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Escherichia coli as indicator of the human Salmonella risk caused by consumption of pork

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Introduction

Salmonella is widespread in the slaughter pig production in Europe, and *Salmonella* from pork constitutes a significant risk for consumers. At slaughter, food chain information do 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. So far there have been no reports describing how the hygiene level at slaughter associates to *Salmonella* risk.

We have collected quantitative hygiene data (*E. coli*) and quantified *Salmonella* on pig carcasses at slaughter. The objective is to establish the correlation between the hygiene level and *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

Carcasses from pigs slaughtered at five Danish pig slaughterhouses were sampled in the period 10 May 2005 to 5 June 2007. Sampling was performed at the slaughterline just before cooling. Carcass swabs (2800 cm^2) from 1,906 carcasses were 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. This mixture was stomached before dilution. 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 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).

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 in which *E. coli* was found to be below the detection limit (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* levels. 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 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 correlation between the concentration of *E. coli* and *Salmonella* is depicted in Figure 1.

The odds of *Salmonella* being present on the carcass were found to increase by 1.87 for every one log_{10}-unit increase of *E. coli*. The risk of *Salmonella* being present on the carcass varied between slaughterhouses.

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 1 show 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 32 cm^2.

Conclusion

This is to our knowledge the first report on estimating consumer risk of salmonellosis from the hygiene level at pig slaughter. The perspective is the ability to establish risk based process hygiene criteria based on this principle.

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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 (the unit 'CFU/ml' corresponds to 'CFU/32cm^2').

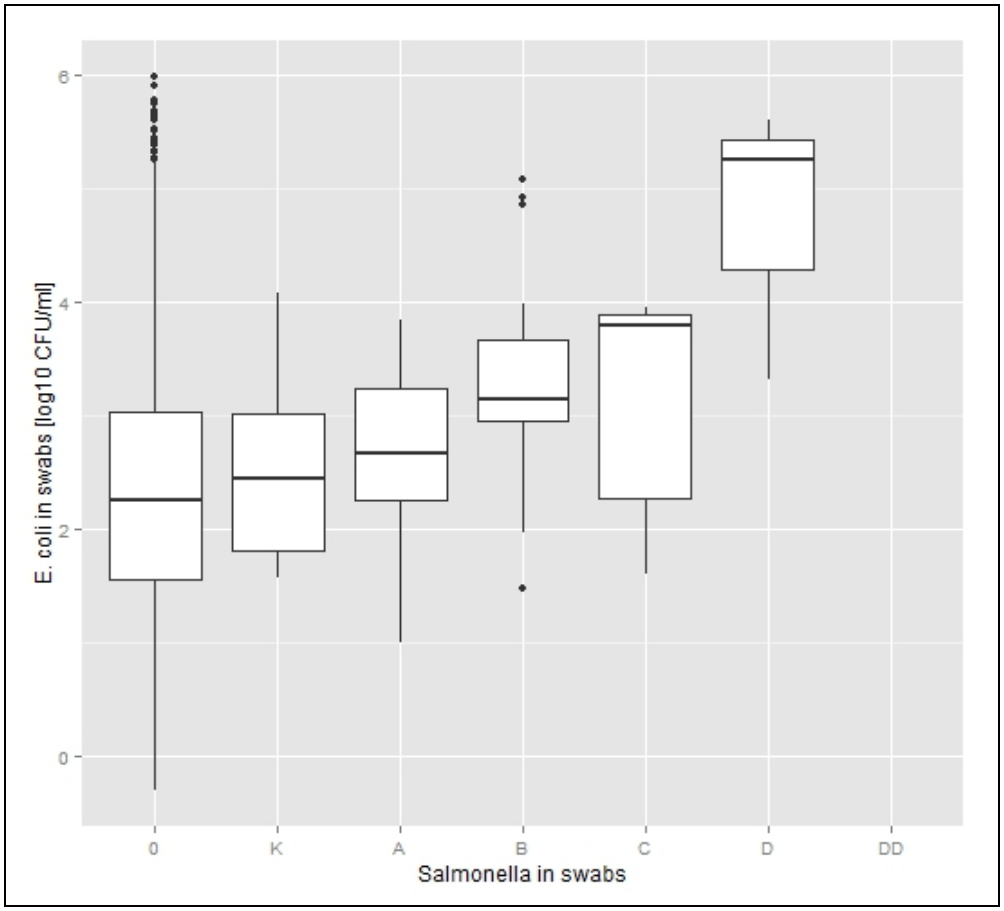

Table 1 – Modelled estimation of the total number of human salmonellosis cases in Denmark in 2006 depending on the maximum level of E. coli on pig carcasses at slaughter.

	Maximal level of *E. coli* on carcass [log_{10} CFU/32 cm^2]					
	0 - 1	**1 - 2**	**2 - 3**	**3 - 4**	**4 - 5**	**5 - 6**
No. of cases	0.0	6.3	1.6	40.7	0.6	51.8
Accumulated no. of cases	0.0	6.3	7.9	48.6	49.2	101.0