_{ill} = 1 - e^{(-rxD)}$$

where $P_{ill}$ = probability of becoming ill.
\[ D = \text{ingested dose (number of cells).} \]
\[ r = \text{probability of becoming ill after ingesting a single cell of } L.\text{monocytogenes.} \]

Despite the fact that in some listeriosis cases have been reported to be related to infective doses close to 100 cfu/serving, they were sporadic; usually, the dose causing disease is higher (>8 log cfu/serving) (Pérez-Rodríguez et al., 2007). The high infective dose of the pathogen is a consequence of the opportunistic nature of the infection (Rodríguez Ferri, 1992). Nevertheless, these values, although high, are reachable due to the capacity of the pathogen to grow at refrigeration temperatures.

**Risk characterisation**

Risk characterisation is a “process for determining the qualitative and/or quantitative evaluation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on a hazard identification, hazard characterisation and exposure assessment” (CAC, 1999). As described above in Exposure Assessment section, exposure to the pathogen is assumed to be the same in both groups (bars and restaurants (BR); geriatric nursing homes, nurseries, schools and hospitals (GNSH)).

To consider the uncertainty in the number of individuals of each population becoming ill as a result of exposure to *Salmonella* and *L. monocytogenes*, the binomial probability distribution was used.

In the case of GNSH population, 100% of individuals was considered to be immunocompromised, whereas in BR population, the percentage of immunocompromised population was 29.4% for *Salmonella* and 18.2% for *L. monocytogenes*, respectively. These percentages represent the high-risk groups associated to each type of microorganism. In the case of *Salmonella*, these high-risk groups are children <12 years and elderly people (>65 years old); in the case of *L. monocytogenes*, high-risk groups are comprised of pregnant women and elderly people (>65 years old).

**Salmonella**

After calculating the median of \( P_{\text{inf}} \) for the different dose levels, a scenario was set up to estimate the number of individuals who would become ill as a consequence of the intake of *Salmonella* cells via contaminated fresh meat preparations.

Two population groups were assumed for BR and for GNSH of 1,000 people each. The binomial distribution enables the calculation of the probability of occurrence of an event, in this case, the probability of finding 0, 1, 2…1000 ill individuals in each population group.

The mass function of the binomial distribution is as follows:

\[
P_x = \binom{n}{x} q^{(n-x)} p^x = \left( \frac{n!}{x! (n-x)!} \right) q^{(n-x)} p^x
\]

where \( n \) is the number of individuals exposed (1,000), \( P_x \) the probability of becoming ill, that is the probability of finding \( x \) infected individuals who become ill, and \( q \) is the probability of finding \( x \) infected individuals who do not become ill. The mean of the binomial distribution is \( n \times p \), whereas the variance is \( n \times p \times q \). The binomial distribution assumes that: (i) the number \( n \) of individuals is
predetermined, (ii) there is independence between individuals, (iii) the individual may be ill or healthy, and (iv) the probability of any individual becoming ill selected at random from the population is the same.

Therefore, in this case, \( n = 1,000 \) and \( p = P_{\text{inf}} \times P_{\text{ill} \mid \text{inf}} \). The value \( p \) of the distribution was the result of multiplying \( P_{\text{inf}} \) (median calculated in the Hazard Characterisation stage) by the probability of becoming ill once infected \( (P_{\text{ill} \mid \text{inf}}) \). \( P_{\text{ill} \mid \text{inf}} \) was assumed to be equal to 0.05 for the immunocompetent population and 0.1 for the immunocompromised population (Buchanan et al., 2000).

As stated previously, in the case of GNSH population, 100% of individuals was considered to be immunocompromised, whereas in BR population, the percentage of immunocompromised population was 29.4%.

Based on this information, the number of ill people was calculated based on probability calculations. Monte Carlo simulation was applied (10,000 iterations) using the ModelRisk v3.0 software (Vose Consulting, Belgium). The results obtained for the BR and GNSH populations for S. Enteritidis and S. Typhimurium are shown in Figures 3 and 4 for different dose levels.

![Figure 3](image-url)

**Figure 3.** Estimates of the number of ill people by S. Enteritidis at different levels of ingested doses; a) 1 cfu; b) 50 cfu; c) 10^3 cfu; d) 10^6 cfu/serving) in bars and restaurants (BR), and , geriatric nursing homes, nurseries, schools and hospitals (GNSH).
The mean, minimum value, maximum value and 95th percentile of the distributions represented in Figures 3 and 4, are shown in Tables 6 and 7, respectively.

The mean, minimum value, maximum value and 95th percentile of the distributions represented in Figures 3 and 4, are shown in Tables 6 and 7, respectively.

**Figure 4.** Estimation of the number of ill people by *S. typhimurium* at different levels of ingested doses; a) 1 cfu; b) 50 cfu; c) $10^3$ cfu; d) $10^6$ cfu/serving) in bars and restaurants (BR), and geriatric nursing homes, nurseries, schools and hospitals (GNSH).

The mean, minimum value, maximum value and 95th percentile of the distributions represented in Figures 3 and 4, are shown in Tables 6 and 7, respectively.

**Table 6.** Mean, minimum, maximum values, and 95th percentile of the final distributions of the estimated number of ill people by *S. Enteritidis* in BR and GNSH populations assuming ingested doses of 1, 10, 50, $10^2$, $10^3$, $10^6$, and $10^8$ cfu/serving.

<table>
<thead>
<tr>
<th>Dose (cfu/serving)</th>
<th>Establishment</th>
<th>Number of ill people</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>BR</td>
<td>52.07</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>83.70</td>
</tr>
<tr>
<td>10</td>
<td>BR</td>
<td>54.55</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>88.99</td>
</tr>
<tr>
<td>50</td>
<td>BR</td>
<td>55.53</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>90.01</td>
</tr>
<tr>
<td>$10^2$</td>
<td>BR</td>
<td>55.81</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>90.17</td>
</tr>
<tr>
<td>$10^3$</td>
<td>BR</td>
<td>56.19</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>91.06</td>
</tr>
<tr>
<td>$10^6$</td>
<td>BR</td>
<td>56.94</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>91.70</td>
</tr>
<tr>
<td>$10^8$</td>
<td>BR</td>
<td>57.00</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>92.00</td>
</tr>
</tbody>
</table>
The results of the dose-response model showed that the probability of infection by *S. Enteritidis* and *S. Typhimurium* was not significantly higher at increasing dose levels. This may be due in part to the high variability found in the infective dose for *Salmonella* serotypes in the different studies. Blaser and Newman (1982) detected infective doses in humans between $10^5$ and $10^{10}$ cfu/serving. On the other hand, doses around $10^1$ - $10^3$ cfu/serving have been found in immunocompetent populations (Vought and Tatini, 1998). In our case, a high degree of infection of the *Salmonella* serotypes studied was observed, since low dose levels may potentially cause illness, being *S. Enteritidis* the pathogen that would result in higher number of ill people. However, RAs are dependent on the type of dose-response model used. In this report, the model used by Teunis et al. (2010) is considered to be the most suitable, as it differentiates between two population groups (immunocompromised and immunocompetent), and it was based on epidemiological information reported from outbreaks caused by foods contaminated by *Salmonella*.

In addition, the results obtained revealed significant differences between the population groups studied (BR and GNSH), as the estimated number of ill people in GNSH was higher by 61% and 64% for *S. Enteritidis* and *S. Typhimurium*, respectively, in comparison with BR.

The high level of infection by *Salmonella*, together with the greater susceptibility associated to GNSH population led to a simulation producing 50 ill people/1,000 individuals exposed from consumption of a dose of *S. Enteritidis* of only 1 cfu/serving. The high-risk character of GNSH population can be evidenced by contrasting the previous estimate with the simulated estimate of the number of ill people in BR population originated by a dose of up to $10^6$ cfu/serving, which was 33 ill people/1,000 individuals.

### Table 7. Mean, minimum, maximum values, and 95th percentile of the final distributions of the estimated number of ill people by *S. Typhimurium* in BR and GNSH populations assuming ingested doses of $1$, $10$, $50$, $10^2$, $10^3$, $10^6$, and $10^8$ cfu/serving

<table>
<thead>
<tr>
<th>Dose Establishment</th>
<th>Number of ill people</th>
<th>Mean</th>
<th>Minimum</th>
<th>95th percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 BR</td>
<td>45.74</td>
<td>24</td>
<td>57</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>GNSH</td>
<td>76.83</td>
<td>50</td>
<td>91</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>10 BR</td>
<td>49.02</td>
<td>26</td>
<td>60</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>GNSH</td>
<td>80.93</td>
<td>51</td>
<td>95</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>50 BR</td>
<td>50.44</td>
<td>26</td>
<td>62</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>GNSH</td>
<td>83.31</td>
<td>52</td>
<td>98</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>$10^2$ BR</td>
<td>52.41</td>
<td>28</td>
<td>64</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>GNSH</td>
<td>84.53</td>
<td>52</td>
<td>99</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>$10^3$ BR</td>
<td>53.05</td>
<td>28</td>
<td>65</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>GNSH</td>
<td>85.63</td>
<td>52</td>
<td>100</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>$10^6$ BR</td>
<td>54.13</td>
<td>30</td>
<td>66</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>GNSH</td>
<td>86.36</td>
<td>52</td>
<td>101</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>$10^8$ BR</td>
<td>55.00</td>
<td>31</td>
<td>67</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>GNSH</td>
<td>88.00</td>
<td>55</td>
<td>102</td>
<td>126</td>
<td></td>
</tr>
</tbody>
</table>
exposed. This means that consumption of a contaminated fresh meat preparation, even at low levels of
Salmonella, would result in all cases in higher number of ill people in GNSH than in BR population.

Therefore, in view of the results obtained, total inactivation of Salmonella should be achieved in
fresh meat preparations prior to consumption (Giovannini et al., 2004). Based on a number of studies
on fresh meat preparations, such as fresh sausages (Mürmann et al., 2011), or other meats such as
cooked chicken (Oscar, 2004), it has been confirmed that inadequate heat treatment may increase the
risk of salmonellosis if raw material is contaminated.

The effectiveness of the treatment will in turn depend on the initial contamination of Salmonella in
the fresh meat preparations. Therefore, the competent Health Authority must proceed to control the
raw material (Mürmann et al., 2011). Contamination level of raw material is higher when it is stored
at abusive temperatures, or for long periods, or when the sanitary conditions are not observed by
suppliers. It should be noted that if a fresh meat preparation is found to have a high contamination
level, a more intense heat treatment should be required in order to guarantee food safety. In this
respect, the type of treatment will have a great influence on the inactivation of Salmonella in fresh
meat preparations. Gonzales-Barrón et al. (2010) performed a RA of S. Typhimurium associated with
the consumption of pork sausages in Ireland. The results of the study indicated that, among the
contaminated batches of sausages, grilled sausages presented higher risk of salmonellosis than fried
sausages, mainly due to the differences in temperature and time applied to each of the assessed
treatments.

In foodservice centres, additionally to the heat treatment, alternative methods for reducing the risk
of infection from Salmonella include a correct defrosting of the product and heat maintenance of the
food after cooking (Bemrah et al., 2003). At the same time, adequate training of the staff involved in
food handling is required, together with more information on the labels of meat products, including
treatment and handling recommendations in order to guarantee food safety to the consumer (Oscar,
2004).

L. monocytogenes

In the case of L. monocytogenes, the number of cases of listeriosis was estimated using the same
methodology as that used in the study of Salmonella, although with the application of the dose-
response model described above for L. monocytogenes (Equation, 6).

The results indicate that doses below $10^4$ cfu/serving did not produce any case of listeriosis in the
two populations studied (GNSH and BR) (Table 8). However, in establishments with high-risk groups
(GNSH), levels above this value were associated with the appearance of the disease. For GNSH, a
maximum incidence of one ill person/1,000 individuals exposed was observed for a dose of $10^5$
cfu/serving, and four ill people for the maximum dose of $10^8$ cfu/serving. On the other hand, for BR,
no listeriosis cases were observed for any of the doses considered, indicating that the level of risk in
this type of establishment was significantly lower than in GNSH, in which the majority of the
population are immunocompromised.
As mentioned above, listeriosis has a significant mortality rate. The study carried out by FDA (2003) estimated the death rate for each of the high-risk groups to \textit{L. monocytogenes}. By applying this rate to our results, a level of $10^8$ cfu/serving would cause death in one out of the four estimated ill people in GNSH. This fact highlights the great susceptibility of GNSH population, together with the fatal consequences that would be produced by an exposure to a high dose of the pathogen.

**Conclusions**

The results obtained reveal the existence of differences between the populations studied with respect to the magnitude of the response to the intake of identical doses of pathogens. The number of ill people estimated is greater in the case of the group from geriatric nursing homes, nurseries, schools and hospitals (GNSH) than in bars and restaurants (BR), both for the \textit{Salmonella} serotypes studied and \textit{L. monocytogenes}. It should be remembered that the estimates by the present this Risk Assessment are based on equal population sizes and equal doses in both populations (GNSH and BR). Therefore, with respect to the size of the population, if higher number of people had been considered in GNSH than in BR, the differences in the number of ill people between the two populations would have been greater.

On the other hand, the effect of the dose varies according to the foodborne pathogen considered (\textit{Salmonella} or \textit{Listeria monocytogenes}). Thus, different doses of \textit{Salmonella} in GNSH or in BR would not produce a significant difference in the number of ill people in both populations. However, this is not the case for \textit{Listeria monocytogenes}, in which only doses above $10^4$ cfu/serving would produce an increase in the number of ill people in GNSH, population considered 100% immunocompromised.

### Table 8. Mean, minimum, maximum values, and 95th percentile of the final distributions of the number of ill people by \textit{Listeria monocytogenes} in BR and GNSH assuming ingested doses of $1 - 10^8$ cfu/serving

<table>
<thead>
<tr>
<th>Dose (cfu/serving)</th>
<th>Establishment</th>
<th>Number of ill people</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BR</td>
<td>Minimum</td>
</tr>
<tr>
<td>$10^4$</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>0</td>
</tr>
<tr>
<td>$10^5$</td>
<td>BR</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>0.001</td>
</tr>
<tr>
<td>$10^6$</td>
<td>BR</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>0.005</td>
</tr>
<tr>
<td>$10^7$</td>
<td>BR</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>0.059</td>
</tr>
<tr>
<td>$10^8$</td>
<td>BR</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GNSH</td>
<td>0.585</td>
</tr>
</tbody>
</table>
References


FDA (2003). Food and Drug Administration. Quantitative assessment of relative risk to public health from


Real Decreto 1376/2003, de 7 de noviembre, por el que se establecen las condiciones sanitarias de producción, almacenamiento y comercialización de las carnes frescas y sus derivados en los establecimientos de comercio al por menor. BOE 273 de 14 de noviembre de 2003, pp: 40094-40101.

Real Decreto 728/2011, de 20 de mayo, por el que se modifica el Real Decreto 1376/2003, de 7 de noviembre, por el que se establecen las condiciones sanitarias de produccion, almacenamiento y comercialización de las carnes frescas y sus derivados en los establecimientos de comercio al por menor. BOE 131, de 2 de junio de 2011, pp: 54461-54462.


Risk assessment of the consumption of fresh meat derivatives by certain population groups with respect to the modification of Royal Decree 1376/2003


