Use of a slaughter hygiene indicator (Escherichia coli) to quantify the risk of human salmonellosis related to pork in Denmark – an approach for risk based meat control?

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Abstract
Food chain information does not per se allow an effective distinction of herds according to shedding of Salmonella. Thus, no effective sorting of pigs at slaughter according to Salmonella risk is possible and hygiene improvement is the only effective mitigating tool so far at the slaughter. From a large study of 1906 slaughter pigs we quantified Salmonella and established quantitative hygiene data (E. coli) on pig carcasses (paired data) at slaughter. Based on the results, we found a positive correlation between level of E. coli and the prevalence of Salmonella positive carcasses. The odds ratio for Salmonella being present on the carcass was found to increase by 1.87 for every one log₁₀ unit increase of E. coli found on the carcass. A simple Salmonella consumer risk model was constructed using the observed levels of E. coli contamination as input and the model, established a positive correlation between slaughter hygiene (E. coli) and consumer risk. Further, we analysed two years’ own control data on Salmonella (prevalence data) and E. coli (quantitative data) from four large slaughterhouses in Denmark and found a similar positive correlation between the E. coli level and the carcass prevalence of Salmonella. The aim of this study was to propose a principle for setting risk based hygiene targets on E. coli on carcasses at pig slaughter. As such we provide input to the discussion on how to develop a risk based meat control procedure, based on statistical process control.

Introduction
The Salmonella is widespread in the slaughter pig production in Europe, and Salmonella from pork constitutes a significant risk for consumers. In recent years the ability of the classical meat control to provide consumer protection against food borne pathogens, has been discussed. A consensus of basing the meat control on food chain information of the pathogens is emerging in Europe. Food chain information do however not per se allow for an effective distinction between pigs from Salmonella positive and negative herds, and improvement of the general slaughter hygiene is the only mitigating tool to use. A modernisation of the meat control could include a risk based statistical process control but so far there have been no reports describing how the hygiene level at slaughter associates to Salmonella risk. We have established quantitative hygiene data (E. coli) and quantified Salmonella on pig carcasses at from 2880 pigs slaughter. Moreover we have analysed two years own control data on E. coli and Salmonella from five large pig slaughter houses in in Denmark. The objective is to establish the correlation between the hygiene level and presence of Salmonella and to provide the first suggestion for a method to set risk based process hygiene criteria at pig slaughter.

Material and methods
Sample collection
1906 carcasses from pigs slaughtered at five large Danish pig slaughterhouses were sampled in the period May 2005 to June 2007. Carcass swabs (2800 cm2) from was taken just before cooling and analysed both quantitatively for E. coli and semi-quantitatively for Salmonella. A total of 75 ml peptone water was added to stomacher bags with carcass swabs containing approximately 12.5 ml of peptone water and tissue fluid. The sample was stomached and one millilitre of 10-fold dilutions were spread on Petrifilm and subsequently incubated at 41.5 °C for 23-25 h. The number of E. coli was determined using Select E. coli Count Plate Petrifilm (3M Microbiology, St. Paul, MN, USA) in accordance with the supplier’s instructions. Cell counts were determined by automated reading using a Petrifilm plate reader MI649 9 (3M Microbiology, St. Paul, MN, USA). From a the ten-fold dilution of the homogenate, a semi-quantitative analysis for Salmonella was performed. All stomached samples were analysed for Salmonella using MSRV agar (ISO 6579, Annex D, Anonymous, 2007). Own control data from the same five slaughter houses were obtained based on swabbing of 300 cm² mandatory for slaughter-houses exporting to USA. Each day one pooled sample of five swab samples were analysed for Salmonella and one sample per 1000 carcasses were analysed for E. coli with a range of 5-12 samples per day depending of slaughterhouse.
Statistical analyses

All statistical analyses were performed with the software R (ver. 2.15.1) and RStudio (ver. 0.96.331). Bacterial counts of *E. coli* were log$_{10}$-transformed to obtain approximately normally distributed data. Samples with *E. coli* below the detection limit of 1 CFU/ml were assigned a value of 0.5 CFU/ml to allow log$_{10}$ transformation.

Means and standard deviations were calculated for the log$_{10}$-transformed *E. coli* data. The corresponding *Salmonella* prevalence was calculated after dichotomisation of the results (0 = *Salmonella* negative; 1 = *Salmonella* positive). A box-and-whisker plot was made to illustrate the correlation between the concentration of *Salmonella* and *E. coli* found in swab samples.

To determine the association between *E. coli* and *Salmonella*, univariable analyses were carried out. Variables with $p \leq 0.25$ were included in a multivariable logistic regression analysis. Selection of explanatory variables for the final model was done by stepwise backwards elimination of the least significant variable until only significant variables remained. In the analysis, $p$-values lower or equal to 0.01 were considered as statistically different. The final explanatory variables were tested for interaction and confounding.

Risk model

The risk model takes into account both the prevalence of *Salmonella* on carcasses and the estimated number of *Salmonella* bacteria present. The number of *Salmonella* bacteria per cm$^2$ was estimated from the observed contamination of *E. coli* on the carcass and the established regression between number of *E. coli* and number of *Salmonella* bacteria on the carcass. A simple exposure model was developed assuming that: 1) the concentration of bacteria per cm$^2$ was even on the whole carcass 2) the whole carcass was consumed raw in 200 gram portions and 3) all 101 human illnesses associated to pork in 2006 in Denmark could be associated to this. Additionally, the risk model included three factors: a correction factor, which adjusted the dose-response relationship provided by FAO/WHO (2002); an underreporting factor (Havelaar et al., 2012) and a factor accounting for preparation of pork which adjusted the model output to the number of registered cases in Denmark in 2006.

Results

The average level of *E. coli* found on the skin of the carcasses was 0.8 log CFU/cm$^2$, from all five slaughterhouses. The corresponding prevalence of *Salmonella* was found to be 2.5%.

The odds ratio for *Salmonella* being present on the carcass was found to increase by 1.87 for every one log$_{10}$ unit increase of *E. coli*. The correspondence between *Salmonella* prevalence and *E. coli* carcass contamination across all 5 slaughterhouses is shown in Table 1.

The correlation between the concentration of *E. coli* and the concentration of *Salmonella* is depicted in Figure 1. By applying the observed *E. coli* and *Salmonella* data to the risk model, it was possible to make an estimate on the relationship between hygiene level measured by *E. coli* and the *Salmonella* consumer risk. Table 2 shows that the number of human cases could have been reduced by approx. 50% (from 101 to 48.6), if the *E. coli* level at slaughter had not exceeded 3-4 log$_{10}$ CFU per 38 cm$^2$.

The analysis of the own control data showed a positive correlation between the number of samples being positive for *E. coli* and a positive analysis for *Salmonella* (Table 3).

Discussion

Our project data showed that it is possible to associate a certain *Salmonella* prevalence and the *Salmonella* concentration to a certain hygiene indicator level (*E. coli*). From the outcome of the risk model, developed on the project data, a risk based
criteria of $3 \times 10^3 \text{ to } 3 \times 10^4 \text{ CFU per } 38 \text{ cm}^2$ could be suggested to significantly reduce the number of human illnesses. However, in order to be operational a risk based process hygiene criteria should be embedded into the own control data from the slaughterhouse. The own control data is few per slaughter day but analysed across a number of slaughter days and across all five slaughterhouses it was possible also to identify a positive correlation between *Salmonella* and level of hygiene. The observed relationship between *Salmonella* and *E. coli* in the own control data opens for the opportunity to set risk based process hygiene criteria at least under the conditions similar to the five slaughterhouses studied.

**Conclusion**
This is to our knowledge the first report, which estimates consumer risk of human salmonellosis from the hygiene level at pig slaughter. This holds the perspective to establish risk based process hygiene criteria based on this principle.

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**References**


**Figure 1** – Box-and-whisker plot of the level of *E. coli* stratified by the concentration of *Salmonella*. The letters represents the following concentration intervals: K < 0.10 CFU/ml, A: 0.10 – 0.91 CFU/ml, B: 0.91 – 10.1 CFU/ml, C: 10.1 – 101 CFU/ml, D: 101 – 909 CFU/ml, DD > 909 CFU/ml.

**Table 2** – Modelled estimation of the total number of human salmonellosis cases in Denmark in 2006 depending on the maximum level of *E. coli* on pig carcasses before cooling at slaughter.

<table>
<thead>
<tr>
<th>Maximal level of <em>E. coli</em> at carcass [log$_{10}$ CFU 38cm$^2$]</th>
<th>0-1</th>
<th>1-2</th>
<th>2-3</th>
<th>3-4</th>
<th>4-5</th>
<th>5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of cases</td>
<td>0.0</td>
<td>6.3</td>
<td>1.6</td>
<td>40.7</td>
<td>0.6</td>
<td>51.8</td>
</tr>
<tr>
<td>Accumulated no. cases</td>
<td>0.0</td>
<td>6.3</td>
<td>7.9</td>
<td>48.6</td>
<td>49.2</td>
<td>101.0</td>
</tr>
</tbody>
</table>

**Table 3** - Relationship between the number of days with a positive *Salmonella* analysis and the prevalence of *E. coli* positive swab samples per day of slaughter for a total of 1,839 days.

| Own control data | Prevalence of *E. coli* positive swab samples per day |
|---|---|---|---|---|---|---|---|
| | 0% | 0,1–25 % | 25,1–50% | 50,1–75% | 75,1–100% |
| *Salmonella* positive days | 9 | 20 | 17 | 2 | 1 |
| *Salmonella* negative days | 710 | 554 | 408 | 97 | 21 |
| *Salmonella* positive days [%] | 1.3 | 3.5 | 4.0 | 2.0 | 4.6 |
| % confidence interval | 0.57–2.36 | 2.14–5.33 | 2.35–6.33 | 0.25–7.11 | 0.12–22.84 |