Prevalence of *Salmonella* in caecal contents of slaughter pigs in Ireland as estimated from meat juice serology data

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Background

- Part of a bigger project assessing the risk factors contributing to the occurrence of *Salmonella* on pork in Ireland.
  - On-farm
  - Slaughterhouse
- Risk assessment models are being built for both stages.
Why the interest in *Salmonella* in pork?

- Pork constitute the source of approximately 5-30% of the cases of human salmonellosis in industrialised countries.

- While considerable redistribution of *Salmonella* occurs during the various slaughter processes, the primary source of *Salmonella* contamination resides in the *Salmonella*-positive pig.
...Background

- *Salmonella* detection in pigs/carcasses can be done:
  - Standard culture and isolation:
    - Faeces, caecum, mesenteric lymph nodes, etc.
  - ELISA serology:
    - Antibodies in meat juice, blood serum.
  - Conventional culture is labour-intensive, time-consuming and expensive, although they provide the best indication of *Salmonella* presence.
...Background

- Serological tests are more convenient and effective for screening antibodies against *Salmonella*

- Thus, national programmes to reduce *Salmonella* in pork are based on serological tests, which include classification of finisher herds.
In Ireland:
National Salmonella Control Programme

Finisher herd\textsubscript{1} 
Finisher herd\textsubscript{2} 
Finisher herd\textsubscript{3} 
Finisher herd\textsubscript{n}

\begin{itemize}
\item Category 1: <10%
\item Category 2: 10-50%
\item Category 3: >50%
\end{itemize}

ELISA cut-off 40\% OD

n=72
n=72
n=72
n=72
A risk assessment slaughterhouse model

Main’s model input is the proportion of sub-clinically infected pigs entering the abattoir (*Salmonella* caecal carriage as detected by *bacterial culture*)

The question is...

- On a herd basis, can we relate bacterial culture to ELISA tests, so that ‘national serology monitoring data’ can be effectively incorporated to risk assessment?

- The elucidation of the association between bacterial culture and ELISA serology in pigs naturally infected with *Salmonella* would be particularly useful if we were to make an inference on sub-clinical *Salmonella* infection of a group of slaughter pigs; and ultimately, to use this prediction to estimate the risk of carcass contamination during slaughter.
The question is...

Slaughterhouse – Processing model

Outputs: Prevalence in pig carcass and pork cuts

- Input 1: Salmonella caecal carriage of slaughter pigs
- Input 2
- Input 3

- Input 4: Herd-level serology data from National Salmonella Control Programme

- Input 5

Holistic on-farm model
However...

- As ELISA serology test measures presence of antibodies, it cannot differentiate between current and past infections.
Objective

- To assess whether the current knowledge on the herd-level association between bacterial culture and ELISA serology provides grounds for the utilisation of meat juice serology data for predicting caecal prevalence of *Salmonella* in pigs entering the abattoir.

- Assessment performed through Monte Carlo simulation.
Methodology

- A database of serology test results was facilitated by DAFF for the years 2005 and 2006.
- It consisted of the number of seropositive meat juice samples \((s)\) out of an annual sample size \((n)\) taken from abattoirs in 3 sampling occasions.
- \(s_{2005}, n_{2005}\), and \(s_{2006}, n_{2006}\) were provided for 436 representative herds.
...Methodology

- Data describing a relation between the proportion of slaughter pigs carrying *Salmonella* and the proportion of seropositive carcasses for a number of sampled herds was employed (Davies et al., 2003).

- Caecal contents for culture
- Diaphragmatic muscle for ELISA

Y: proportion of *Salmonella*-positive caecal contents
X: proportion of seropositive carcasses

19-22 carcasses were sampled per herd

20 herds = 20 (X, Y)
Methodology

- In order to add uncertainty to the regression \( Y = \text{Normal} (mX + c, \sigma) \)
- The 20 data pairs \((X, Y)\) were bootstrapped for 20,000 iterations, and 20,000 values of \(m, c\) and \(\sigma\) were obtained.
- Parametric distributions were fitted to \(m, c\) and \(\sigma\)

\[
m = \text{Weibull}(5.992, 1.945)
\]

\[
c = \text{LogLogistic}(0.137, 0.225, 5.340)
\]

\[
\sigma = \text{Weibull}(0.609, 0.177)
\]
...Methodology

- For every herd, the true seroprevalence ($SP$) was modelled as a Beta distribution
  \[ SP_{2005} = \text{Beta}(s_{2005}+1, n_{2005}-s_{2005}+1) \]
- Using Bayesian analysis, $SP_{2005}$ was used as a prior distribution, and revised with the new values of $s_{2006}$ and $n_{2006}$.
- Thus, a final estimation of $SP_i$ was done for every herd.
- Using the herd-level relationship data, the prevalence of *Salmonella* in caecal contents of slaughter pigs ($Pc_i$) was calculated for every herd, as
  \[ P_{c_i} = \text{Normal} \left( n \times SP_i + c, \sigma \right) \]
...Methodology

- 100 values were sampled from the $P_{c_i}$ distributions within each herd category, and histograms were built for $P_{cat1}$, $P_{cat2}$ and $P_{cat3}$.

- The overall proportion of slaughter pigs that would carry *Salmonella* in caecal contents ($P_c$) was estimated as the weighted average of $P_{cat1}$, $P_{cat2}$ and $P_{cat3}$ with the number of pigs per category $n_{cat1} = 1102903$, $n_{cat2} = 995112$ and $n_{cat3} = 305700$.

- Simulation using @Risk (Palisade) for 10,000 iterations.
Approximation to the actual prevalence of Salmonella in caecal contents of slaughter pigs in Ireland

○ For validation: Sources of information of prevalence of Salmonella in caecal contents of pigs sampled in Irish abattoirs

<table>
<thead>
<tr>
<th>Source</th>
<th>Positive samples</th>
<th>Total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duggan et al. (2008)</td>
<td>87</td>
<td>193</td>
</tr>
<tr>
<td>Quirke et al. (2001)</td>
<td>61</td>
<td>419</td>
</tr>
<tr>
<td>UCD study (2000)*</td>
<td>85</td>
<td>471</td>
</tr>
<tr>
<td><strong>Pooled data</strong></td>
<td><strong>233</strong></td>
<td><strong>1083</strong></td>
</tr>
</tbody>
</table>

○ Thus, the approximation to the actual proportion of slaughter pigs positive for Salmonella in caecal contents was given by

\[ \text{Beta} \left( 233 + 1, 1083 - 233 + 1 \right) \]
Results

- The meat juice serology data indicated moderate exposure to *Salmonella*, with 7.0%, 20.4% and 44.3% of tissue fluid samples from Category 1, 2 and 3, positive for *Salmonella* antibodies at 40% OD.
...Results

- Estimates of Salmonella prevalence in caecal contents of slaughter pigs as found by simulation:
The Pc value estimated by simulation (0.222) was very close to the mean incidence value of the caecal surveys’ validation data (0.215).

Estimated Pc similar to national abattoir surveys in:
- UK → 23%
- France → 24.8%
However...

○ The higher spread of the simulation’s output (high level of uncertainty about $P_c$) arose partly from the spread of the paired data utilised for the regression, which consequently produced wide distributions for the parameters $m$, $c$ and $\sigma$.  

Estimated prevalence of *Salmonella* in caecal contents of slaughter pigs in Ireland

- $X \leq 0.0694$ (2.5%)
- $X \leq 0.4307$ (97.5%)
- Mean: 0.222

Probability density function

- Estimated $P_c$
- Fitted distribution
- Caecal survey 95%CI
Discussion

- This is a consequence of the different stages of *Salmonella* infection that these two diagnostic tests measure,
  - ‘false positives’ → seropositivity may reflect historical and cleared infections
  - ‘false negatives’ → infected pigs may be sampled before mounting a detectable antibody response at 40% OD.
...Discussion

- While the association between serological response and culture results at herd level has been evidenced, other parameters of *Salmonella* transmission should be taken into account by a dynamic on-farm model so that the prevalence of *Salmonella* caecal carriage could be more accurately predicted.
Conclusions

- Through this simulation exercise, a second purpose for a systematic monitoring by serological testing is to be conveyed: The possibility to estimate sub-clinical infection (Salmonella caecal carriage) in a batch to be slaughtered on the basis of serological examination of slaughter pigs.

- Although the existing national control programmes are based on serology tests in which antibodies against Salmonella are measured, it is the presence of Salmonella in a batch what is important regarding contamination of carcasses, and therefore, in order to produce more accurate estimates of sub-clinical infection (caecal prevalence), further elucidation of this association should be attained by a dynamic on-farm risk assessment model.
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Thanks for your attention

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