Qualitative and quantitative risk assessment for human salmonellosis due to multi-resistant Salmonella Typhimurium DT104 from consumption of Danish dry-cured pork sausages

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Received 5 April 2001; accepted 16 September 2001

Abstract

Salmonella Typhimurium DT104 (DT104) is unwanted in products for human consumption due to its antibiotic resistance and ability to cause disease. We intended to set up an improved monitoring and management program to aid in deciding when to use pork contaminated with DT104 for production of sausages without jeopardizing consumer safety. We started by carrying out two assessments of the risk for human health associated with consumption of sausages produced by: (1) Danish pork from average slaughter days; (2) imported pork (IMP) with average prevalence of DT104. The assessments showed that, if Salmonella is present, it is usually in lower numbers (\leq 50 per 400 cm\textsuperscript{2} surface). Additionally, during processing, the numbers will be reduced by at least 2 log-units. In Danish (DK) pork, DT104 constitutes 0.2–1.0\% of the Salmonella isolates reported, while in imported pork (IMP), 18\%. We estimated that out of one million, 25 g servings of DK dry-cured sausages, up to two DT104 bacteria could be found in each of 245 servings. Out of one million servings of 25 g IMP dry-cured sausages, up to two DT104 bacteria would occur in each of 19,260 servings. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Risk assessment; Salmonella Typhimurium DT104; Meat; Sausages; Processing; Monte Carlo simulation

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1. Introduction

*Salmonella* Typhimurium DT104 (DT104) is typically a multi-resistant strain of *Salmonella*, and is unwanted in ready-to-eat products because of the risk for human health. In 1996, DT104 was isolated for the first time in Danish livestock. Between 1996 and 2000, 95 swine or cattle herds were found positive for DT104 (Anon., 2000a). Recently, DT104 was also observed in Danish poultry (www.foedevaredirektoratet.dk/kontrolinfo). Despite depopulation of affected swine herds, it has not been possible to stop the dissemination of the bacteria. It is assumed that DT104 nowadays is sub-clinically present in a considerable proportion of Danish animals, and that eradication is impossible (Anon., 2000a). Therefore, the Danish DT104 policy is currently under reconsideration to optimise resource allocation without jeopardising consumer safety (Anon., 2000a). Additionally, imported pork can contain DT104 in larger amounts, because only the Scandinavian countries have national surveillance strategies to decrease the prevalence of *Salmonella*.

According to current Danish law, meat with DT104 (assumed prevalence ≥ 0.01%) has to be heat treated but in case of sporadic findings of DT104 (assumed prevalence < 0.01%), the meat may go directly to the consumer (Anon., 2000a). However, no monitoring is able to identify all meat contaminated with DT104. Therefore, we were interested in setting up a general monitoring and management program which would include an indication when pork could be used for sausage production as a way to deal with DT104. To get a scientific basis for this, we assessed whether dry-cured sausages produced by pork with DT104 could jeopardise consumer safety. One assessment was made for dry-cured sausages produced using Danish pork and one was made for sausages produced using imported pork.

2. Material and methods

The guidelines for conducting risk assessment described by Codex Alimentarius (Anon., 1999) were followed as closely as possible. However, data were not available for all items (e.g., dose-response for development of disease when consuming DT104). Initially, a qualitative risk assessment was carried out; and next, a quantitative risk assessment modelling the prevalence of DT104 in servings of dry-cured sausages (as data of sufficient quality were available).

2.1. Hazard identification

*S. Typhimurium* is unwanted in foods because it causes disease in man. The phage type DT104 is even more unwanted because of its antibiotic resistance (potentially aggravating the course of disease in people).

DT104 was observed for the first time in 1984 in the UK (Threlfall et al., 1996). An English study showed that DT104 caused a more-severe disease than usually observed in infections with *S. Typhimurium* (Wall et al., 1994). Other studies from the USA and UK have not been able to confirm this (Davies et al., 1996; Hosek et al., 1997). DT104 does not seem to be more invasive than other types of *S. Typhimurium* (ILSI, 2000). Most *Salmonella* infections are self-limiting, and according to The National Central Laboratory
of the Danish Health System, treatment failures due to the antibiotic resistance have not been noticed. Reduced treatment efficacy can be expected in the cases of mono-therapy with, e.g. ampicillin, or if the bacteria have reduced sensitivity to quinolones, aminoglycosides, or cephalosporines (Anon., 2000a).

In Denmark, S. Enteritidis was the most common cause of salmonellosis in 1999. The second most common cause was S. Typhimurium, of which one-quarter was DT104. In 1999, 3268 cases of human salmonellosis were reported, and perhaps 300 of these were related to pork (Anon., 2000b). During the years 1997–1999, a relatively constant number (around 40 per year) of sporadic cases of human DT104 with unknown cause have been reported. Some of these cases may have been caused by imported food, in particular, pork and poultry products (Anon., 2000a). Apart from the sporadic cases, three minor outbreaks have been observed: one related to a private slaughterhouse (Mølbak et al., 1999), the second to a restaurant (cross-contamination most likely with imported pork), and the third to a butcher (isolated from roast beef) (Anon., 2000b).

2.2. Exposure assessment

Risk factors for acquiring DT104 include consumption of contaminated food like poultry, pork, and beef, as well as contact with diseased production animals and pets (Wall et al., 1994; Davies et al., 1996). In the present assessment, we focused on pork and excluded the live pig. We looked on the prevalence of Salmonella and specifically DT104, as well as the possibility of growth in pork and dry-cured sausages. Cross-contamination occurring at the consumer level was not addressed.

2.2.1. Prevalence in pork

Since 1995, Denmark has conducted a national Salmonella surveillance and control program in swineherds producing >100 finishers per year. Basically, it consists of strategies within feed mills, breeding and multiplier herds, piglet-producing herds, and finisher herds. Finisher herds are surveyed serologically. Every month, finisher herds are assigned to one of three levels according to the seroprevalence during the previous 3 months (Mousing et al., 1997). Among approximately 16,000 finisher herds surveyed in Denmark in 1999, 1368 herds had an increased antibody seroprevalence level. These herds subsequently were enrolled in a bacteriologic follow-up, and DT104 was identified in 11 of the 1368 herds (Anon., 2000b). However, in year 2000, DT104 was found in 43 herds (Anon., 2001).

Since, 1 January 1998, approximately 25,000 samples of Danish pork have been analysed yearly in the national surveillance of fresh pork. Additionally, 37,000 pooled export samples (each pool consists of five samples) have been examined per year. Salmonella was isolated in approximately 1% of the samples (Anon., 2000b), and DT104 in 0.002% of the samples. If each of these positive pooled samples consisted of not just one but five positive samples, a maximum observed prevalence of DT104 would have been 0.01% (Olsen, 2001, pers. commun.). Data from two other projects—with an over-representation of herds with higher prevalence of Salmonella (Sørensen et al., 2000; Olsen, 2001)—demonstrate that when Salmonella is present, most commonly it is in lower numbers (≤50 per 400 cm² surface) (Table 1). Finally, none of 3500 carcasses from slaughter days in which a known DT104 herd delivered finishers was positive for DT104.
In July 1998, surveillance was initiated for DT104 in imported fresh or frozen pork (Anon., 1998). Up to the end of 1999, 1104 samples have been tested; 7.7% were positive for Salmonella, and 1.4% for DT104 (www.foedevaredirektoratet.dk/kontrolinfo) (18% of the Salmonella isolates). Preliminary data for year 2000 are in line with the previous data: Salmonella was present in 140 out of 629 (21%) examined batches, and DT104 in 20 (3%).

In conclusion, DT104 seldom is found in Danish finisher herds; however, lately there has been an increase in the prevalence. There is a sporadic prevalence of DT104 in Danish pork, whereas imported pork—compared to Danish pork—has a higher prevalence of Salmonella (and specifically of DT104) so far. When DT104 is present, it is usually in lower numbers (≤50/400 cm²) (Table 2), even on carcasses originating from herds with the highest Salmonella seroprevalence. This is expected to be the case for imported pork, too.

2.2.2. Possibilities of growth in pork

Inadequate cooling as well as storage and transportation of pork at temperatures >7 °C can cause growth of Salmonella (ICMSF, 1998).

2.2.3. Prevalence in dry-cured sausages

Danish dry-cured sausages are produced with starter culture (bacteria) or glucono-δ-lacton (GDL) (chemical) to control the pH decline. The process is initiated at a temperature of 20–24 °C and 90–95% relative humidity. At the end of processing, most of the products contain 10–14% salt in water, and usually, 20–25% of the weight is reduced by drying. Final pH is approximately 4.8–5.0. Because, no heat treatment is involved in the production, there is a risk that Salmonella might be present in the final product.

During 1998–2000, the Danish authorities have surveyed retail outlets and analysed 960 samples of pork sausages either in their routine surveillance or screening; Salmonella

<table>
<thead>
<tr>
<th>No. of Salmonella bacteria</th>
<th>Salmonella prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Input data: distribution of positive samples</td>
</tr>
<tr>
<td></td>
<td>1 g of caecal content</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>500</td>
<td>12</td>
</tr>
<tr>
<td>5000</td>
<td>25b</td>
</tr>
<tr>
<td>50000</td>
<td>25b</td>
</tr>
</tbody>
</table>

a Only two dilutions were analysed; the highest titres were >50 (Olsen et al., 2000).
b Only three dilutions were analysed; the highest titres were >500 (Sørensen et al., 2000).
Table 2
Description of input data used for simulating the number of DT104 in Danish dry-cured sausages

<table>
<thead>
<tr>
<th>Variable</th>
<th>Input data</th>
<th>Derived distribution</th>
<th>Origin of information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of <em>Salmonella</em> in Danish pork</td>
<td>1.0%: all pork; 1.6%: sausage pork; 5105 samples analysed: 83 <em>Salmonella</em>-positive slaughterhouse variation</td>
<td>Pert distribution: minimum = 0.0%; mode = 0.5%; maximum = 8.0%</td>
<td>Routine surveillance data from the Danish slaughterhouses</td>
</tr>
<tr>
<td>Prevalence of <em>Salmonella</em> in imported pork</td>
<td>1104 samples analysed: 85 <em>Salmonella</em>-positive</td>
<td>Beta distribution: $r = 85; n = 1104; p = 7.7%</td>
<td>Import control data, please see: <a href="http://www.foedevaredirektoratet.dk/kontrolinfo">www.foedevaredirektoratet.dk/kontrolinfo</a></td>
</tr>
<tr>
<td>Size of sausage production</td>
<td>Usually: many small pieces of pork primarily from the fore-end</td>
<td>Fixed distributions 960 pieces of 250 g</td>
<td>Expert opinion from establishments producing sausages</td>
</tr>
<tr>
<td>Pathogen reduction associated with sausage production</td>
<td>minimum = 1.8 log-units; maximum = 5.3 log-units</td>
<td>Pert distribution: minimum = 2.0 log-units; mode = 2.2 log-units; maximum = 3.0 log-units</td>
<td>Data from pilot experiments</td>
</tr>
<tr>
<td>Relative distribution of DT104 compared to <em>Salmonella</em></td>
<td>0.2–1.0%, Danish pork; 18.0%, imported pork</td>
<td>Fixed distributions: 1%, Danish; 18%, imported</td>
<td>Routine surveillance data from the Danish slaughterhouses and import control data</td>
</tr>
<tr>
<td>Serving sizes (g)</td>
<td>Child: 10; adult: 25; party: 40</td>
<td></td>
<td>Expert opinion</td>
</tr>
<tr>
<td>Prevalence of <em>Salmonella</em> in sausages (used for validation of model results)</td>
<td>1618 negative samples in manufacturers’ own check programme; 300 negative samples from sausage production with DT104 pork; positive samples have been found in a retail outlet only once, and here, cross-contamination could not be ruled out (960 samples were analysed)</td>
<td></td>
<td>Routine surveillance data from the Danish slaughterhouses and retail outlets</td>
</tr>
</tbody>
</table>
was isolated only once, and here, cross-contamination in the shop could not be ruled out (The Danish Zoonosis Center’s database). Likewise, 1618 samples were negative in the manufacturers’ own check program. Moreover, two production runs of dry-cured sausages made out of pork that tested positive for DT104 were investigated. All 300 samples, each of 100 g, were *Salmonella* negative (pers. commun. Olsen et al., 2000). Additionally, a survey of salted and smoked ready-to-eat pork products was carried out during 1998 and 1999. In total, 545 samples were cultured and none was positive for *Salmonella* (www.fdir.dk/publikationer/publikationer/publikationer/mad&mikro5/kap3.html).

Insufficient fermentation has been associated with the presence of *Salmonella* in sausages (ICMSF, 1998). Outbreaks due to contaminated sausages have been reported from the USA, where *S. Typhimurium* was isolated from semi-dry fermented beef-sausage products. This was probably a result of a very high prevalence in the raw meat or due to processing failures (Sauer et al., 1997). In Italy, *Salmonella* has been isolated from sausages too—primarily in fresh pork sausages, but also in dry-cured (Prencipe et al., 2000). These products had gone through either a natural fermentation or no fermentation at all (fresh sausages). Hence, the reported outbreaks have probably been caused by a high *Salmonella* burden of the raw meat and/or processing failures—perhaps improper acidification and inadequate addition of e.g. smoke, NO₂, and salt.

2.2.4. **Possibilities of growth in dry-cured sausages**

Due to the lack of available data specifically for *Salmonella*, data for *E. coli* have been used to describe the pathogen reduction associated with dry-cured sausage processing. According to Ihnot et al. (1998), DT104 is more susceptible to conditions in well-controlled sausage production and subsequent storage than *E. coli*. Likewise, *S. Kentucky* and *S. Typhimurium* are more susceptible to well-controlled conditions in the production of dry-cured sausages than *E. coli* O157 (Nissen and Holck, 1998; Ellajosyula et al., 1998), and *S. Typhimurium* is more susceptible to acid than *E. coli* O157 (Stiebing et al., 2000). It was claimed recently that DT104 might be slightly more tolerant to acid and heat than any other types of *Salmonella*, but the difference was not important on a practical level (ILSI, 2000). In conclusion, we judged it was reasonable (i.e. conservative) to use data for *E. coli* to describe the *Salmonella* reduction associated with production of dry-cured sausages.

The Danish Meat Research Institute (DMRI) has investigated the survival of non-pathogens (*E. coli* O26, O111, and O157) in fermented sausages and GdL-acidified sausages (Dalsgaard, 1998). Drying and smoking reduced the number of such *E. coli* by 2.0 log-units, and after 16 weeks of chill storage, the total reduction was 3.3 log-units. Other investigations from DMRI demonstrated a 1.8–2.8 log-units reduction in the number of *E. coli* at different product parameters and with 18% weight loss, whereas 30% weight loss resulted in 2.8–4.5 log-units reduction (Table 3) (Lammert and Frøstrup, 2000). In conclusion, around 2–3 log-units reduction of *Salmonella* can be expected during traditional Danish pork dry-cured sausage production.

2.2.5. **Consumer habits**

In Denmark, sausages usually are consumed at lunchtime; less commonly they form part of a dinner (a so-called “sausage table”). At lunch, approximately 10 g are consumed as a
child serving, 25 g as an adult serving, and 40 g as a party serving. At a sausage table, the total consumption is not only higher, but it also consists of a variety of sausage types.

2.3. Human exposure

The qualitative assessment indicates that use of Danish pork (where DT104 is occasionally present) for sausage production does not represent an unacceptable risk for human health (not even for high risk groups), because DT104 will only seldom be present—and then in low numbers, for three reasons:

1. *Salmonella* has low prevalence (qualitatively and quantitatively) in pork.
2. DT104 only constitutes a limited proportion of the *Salmonella* isolates.
3. During production of sausages, any *Salmonella* will be reduced by around 2–3 log-units.
4. *Salmonella* has never been found in producers’ own control samples of dry-cured sausages.
5. Only once has DT104 been found in a retail outlet, and cross-contamination was strongly suspected.

Imported pork has higher *Salmonella* prevalence (95% CI of beta distribution: 6.5–9.1%) and DT104 is a larger proportion of the isolates, compared to Danish pork (95% CI of binomial distribution: 1.2–2.0%). Nevertheless, in Denmark, DT104 have never been found in any sausage produced from either Danish or imported pork. However, DT104 probably will occur occasionally in negligible numbers due to the low background prevalence in pork.

2.4. Quantitative risk assessment

2.4.1. Simulation with @Risk

The software program @Risk (ver. 4.0 Palisade Corporation, Newfield, NY) was used to simulate the number of *Salmonella* in dry-cured sausages. Monte Carlo sampling with

<table>
<thead>
<tr>
<th>Origin of pork</th>
<th>Serving size (g)</th>
<th>1 DT104 bacteria</th>
<th>2 DT104 bacteria</th>
<th>3 DT104 bacteria</th>
<th>4 DT104 bacteria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danish</td>
<td>10</td>
<td>103</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>103</td>
</tr>
<tr>
<td>Danish</td>
<td>25</td>
<td>235</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>245</td>
</tr>
<tr>
<td>Danish</td>
<td>40</td>
<td>355</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>370</td>
</tr>
<tr>
<td>Imported</td>
<td>10</td>
<td>7866</td>
<td>270</td>
<td>18</td>
<td>0</td>
<td>8154</td>
</tr>
<tr>
<td>Imported</td>
<td>25</td>
<td>18216</td>
<td>1044</td>
<td>90</td>
<td>0</td>
<td>19350</td>
</tr>
<tr>
<td>Imported</td>
<td>40</td>
<td>25884</td>
<td>2952</td>
<td>324</td>
<td>54</td>
<td>29214</td>
</tr>
</tbody>
</table>

*a* We assumed that DT104 constitutes 1% of the *Salmonella* isolates identified in Danish pork, and 18% of the isolates identified in imported pork.
10,000 simulations was used. Additionally, @Risk was used to estimate the number of human cases due to consumption of contaminated dry-cured sausages.

2.4.2. Number of meat pieces in a batch

Batch sizes in Denmark range from 200 to 250 kg, and meat cuts and pieces of lard for sausage production weight of 150–350 g. For the analysis, we used 240 kg as a fixed batch size, and 250 g as a fixed weight for a piece of meat or fat. One simulated batch was composed of 960 pieces of meat and fat (hereafter, the term “meat” is used for both types of ingredients) (Table 2).

2.4.3. Qualitative prevalence of Salmonella

Data on DT104 specifically are sparse. Therefore, to describe the prevalence (qualitatively and quantitatively), we used data for Salmonella in general. Because different types of meat might be used (and the Salmonella prevalence differs accordingly), we used data from the Danish pork surveillance of the group which supplies most of the cheap sausage pork. In 1999, 5105 samples were taken from this category of meat and 83 (1.6%) were positive for Salmonella (Table 2). This prevalence is 1 year average including several slaughterhouses. We noted that the variation between slaughterhouses could be described by a pert distribution with minimum 0%, mode 0.5%, and maximum 8% (Table 2). For imported pork, a beta distribution was used (n = 1104 samples analysed; r = 85 positive for Salmonella; average prevalence = 7.7%) (Table 2). No data on slaughterhouse variation for imported pork was available.

2.4.4. Quantitative prevalence of Salmonella

Quantitative data were not available for sausage meat. Instead, data from two other projects were used to assess the quantitative occurrence of Salmonella on a piece of meat (Olsen, 2001; Sørensen et al., 2000) (Table 1). The first project, the head project, only covered herds with the highest seroprevalences (Level 3 herds). Results were based on swabbing 400 cm$^2$ of the outer surface of the fore-end, and the positive samples were only analysed two dilutions; the highest titres were >50 (Table 1). When cutting up a carcass, bacteria from the outer surface may be spread to other parts of the carcass. In the risk analysis, we assumed that the bacterial load of one piece of meat was equal to the total load of 400 cm$^2$ swabbed outer surface. In the second project (the Levels 1–2–3-investigation), herds with higher seroprevalences were over-represented (Levels 2 and 3). The number of Salmonella per gram caecal content was determined, and the positive samples were analysed for only three dilutions; the highest titres were >500 (Table 1). During slaughter, splashes of faeces may hit the carcass. We assumed that one piece of meat could harbour approximately the same number of Salmonella as 1 g of caecal content.

Based on this information and the fact that approximately 1.6% of the samples of sausage meat were positive, a discrete distribution was derived for a piece of Danish meat of 250 g (Table 1). This distribution was combined with the distribution describing slaughterhouse variation. We assumed that the quantitative distribution of positive meat pieces of imported pork corresponds to Danish meat (Table 1). A random sampling was simulated for 960 meat pieces, each with the before-mentioned probability of harbouring Salmonella. The total number of Salmonella occurring in one batch was calculated.
as the sum of *Salmonella* on all 960 meat pieces for Danish and imported pork, respectively.

2.4.5. *Pathogen reduction and number of Salmonella per serving*

The pathogen reduction associated with dry-cured sausage processing was included in the model. A pert distribution was used with minimum 1/100 (2 log-units), mode 1/150 (2.18 log-units), and maximum 1/1000 (3 log-units), corresponding to the log reductions obtained in pilot experiments (Dalsgaard, 1998; Lammert and Frøstrup, 2000) (Table 2). The number of *Salmonella* was calculated per gram and per 10, 25, or 40 g (representing servings for a child, an adult, or a party) (Table 2). A Poisson distribution was used to describe how often a specific number of bacteria could be expected (prevalence of bacteria is a discrete event).

2.4.6. *Transformation into annual number of servings with DT104 in Denmark*

The results were transformed into number of servings containing DT104 per one million servings. We assumed that in Danish pork, DT104 constitutes 1.0% of the identified *Salmonella* isolates (surveillance results indicate 0.2%), and for imported pork 18% (same as surveillance results). Based on the annual consumption (4500 t) of Danish sausages in Denmark, the number of servings with DT104 was calculated.

2.4.7. *Number of potential human cases of diarrhoea due to contaminated sausages*

The annual number of potential human cases due to consumption of dry-cured sausages made of Danish pork was estimated. A binomial distribution was used, where the annual number of average servings with DT104 equalled the number of trials. The risk of a positive outcome was determined by the attack risk, i.e. the number of cases in proportion to the number of exposed persons. Data were used from a German outbreak caused by potato chips contaminated with low numbers of *Salmonella* (where *S. Saintpaul*, *S. Rubislaw*, *S. Javiana* were isolated); that attack risk was 1/10,000 (Lehmacher et al., 1995).

2.4.8. *Scenario analyses*

A scenario analysis was carried out to illustrate the effect of a lower pathogen reduction (pert distribution with minimum 1/10, mode 1/150, and maximum 1/1000). Similarly, a scenario analysis was carried out assuming a 10 times higher prevalence than in normal Danish pork. This analysis also served as a scenario analysis for imported pork (assuming a *Salmonella* prevalence twice as high as in normal imported pork).

3. Results

3.1. *Quantitative risk assessment*

3.1.1. *Number of Salmonella per gram sausage and per serving*

The simulations predicted that on an average, 0.0010 (90% CI: 1.32 × 10⁻⁷–3.56 × 10⁻³) and 0.005 (95% CI: 1.18 × 10⁻³–1.05 × 10⁻²) *Salmonella* bacteria would be present in 1 g dry-cured sausage made by Danish (DK) and imported pork (IMP), respectively.
We assumed that the bacteria were distributed evenly due to the chopping and extended stirring. This implies that the vast majority of servings will not harbour *Salmonella* bacteria. For DK dry-cured sausages, a maximum of two *Salmonella* bacteria could be expected in a serving (100th percentile: 2). In comparison, a serving of IMP dry-cured sausages would harbour a maximum of four *Salmonella* bacteria (100th percentile: 4) (Table 3). In conclusion, servings of sausage made of imported pork may harbour a slightly higher number of *Salmonella* than sausages made of Danish pork. Table 3 presents the estimated number of servings with DT104 per one million servings of 10, 25, or 40 g. It is noted, that DT104 occurs more often in IMP sausages compared to DK sausages. If all sausages produced by Danish pork were consumed as dry-cured sausages, there would be 43,875 average servings with one (or less often two) DT104 bacteria out of the 180 million servings consumed.

3.1.2. Scenario analyses

If the pathogen reduction was lower than expected, the average number of *Salmonella* bacteria per gram would increase to 0.0015 (90% CI: $1.59 \times 10^{-7}$–$5.59 \times 10^{-7}$), and at maximum two *Salmonella* bacteria would occur in a serving of 10 g (100th percentile: 2), and five in a serving of 25 or 40 g (100th percentile: 5). If the *Salmonella* burden in raw meat was 10 times higher than usual in Danish pork, the average number of *Salmonella* per gram would increase to 0.01 (90% CI: $4.60 \times 10^{-4}$–$2.96 \times 10^{-2}$), and at maximum three *Salmonella* would occur in a serving of 10 g (100th percentile: 3), five in a serving of 25 g (100th percentile: 5), and seven in a serving of 40 g (100th percentile: 7).

![Fig. 1](image_url). Predicted distribution of potential number of annual cases of diarrhoea in humans in Denmark due to consumption of dry-cured sausages made of Danish pork with possible presence of DT104—if one or two DT104 bacteria can produce disease in man.
3.2. Number of potential cases of diarrhoea if one or two DT104 bacteria were capable of producing disease

The distribution of the potential number of cases is shown as the number of years out of 100 in which the indicated number of cases would be observed (Fig. 1). Most commonly, four cases per year and a maximum of 10 cases per year can be expected.

4. Discussion

4.1. Quantitative risk assessment

The model assumed that 960 meat pieces were included in a batch of normal sausage production. However, in recipes for more-expensive types of sausages, the main ingredient is entire fore-ends. These types of sausages therefore consist of fewer and larger meat pieces. Hence, the Salmonella burden may be lower in the expensive sausage products than in the cheap ones, due to the smaller exposed surface area. Therefore, we chose the worst case with the highest burden of Salmonella. The distribution of the quantitative content of Salmonella on a piece of meat was derived from two other projects. Discussions with experts in the field assured that the Salmonella burden was not underestimated. To the contrary, the experts stated that the distribution used might have overestimated the prevalence. However, we believe that this accounts for a single day with an even-higher Salmonella burden than usual. In case new and more suitable data become available, these will be incorporated into the model.

We used data for E. coli to describe the Salmonella reduction associated with sausage processing. According to literature and discussions with experts, this was reasonable and even conservative.

We assumed that the Salmonella bacteria were evenly distributed due to the chopping and extended stirring. Although, clustering can happen, no data were available—so, it was not modelled. The Poisson distribution takes some clustering into account. However, the maximum number of Salmonella occurring in a serving might still be somewhat higher than the 100th percentile estimated by the model. On the other hand, the above-mentioned conservative estimates for the prevalence of Salmonella, and Salmonella reduction counteract this possibility.

When carrying out scenario analyses, plausible scenarios should be modelled—ones which could occur without being noted by the producers’ own routine control check. (Extreme scenarios will be caught by routine control and are not of interest.) We modelled the effect of a lower pathogen reduction, allowing for a minimum of 1 log reduction instead of a minimum of 2 log-units. We do not believe an even-lower reduction will take place without being noted. We also modelled the effect of a Salmonella burden 10 times as high as in the main model of Danish pork. This scenario is likely to occur (e.g. due to use of imported pork, which based on past data has a higher prevalence of Salmonella). Both scenario analyses indicated that the results would not change substantially—our results are robust.

When estimating the annual number of servings of sausages made by Danish pork containing DT104 consumed in Denmark, we assumed that the relative relationship...
between DT104 and all other types of *Salmonella* in Danish pork is 1%. However, surveillance data from 1999 to October 2000 have shown that in fact, DT104 only constituted 0.2–1.0% of the *Salmonella* identified in Danish pork (depending upon whether more samples in a pool of five are positive). Hence, the estimates we used are conservative.

Simulations provide results as distributions; depending on the specific circumstances, minimum, average or maximum values are of interest. For the number of *Salmonella* bacteria occurring in a serving of dry-cured sausages, we focused on the 100th percentile for the Poisson distribution because this number gave the maximum number to expect in a serving. We interpret this maximum number as the number expected to occur if pork with “low” prevalence of DT104 is used. The simulations predicted that the vast majority of servings do not harbour any *Salmonella* and if *Salmonella* is present, it is in numbers probably below the detection limit. This is confirmed by data from the manufacturers’ own check program, retail outlet investigations, and productions runs where DT104 pork has been used (Table 2).

### 4.2. Number of potential cases if a few DT104 is enough to produce disease

The most difficult question is how to interpret the results of the analysis: can a person get ill from consuming only one or two DT104 in a serving? No data exist describing the dose-minimum for DT104; however, in general, the higher the dose, the higher the attack risk (Pegues et al., 1995). Data from an outbreak in Germany had 4–45 *Salmonella* bacteria in a serving (0.04–0.45 g–1). Our modelling of DK dry-cured sausages showed a maximum of one or two *Salmonella* in a serving (on average 0.0010 *Salmonella* per gram) 40–450 times lower than observed in the German outbreak. For IMP dry-cured sausages, there would be 8–90 times fewer *Salmonella* organisms than in the German outbreak. The attack risk we used (1/10,000) might be too high. On the other hand, DT104 might have a higher attack risk than other serovars of *Salmonella* due to its antibiotic resistance (consider people who currently are under antibiotic treatment, e.g. patients with lack of gastric acidity, neonates, patients with achlorhydria, gastric surgery) (Pegues et al., 1995).

Usually, substantially higher numbers of *Salmonella* are required to produce disease in man (Blaser and Newman, 1982; Pegues et al., 1995; Kothary and Babu, 2001). However, others have reported disease from consumption of low numbers of *Salmonella*, e.g. in ice (Hennessy et al., 1996) or chocolate (Kapperud et al., 1990). Unfortunately, in those papers, attack risks were not estimated. In the ice outbreak, the authors stated that an average of 0.093 *Salmonella* were present per gram. This is nine times higher than what our model for Danish pork predicted, and twice as much as the model prediction for imported pork. The scenario analysis which assumed a 10 times higher DK pork *Salmonella* prevalence (approximately, twice the prevalence in imported pork) had a distribution where the maximum number of *Salmonella* was closer to the numbers observed in the reported outbreaks. For the chocolate outbreak, in about 90% of the positive samples, ≤10 *Salmonella* were present per 100 g. However, those authors did not discuss whether the samples with ≤10 *Salmonella* were in fact infective.

The predicted annual number of cases due to consumption of Danish sausages was that typically there would be four cases. According to the National Central Laboratory of
the Danish Health System, around 40 sporadic human DT104 cases with unknown source of infection are reported annually. If it is assumed that one or two DT104 bacteria were capable of causing disease, then four out of the observed 40 cases will be due to consumption of sausages. The question is whether this is realistic. The National Central Laboratory has judged that some of these cases are attributable to consumption of imported pork and poultry; these products are known for having substantially higher risk of harbouring *Salmonella*—and DT104, in particular, than similar Danish products. To sum up, it is not clear whether only a few DT104 is sufficient to produce disease in people.

4.3. Risk management

The results show that two main factors determine whether sausages represent a risk of DT104 for humans: the prevalence of DT104 in pork, and the second is the pathogen reduction associated with the production. Hence, risk management should be based on this.

5. Conclusions

Our assessment predicted the following.

- When *Salmonella* is present in raw pork, it is usually in low numbers.
- During processing, any *Salmonella* will be reduced by around 2 to 3 log-units.
- Dry-cured sausages produced by Danish pork or pork imported to Denmark will only seldom harbour remaining DT104 and if at all, only in low numbers (1–4 bacteria per serving).

It is not known whether these low numbers of DT104 in the sausage are capable of producing disease in man. We conclude that dry-cured sausages produced by pork with “low” prevalence of DT104 do not seem to constitute an unacceptable risk for humans. However, if pork with “high” prevalence of DT104 is used for production of dry-cured sausages, disease might occur. It is necessary to set limits between sporadic, low, and high prevalence. These limits are geographical, cultural and political dependent, and should be decided as a co-effort between the producers and the food-safety authorities.

Acknowledgements

We acknowledge Inger Guldbæk from Tulip, Marianne Fuglsang from 3-Stjernet, and Lene Lund Sørensen and Søren Andersen from The Danish Bacon and Meat Council, Tine Hald and Flemming Bager from the Danish Zoonosis Center, Kåre Moølbak from Statens Serum Institut, as well as Søren Aaboe and Helle Sommer from the Danish Food Administration for discussions about building the model and assessing the risk.
References


