An assessment of the use of meat juice serology monitoring data for estimating prevalence of caecal Salmonella carriage of Irish slaughter pigs

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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 $1$

Finisher herd $2$

Finisher herd $3$

Finisher herd $n$

n=72

Category 1: <10%

Category 2: 10-50%

Category 3: >50%

ELISA cut-off 40% OD
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)

Source: Gonzales Barron, Soumpasis, Butler, Prendergast, Duggan, Duffy (2008). Accepted in JFP
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

Herd-level serology data from National Salmonella Control Programme

Input 3

Input 2

Input 4

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 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.
- Estimation of prevalence of *Salmonella* in caecal contents of slaughter pigs was performed through two separate simulations utilising
  - herd-level data (paired data describing relationship between seroprevalence and *Salmonella* prevalence in caecal contents of slaughter pigs for 20 herds),
  - animal-level data (agreement data between serology results and culture of caecal contents from 2403 slaughter pigs).
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: Herd-level data

- 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).

![Diagram](image)

- **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: Herd-level data

- In order to add uncertainty to the regression, the 20 data pairs $(X, Y)$ were bootstrapped for 20,000 iterations, and 200,000 values of $m$, $c$ and $\sigma$ were obtained.
- Parametric distributions were fitted to $m$, $c$ and $\sigma$.

$$Y = \text{Normal}(mX + c, \sigma)$$
...Methodology: Herd-level data

- 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
  \[
  Pc_i = \text{Normal} (n \times SP_i + c, \sigma)
  \]
...Methodology: Herd-level data

- 100 values were sampled from the $P_{ci}$ 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.
Methodology: Animal-level data

- Data describing the extent of agreement between the *Salmonella* culture results of caecal contents and meat juice ELISA results for a cut-off of 40% OD was employed (Davies et al., 2004).
The uncertainty about the prevalence of *Salmonella* in caecal contents of slaughter pigs coming from every herd (*Pci*) was estimated through binomial probabilities using Bayesian analysis.

For instance, for one herd

\[ n_{2005} = 72, s_{2005} = 9 \]

\[ n_{2006} = 72, s_{2006} = 8 \]
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: Serology data

- 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: Herd-level 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 \( Pc \)) arose partly from the spread of the paired data utilised for the regression, which consequently produced wide distributions for the parameters \( m, c \) and \( \sigma \).
...Results: Herd-level simulation

- 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.
Results: Animal-level simulation

- Using rSe and rSp from the literature (under field conditions):

  - Still high level of uncertainty

  - The animal-level simulation produced a higher estimate of Pc (0.312) because of the low rSe value (0.289), very common under field conditions.
...Results: Animal-level simulation

- Simulation’s resulting trend of a relationship between *Salmonella* seroprevalence and *Salmonella* prevalence in caecal contents

- The underlying trend produced by the animal-level simulation was very different from the one assumed in the herd-level simulation (linear relationship).

- It tended to overestimate the within-herd the within-herd caecal prevalence values for high seroprevalence values
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 to reduce level of uncertainty.

- A dynamic on-farm model should be able to predict prevalence of *Salmonella* caecal carriage more accurately.
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.
...Conclusions

- The herd-level simulation seemed to me more appropriate than the animal-level simulation, as better association between serology and caecal culture has been demonstrated at herd level, while at pig level, agreement was not always demonstrable.
Acknowledgments

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Thanks for your attention