A European Union-wide slaughterhouse baseline survey on the prevalence of *Salmonella* in slaughter pigs

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Outline

- Baseline Survey
- Material and methods
- Prevalence estimations
- Associated factor analysis
- Conclusions and Recommendations
- Acknowledgments
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• Baseline Survey
  • Material and methods
  • Prevalence estimations
  • Associated factor analysis
  • Conclusions and Recommendations
• Acknowledgments
Baseline survey
Chart flow

ad hoc EFSA WG
Survey Design

→ European Commission ← Member States
Decisions

← Member States
Survey Implementation

Laboratory Analyses

European Commission
Data set Validation

EFSA+
ad hoc expert WG
Statistical Analysis

EFSA Reports
Outline

• Baseline Survey

• **Material and methods**

• Prevalence estimations

• Associated factor analysis

• Conclusions and Recommendations

• Acknowledgments
Material and methods (1)
Objectives and sampling procedure

- **Primary objectives**
  - Prevalence of slaughter pigs infected with *Salmonella* in lymph nodes
    - at the EU level
    - at the MS level
  - Prevalence of *Salmonella*-contaminated carcasses of slaughter pig
    - at the level of a group of voluntary MSs
    - for each MS individually

- **Sampling design**
  - **Units:** individual carcasses of slaughter pigs
  - **Sample size:** proportional to the live pig population (from 384 to 2,400 carcasses)
  - **Site:** slaughterhouses representing at least 80% of national throughput (2005)
  - **Sample:**
    - Stratified by slaughterhouses, and
    - Proportional to the slaughterhouse throughput (2005)
    - Random selection of sampling days and carcasses
    - Collected by the Competent Authorities from October 2006 to September 2007
Material and methods (2)

Samples from pigs

- **Ileo-caecal lymph node samples**
  - At least 5 lymph nodes and \( \geq 15 \) gr. per carcass
  - 25 MSs + Norway

- **Carcass swabs**
  - After evisceration and before chilling
  - One sponge per carcass
  - Swabbed at four sites (100 cm\(^2\) per site):
    - Hind limb, medial
    - Mid-dorsal region
    - Abdomen, lateral
    - Jowl
  - 13 MSs on a voluntarily basis
  - 384 pigs per MS were randomly sub-sampled from the included pigs
Material and methods (3)
Samples from pigs and questionnaire

• **Sense of the samples**
  - Lymph node
    - A sensitive test at the individual animal level
    - *Salmonella* infection of slaughter pigs at primary production
  - Carcass swabs
    - *Salmonella* surface contamination of the carcass
    - Contaminated carcass is likely to be a greater risk to public health as the carcass is the start of the food chain

• **Questionnaire**
  - Factors potentially associated with *Salmonella* positivity
  - Collected at the time of sampling by the competent authority
Material and methods (4)

Analyses and serotyping of samples

- **Analyses**
  - Samples sent to laboratories within 36 h. after sampling
  - Samples analysed within 24 hours following receipt, and
  - No later than 96 hours following the time of sampling

- **Salmonella National Reference Laboratories (NRL)**
- **Other laboratories**
  - involved in official controls and under supervision of the NRL

- **Normalised detection method**
  - Annexe D of ISO 6579 Standard
  - Pre-enrichment in BPW
  - Enrichment on MSRV medium plates

- **Serotyping**
  - According to the Kauffmann-White scheme
Material and methods (5)
Statistical analysis

- **Descriptive analysis**
  - “survey protocol” vs. “collected sample”

- **Estimation of prevalences**
  - GEE model
  - Standardised weights
    - clustering and disproportionate stratified sampling design
      - MS-level weight (reciprocal of the sampling proportion for throughputs)
      - SH-level weight: WY2 (reciprocal of the sampling proportion for pigs)
  - Observed prevalences
    - no correction made for imperfect Se and Sp
  - 4 outcomes were considered:
    - *Salmonella* spp.
    - *S. Derby*
    - *S. Typhimurium*
    - ‘Other *Salmonella* serovars’
Outline

- Baseline Survey
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- **Prevalence estimations**
- Associated factor analysis
- Conclusions and Recommendations
- Acknowledgments
• **Data validation and cleaning**
  – 0.7% (141/19,300) of records were excluded from the dataset

• **Validated dataset**
  – 19,159 carcasses
    → 19,071 lymph node samples
    → 5,736 carcass swabs
  – 943 slaughterhouses
Results (2)
Slaughter pig infection by *Salmonella* spp.

- Spain
- Greece
- Portugal
- Luxembourg
- The United Kingdom
- France
- Bulgaria
- Italy
- Ireland
- Belgium
- Cyprus
- Germany
- EU
- Hungary
- The Netherlands
- Denmark
- Slovenia
- Czech Republic
- Latvia
- Poland
- Slovakia
- Estonia
- Austria
- Lithuania
- Sweden
- Norway
- Finland

EU: 10.3% (CI<sub>95%</sub> 9.2 – 11.5)
Results (3)
Salmonella serovars distribution

- **Lymph nodes**
  - **87 serovars** isolated from 2,600 positive lymph nodes (25 MSs + Norway)

<table>
<thead>
<tr>
<th>10 Most Frequent Serovars</th>
<th>N</th>
<th>%</th>
<th>Nb. of countries with serovars</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>1,040</td>
<td>40.0</td>
<td>25</td>
</tr>
<tr>
<td>S. Derby</td>
<td>380</td>
<td>14.6</td>
<td>20</td>
</tr>
<tr>
<td>S. Rissen</td>
<td>151</td>
<td>5.8</td>
<td>5</td>
</tr>
<tr>
<td>S. 4,[5],12:i:-</td>
<td>128</td>
<td>4.9</td>
<td>8</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>126</td>
<td>4.8</td>
<td>19</td>
</tr>
<tr>
<td>S. Anatum</td>
<td>63</td>
<td>2.4</td>
<td>10</td>
</tr>
<tr>
<td>S. Bredeney</td>
<td>51</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>S. Infantis</td>
<td>49</td>
<td>1.9</td>
<td>16</td>
</tr>
<tr>
<td>S. London</td>
<td>33</td>
<td>1.3</td>
<td>9</td>
</tr>
<tr>
<td>S. Brandenburg</td>
<td>31</td>
<td>1.2</td>
<td>7</td>
</tr>
</tbody>
</table>
Results (4)
Slaughter pig infection by S. Typhimurium

The EU: 4.7% (CI_{95%} 4.1 - 5.3)
0.3%

Luxembourg, The United Kingdom, Spain, Ireland, Portugal, Belgium, France, Germany, The Netherlands, EU, Denmark, Greece, Hungary, Bulgaria, Czech Republic, Italy, Poland, Lithuania, Sweden, Estonia, Cyprus, Slovakia, Slovenia, Austria, Latvia, Norway, Finland.
Results (5)
Carcass contamination by *Salmonella* spp.

13-MS group: 8.3% (CI<sub>95%</sub> 6.3 – 11.0)

20%

2%
Results (6)
Salmonella serovars distribution

• Carcass swabs
  – 30 serovars isolated from 387 positive carcasses (13 MSs)

<table>
<thead>
<tr>
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<th>N</th>
<th>%</th>
<th>Nb. of countries with serovars</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>191</td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td>S. Derby</td>
<td>94</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>S. Infantis</td>
<td>13</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>S. Bredeney</td>
<td>8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>S. Brandenburg</td>
<td>7</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>S. Reading</td>
<td>6</td>
<td>1.55</td>
<td>2</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>5</td>
<td>1.29</td>
<td>3</td>
</tr>
<tr>
<td>S. Kedougou</td>
<td>5</td>
<td>1.29</td>
<td>2</td>
</tr>
<tr>
<td>S. 4,[5],12:i:-</td>
<td>5</td>
<td>1.29</td>
<td>1</td>
</tr>
<tr>
<td>S. Agona</td>
<td>4</td>
<td>1.03</td>
<td>3</td>
</tr>
</tbody>
</table>
Results (7)
Carcass contamination by S. Typhimurium

13-MS group: 3.9% (CI_{95%} 2.8 – 5.5)

11.7%

0.4%
Outline

• Baseline Survey
• Material and methods
• Prevalence estimations
• **Associated factor analysis**
• Conclusions and Recommendations
• Acknowledgments
• To investigate the effect of potential factors on:
  – *Salmonella* infection of slaughter pigs in the ileo-caecal lymph nodes
  – *Salmonella* surface contamination of slaughter pig carcasses

• Factors:
  – Sampling process sensitivity
  – Sample positivity: lymph node infection / carcass contamination

• To investigate the *Salmonella* serovar distribution in slaughter pigs across the EU

Report
Factors tested

• Factors related to the sampling process sensitivity
  1. Weight of the lymph node sample
  2. Number of lymph nodes in the sample
  3. Time between sampling and testing (in days)

• Factors related to lymph node infection
  1. Month of sampling
  2. Hour of sampling in the slaughterhouse
  3. Weight of carcasses
Associated factor analysis
Statistical analysis

• **Preliminary steps**
  • Descriptive analysis of the factors
    • Graphical visualisations according to *Salmonella* status
  • Categorisation of variables
    • Time between sampling and testing: shape of distribution
    • Month of sampling → Quarter of sampling: seasonal effect

• **Random effect logistic model**
  • To account for slaughterhouse clusters
    • Random intercept for slaughterhouse
  • To deal with potential confounding between certain factors and countries
    • Fixed effect of the country
## Associated factor analysis

Model of *Salmonella* infection of pigs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Random effect logistic model&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of the lymph node samples&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-24gr</td>
<td></td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>25-34gr</td>
<td></td>
<td>1.3</td>
<td>1.1 - 1.6</td>
</tr>
<tr>
<td>35-44gr</td>
<td></td>
<td>1.2</td>
<td>0.8 - 1.7</td>
</tr>
<tr>
<td>≥ 45gr</td>
<td></td>
<td>1.9</td>
<td>1.2 – 3.0</td>
</tr>
<tr>
<td>Time (in days) between the date of sampling and testing in the laboratory&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 2 days</td>
<td></td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3 to 4 days</td>
<td></td>
<td>1.2</td>
<td>1.04 – 1.4</td>
</tr>
<tr>
<td>5 to 7 days</td>
<td></td>
<td>0.99</td>
<td>0.65 – 1.5</td>
</tr>
</tbody>
</table>

- OR > 1.0 implies increased risk among pigs exposed
- OR < 1.0 implies a reduced risk among pigs exposed
• Impact of the lymph node weight on the likelihood of detection of *Salmonella*

• The difficulties in standardisation of the lymph node sampling process should be considered when defining *Salmonella* control programme
Factors tested

• Factors related to the sampling process sensitivity
  1. Time between sampling and testing (in days)

• Factors related to carcass surface contamination
  1. *Salmonella* status of live slaughter pig (lymph nodes)
  2. Month of sampling → Quarter of sampling
  3. Hour of sampling in the slaughterhouse
  4. Weight of the carcasses
## Associated factor analysis

**Salmonella on carcasses: Model**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Random effect logistic model&lt;sup&gt;a, b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
</tr>
<tr>
<td><strong>Time (in days) between the date of sampling and testing in the laboratory</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0 day</td>
<td>0.51</td>
</tr>
<tr>
<td>1 day</td>
<td>1</td>
</tr>
<tr>
<td>2 days</td>
<td>1.009</td>
</tr>
<tr>
<td>3 to 7 days</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Lymph node infection of the live slaughter pig</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>Quarter of sampling</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Oct. – Dec. 2006</td>
<td>0.51</td>
</tr>
<tr>
<td>Jan. – Mar. 2007</td>
<td>0.58</td>
</tr>
<tr>
<td>Apr. – Jun. 2007</td>
<td>1.002</td>
</tr>
<tr>
<td>Jul. – Sept. 2007</td>
<td>1</td>
</tr>
</tbody>
</table>

- Significant random slope of “LN infection” for the slaughterhouse
Associated factor analysis
Salmonella on carcasses: Model

Positive carcass probability

Basic risk

Lymph node infection

Sampling-testing time

Study quarter

Member State

\[ Y_{cs} = (\mu + m_s) + (\beta_{ln} + B_s).LN + \beta_d . Delay + \beta_q . Q + \beta_{ms} . MS + \epsilon_{cs} \]

Slaughterhouse level part \( \sim N(0, \sigma_B) \)

Slaughterhouse level part \( \sim N(0, \sigma_m) \)
Associated factor analysis

*Salmonella* on carcasses: “slaughterhouse effect”

Proportion (%) of *Salmonella* spp. positive pigs

Proportion (%) of *Salmonella* spp. contaminated carcasses

Box plots showing the distribution of the proportion (%) of *Salmonella* spp. positive pigs across different categories.
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• Association between *Salmonella* infection of slaughter pigs and carcass contamination

• The risk of carcass contamination varied significantly between slaughterhouses

• Certain slaughterhouses were more capable of controlling and preventing *Salmonella* contamination than others

• At EU level, the April-September 2007 survey period was more at risk → to be verified in individual MSs

• Sampling and testing procedure impacted on the likelihood of detection of *Salmonella*
Associated factor analysis
Recommendations

- Harmonisation of sampling and testing procedures should be considered of importance at the national and EU level
- Relevant studies on the survival rates of *Salmonella* in different relevant matrices

- Further analytical studies at the national level
- As pig infection and slaughterhouse process have both an impact on risk of carcass contamination:
  - Integrated control programme may prove to be a feasible and cost-effective option
  - MSs are encouraged to guarantee *Salmonella* controls at primary production as in the slaughterhouses
  - EU pig meat industry is invited to pay increased attention to slaughter hygiene and other relevant factors affecting *Salmonella* contamination of carcasses
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• Acknowledgments
Acknowledgements

• The competent authorities of MSs
• Ad hoc expert working group
  Thomas Blaha, Kristen Barfod, Alex Cook, Pedro Rubio Nistal, Micheál O’Mahony, Arjen W. van de Giessen, Kris De Smet

• More information available on EFSA web site
  • Part A Report on prevalences
  • Part B Report on associated factors analysis
Thank you for your attention!