Salmonella
and
Salmonellosis

Doris Mueller-Doblies Dr. med. vet. MRCVS
Bacteriology Department
AHVLA Weybridge, New Haw, Addlestone, UK
Overview

- Background on *Salmonella*
- Human salmonellosis cases in the EU – trends
- *Salmonella* in food of animal origin
- EU legislation on *Salmonella* control in chickens
- *Salmonella* prevalence in breeding, laying and broiler chickens across the EU
- Vaccination of chickens against *Salmonella*
Salmonella

- Family Enterobacteriaceae
- Gram negative, straight rods, 0.7 - 1.5 x 2 - 5 µm
- Usually motile - flagella
- Nomenclature has been controversial
- Names were given according to clinical picture and hosts-specificity first, then by geographical locations
- Serological analysis according to the White-Kauffman-LeMinor scheme was adopted in 1946
- Different molecular techniques have been developed, aiming at replacing serotyping in the future
- Molecular techniques are already routinely used in Canada and Denmark
Nomenclature

**Family:** *Enterobacteriaceae* (first letter capitalised, italicised)

**Genus:** *Salmonella* (first letter capitalised, italicised)

2 species: *S. enterica* and *S. bongori* (not capitalised, italicised)

*S. enterica* - 6 subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, *indica* (not capitalised, italicised)

Most zoonotic *Salmonella* belong to the subspecies *enterica*

**Serovar** (serotype or ser.): e.g. Typhimurium (first letter capitalised, not italicised)

Example: *Salmonella enterica enterica* ser. Typhimurium or *S. Typhimurium*

>2600 serovars known
Antigenic diversity

- Three types of antigens:
  - **Somatic (O) antigens** (the specific part of the bacterial lipopolysaccharide)
  - **Flagellar (H) antigens** (protein subunits)
  - **Surface (Vi) antigen:**
    capsular polysaccharide, found only in serovars Typhi, ParatyphiC and some Dublin strains
H antigens are typically biphasic; two genetic systems located distantly on the chromosome help the organism to evade the host’s immune defence.

- Alternating gene expression means they can switch from one “phase” to another “phase”.
- Monophosphasic variants express only one phase antigen.
Monophasic variants of S. Typhimurium

S. Typhimurium - antigenic formula

1,4,[5],12 : i : 1,2

Somatic (O) antigens  Flagellar (H) antigens

Phase 1 antigen

Phase 2 antigens

Current epidemic monophagic strains of S. Typhimurium only express phase 1 flagellar antigen: 1,4, [5], 12 : i : -
Pathogenesis of salmonellosis

- Passage through stomach, colonization of small intestine
- Invasion of M-cells and entry into Peyer’s patches
- Drainage to mesenteric lymph node
- Spread via thoracic duct into great vein
- Blood
- Survival and multiplication within macrophages of liver and spleen

(Baeumler et al., *Salmonella* in domestic animals, Wray&Wray 2000)
Salmonellosis - Clinical picture in humans

• Most common form is a self-limited, uncomplicated gastroenteritis (diarrhea, abdominal cramps and fever)
• Enteric fever (including typhoid fever) - life-threatening systemic illness requiring prompt antibiotic therapy
• Reactive Arthritis, Irritable Bowel Syndrome
• Asymptomatic carrier state can occur
Salmonellosis - Clinical picture in domestic animals

- Sub-clinical infections are common
- Infection can easily spread between animals in a herd or flock without detection
- Animals may become intermittent or persistent carriers; asymptomatic carrier state can occur
- Infected cattle, sheep and horses may show fever, diarrhoea and abortion
- In calf herds, *Salmonella* may cause outbreaks of diarrhoea and septicaemia with high mortality
- Clinical signs are less common in pigs and goats
- Poultry usually show no obvious signs of infection with zoonotic serovars

(EFSA 2014)
Reported notification rates of zoonoses in confirmed cases in the EU, 2012

- Campylobacteriosis: (N = 214,268)
- Salmonellosis: (N = 91,034)
- VTEC infections: (N = 5,672)
- Listeriosis: (N = 1,642)
- Q fever: (N = 643)
- West Nile fever: (N = 233)
- Brucellosis: (N = 328)
- Trichinellosis: (N = 301)
- Tuberculosis caused by M. bovis: (N = 125)
- Rabies: (N = 2)

Notification rate per 100,000 population: 22.2/100,000

(EFSA 2014)
Distribution of all food-borne outbreaks, 2012

- Unknown
- Salmonella
- Bacterial toxins
- Viruses
- Campylobacter
- Other causative agents
- Other bacterial agents
- Escherichia coli, pathogenic
- Parasites
- Yersinia

Number of outbreaks

Strong evidence outbreaks
Weak evidence outbreaks

(EFSA 2014)
Total number of salmonellosis cases 2008 - 2012

(Data from EFSA)

-30.8% in 5 years
Confirmed cases of human salmonellosis/100,000 in 2012

(Data from EFSA)
Change in the number of confirmed salmonellosis cases 2008 - 2012

(Data from EFSA)
Notification rates and origin of infection in human salmonellosis

(EFSA 2014)
Distribution of the 10 most common serovars in humans in the EU 2012 (n=82,409)
>70% of cases caused by **S. Enteritidis (SE)**, **S. Typhimurium (ST)** or monophasic variants of ST

- Infections with SE mainly linked to eggs and egg products
- ST infections – pigs; cattle and poultry meat; environmental contamination; contact with companion animals; wild bird related
Human salmonellosis cases by serovar (2)

- **Salmonella Infantis** cases continued to increase by 14.5 % (from 2.1 to 2.5 %).
- **S. Stanley** multi-country outbreak, affecting at least seven MSs - linked to the turkey production chain
- **S. Thompson** outbreak in NL – linked to salmon as possible source?
- **S. Panama** – one outbreak in Germany and one in Italy
Human salmonellosis cases by serovar (3)

S. Enteritidis
S. Typhimurium
monophasic variants of ST
S. Infantis: +53.5% since 2008

(Data from EFSA)
Salmonellosis continued to decrease in 2012.

A total of 91,034 confirmed cases.

30.8% decrease compared to 2008.

EU notification rate 22.2 cases per 100,000 population.

Highest notification rates reported by the Czech Republic and Slovakia (≥85 cases per 100,000).

Lowest rates reported by Portugal, Greece and Romania (≤4 per 100,000).
## Compliance with food safety Salmonella criteria

<table>
<thead>
<tr>
<th>Food categories(^1)</th>
<th>Total single samples</th>
<th>Total batches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample weight</td>
<td>N</td>
</tr>
<tr>
<td>Minced meat and meat preparations intended to be eaten raw</td>
<td>25 g</td>
<td>619</td>
</tr>
<tr>
<td>Minced meat and meat preparations from poultry intended to be eaten cooked</td>
<td>25 g or not stated</td>
<td>2,246</td>
</tr>
<tr>
<td>Minced meat and meat preparations from other species than poultry intended to be eaten cooked</td>
<td>10 g or 25 g</td>
<td>5,479</td>
</tr>
<tr>
<td>Mechanically separated meat</td>
<td>25 g</td>
<td>3</td>
</tr>
<tr>
<td>Fresh poultry meat(^3)</td>
<td>25 g</td>
<td>2,701</td>
</tr>
<tr>
<td>Live bivalve molluscs and live echinoderms, tunicates and gastropods</td>
<td>25 g</td>
<td>6</td>
</tr>
<tr>
<td>Egg products</td>
<td>25 g or 120 g</td>
<td>476</td>
</tr>
<tr>
<td>RTE foods containing raw eggs</td>
<td>25 g</td>
<td>25</td>
</tr>
</tbody>
</table>

*EFSA 2014* # as laid down by EU Regulation 2073/2005 and 1441/2007, 2012
Weighted prevalence of *Salmonella*-positive broiler meat samples

Include only MSs that reported data for at least six years: Austria, Belgium, Finland, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Netherlands, Portugal, Slovenia and Spain.

95% confidence interval

(EFSA 2014)
## Salmonella in table egg samples, 2012

### Country Description  Sample unit Sample weight 2012 N N pos % pos

<table>
<thead>
<tr>
<th>Country</th>
<th>Description</th>
<th>Sample unit</th>
<th>Sample weight</th>
<th>2012</th>
<th>N</th>
<th>N pos</th>
<th>% pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>Domestic production</td>
<td>Single</td>
<td>300 g</td>
<td></td>
<td>51</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Belgium</td>
<td></td>
<td>Batch</td>
<td>25 g</td>
<td></td>
<td>118</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bulgaria</td>
<td></td>
<td>Batch</td>
<td>-</td>
<td></td>
<td>57</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>Eggs - table eggs - shell, domestic production</td>
<td>Single</td>
<td>25 g</td>
<td></td>
<td>605</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Eggs - table eggs - white, domestic production</td>
<td>Single</td>
<td>25 g</td>
<td></td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Eggs - table eggs - yolk, domestic production</td>
<td>Single</td>
<td>25 g</td>
<td></td>
<td>641</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Eggs - table eggs, domestic production</td>
<td>Single</td>
<td>25 g</td>
<td></td>
<td>6,464</td>
<td>5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Hungary</td>
<td></td>
<td>Single</td>
<td>10 eggs</td>
<td></td>
<td>655</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Latvia</td>
<td></td>
<td>Single</td>
<td>25 g</td>
<td></td>
<td>90</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Lithuania</td>
<td>Eggs - table eggs - whole</td>
<td>Batch</td>
<td>-</td>
<td></td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Netherlands</td>
<td></td>
<td>Batch</td>
<td>25 g</td>
<td></td>
<td>3,734</td>
<td>2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Romania</td>
<td></td>
<td>Batch</td>
<td>25 g</td>
<td></td>
<td>39</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Slovakia</td>
<td></td>
<td>Batch</td>
<td>25 g</td>
<td></td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td>Single</td>
<td>25 g</td>
<td></td>
<td>257</td>
<td>8</td>
<td>3.1</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td>Single</td>
<td>25 g</td>
<td></td>
<td>265</td>
<td>17</td>
<td>6.4</td>
</tr>
<tr>
<td>Total (16 MSs)</td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>18,843</strong></td>
<td></td>
<td><strong>28</strong></td>
<td><strong>17</strong></td>
<td><strong>0.1</strong></td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td><strong>Single</strong></td>
<td><strong>11,523</strong></td>
<td></td>
<td><strong>17</strong></td>
<td><strong>0.1</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Batch</td>
<td><strong>Batch</strong></td>
<td><strong>7,320</strong></td>
<td></td>
<td><strong>6</strong></td>
<td>&lt;0.1</td>
<td></td>
</tr>
</tbody>
</table>

Note: Only investigations covering 25 or more samples are included.

(EFSA 2014)
SE cases in chickens and humans in the UK

- Human SE cases
- Chicken SE isolations
- Chicken SE PT4 isolations
- 9R and auxotrophic vaccines
- Live metabolic drift mutant vaccines
- Layer NCP 2008

Graph showing the number of chicken incidents and human cases over time.
EU legislation – National Control Programmes (NCPs)
Comprehensive Legislation Package

Risk Assessment

Zoonoses Directive


Food Hygiene Legislation

Reg. 178/2002
Reg. 852/2004
Reg. 853/2004
Reg. 854/2004
Reg. 882/2004
Reg. 2073/2005
(Reg. 1086/2011)

Risk Management

Reg. 2160/2003
Reg. 1177/2006
Reg. 1237/2007 etc...
Feedstuffs: microbiological criteria – no legislation yet

Breeding hens
Target: <1% flocks / year 5 serovars

Breeding turkeys
Target: <1% flocks / year SE/ST

Breeding pigs
Target to be decided

Laying hens
Target: annual reduction to max 2% SE/ST

Broilers
Target: <1% flocks / year SE/ST

Meat turkeys
Target: <1% flocks / year SE/ST

Slaughter pigs
Target to be decided

Heat treatment

Slaughter: Process hygiene criterion (n= 50, c= 5)

Processing

Eggs:
Trade restricted SE/ST

Eggs products:
Absence in 25g, all serovars

Fresh meat:
Absence in 25g, SE/ST

Meat products, preparations, minced meat:
Absence in 25g, all serovars

Meat products, preparations, minced meat:
Absence in 10 g. Raw consumption:
25g, all serovars
Legal Basis – EU Legislation

• Primary / framework legislation

• Sector specific requirements laid out in EU Regulations:
  – Regulation 200/2010 (breeding chickens) – from 2007
  – Regulation 517/2011 (laying chickens) – from 2008
  – Regulation 200/2012 (broilers) – from 2009
  – Regulation 1190/2012 (turkeys) – from 2010
NCPs – Key points

• Emphasis is on *Salmonella* of most public health importance – agreed reduction targets per sector
• Mandatory risk management measures in case of detection of +ve result (SE/ST/mST)
• All ‘commercial’ poultry producers included in the programme:
  • All breeding flocks (> 250 birds)
  • all flocks producing meat/eggs except:
    • private domestic use
    • small quantities direct to final consumer/ retail outlet
    • [defined by the Member State – risk based]
• Annual report to Brussels and periodic FVO audit
Progress of the National Control Programmes

- **Breeders**
  - Target
  - Consult & develop
  - Operational
  - Review
  - Implement

- **Layers**
  - Survey
  - Target
  - Consult & develop
  - Operational
  - Review
  - Implement

- **Broilers**
  - Survey
  - Target
  - Consult & develop
  - Operational
  - Review

Breeding chickens

Prevalence of *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Virchow* and *S. Hadar*-positive adult flocks 2007 - 2012

Target 1%

(EFSA 2014)
Prevalence of SE, ST, S. Infantis, S. Virchow and S. Hadar-positive breeding flocks 2012

(EFSA 2014)
Laying hens

Prevalence of S. Enteritidis and/or S. Typhimurium-positive laying flocks 2008 - 2012

(EFSA 2014)
Prevalence of SE and/or ST-positive laying flocks 2012

(EFSA 2014)
Prevalence of S. Enteritidis and/or S. Typhimurium-positive broiler flocks 2009 - 2012

Target 1%

(EFSA 2014)
Prevalence of SE and/or ST-positive broiler flocks 2012

(ESFA 2014)
Main sources of Salmonella

Replacement birds (breeding/rearing flock infection, hatchery contamination (major source for broilers and turkeys), transport)

Contaminated feed (main source for broilers)

Contaminated Poultry Housing (poor disinfection or pest control after previous infected flock)

People and equipment movements (especially on multi-age or species farm)

Primary and secondary wildlife vectors (e.g. wild birds, badgers, game birds)

Domestic and feral animals

Infected people?

Contaminated water?

Contaminated bedding?
Relevant key control measures…

…….to reduce the *Salmonella* prevalence in the chicken industry:

- **Control measures had to start at the top of the breeding pyramid and were then also implemented to some extent in the production sectors**

**Reduce or eliminate introduction of *Salmonella* onto a farm:**
- Vaccination of breeding flocks
- Slaughter / Threat of slaughter of breeding flocks
- Improved hatchery hygiene
- Improved biosecurity
- Improved control over feed quality

**Reduce or eliminate carry-over of infection on farms:**
- Improved cleaning and disinfection procedures after each crop
- Improved general hygiene and biosecurity during crops
- Improved rodent and wildlife control
Salmonella vaccines

- Live versus killed vaccines
- Suitable for breeding and laying hens

- Different approach towards vaccination of laying flocks in different European countries:
  - Compulsory in Austria, Belgium, the Czech Republic, Germany, and Hungary
  - Allowed and recommended in Bulgaria, Belgium, Cyprus, Estonia, France, Greece, Italy, Latvia, Lithuania, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, and the UK
  - Banned in Denmark, Finland, Sweden and Ireland (Galis et al., 2013)
# Salmonella vaccines currently used in the UK

<table>
<thead>
<tr>
<th>Name</th>
<th>Type of Vaccine</th>
<th>Administration Route</th>
<th>Timing</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salenvac T</td>
<td>killed SE/ST</td>
<td>IM injection</td>
<td>12 weeks &amp; 16 weeks</td>
<td>Alhydrogel adjuvant, high circulating/maternal antibody</td>
</tr>
<tr>
<td>Avipro Salmonella Vac E</td>
<td>live SE</td>
<td>Water</td>
<td>D.O. ; 7w ; 3w pre-lay</td>
<td>metabolic drift mutant</td>
</tr>
<tr>
<td>Avipro Salmonella Vac T</td>
<td>live ST</td>
<td>Water</td>
<td>D.O. ; 7w ; 3w pre-lay</td>
<td>metabolic drift mutant</td>
</tr>
<tr>
<td>Avipro Duo</td>
<td>Live SE/ST mix</td>
<td>Water/spray</td>
<td>D.O. ; 7w ; 3w pre-lay</td>
<td>metabolic drift mutants</td>
</tr>
<tr>
<td>Gallivac SE (2 dose course – v. normal challenge)</td>
<td>live SE</td>
<td>Water/spray</td>
<td>D.O. ; +2w</td>
<td>auxotrophic mutant</td>
</tr>
<tr>
<td>Gallivac SE (3 dose course – v. high challenge/ST)</td>
<td>live SE</td>
<td>Water/spray</td>
<td>D.O. ; 2w ; 3w pre-lay</td>
<td>auxotrophic mutant</td>
</tr>
<tr>
<td>Gallimune SE + ST</td>
<td>killed SE/ST</td>
<td>IM injection</td>
<td>From 6w, &amp; 16 w</td>
<td>mineral oil adjuvant</td>
</tr>
</tbody>
</table>
9R vaccine

• Rough strain of *Salmonella* Gallinarum, developed in England from a number 9 field virulent smooth strain
• Based on the knowledge that rough mutants lose their virulence capability
• Has been used extensively in countries where fowl typhoid is endemic
• Currently in use in some countries to protect layer flocks against *S. Enteritidis*
# Main problems with vaccine administration

<table>
<thead>
<tr>
<th>Killed vaccines</th>
<th>Live vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Operating speed too fast</td>
<td>• Insufficient water tank capacity</td>
</tr>
<tr>
<td>• Vaccine gun filling/calibration problems</td>
<td>• Failure to thirst birds</td>
</tr>
<tr>
<td>• Wrong injection site – leakage</td>
<td>• Poor draining and priming of water lines</td>
</tr>
<tr>
<td>• Missed doses</td>
<td>• Failure to check vaccine distribution (dye)</td>
</tr>
<tr>
<td></td>
<td>• Antimicrobial use in the hatchery</td>
</tr>
</tbody>
</table>

- Insufficient water tank capacity
- Failure to thirst birds
- Poor draining and priming of water lines
- Failure to check vaccine distribution (dye)
- Antimicrobial use in the hatchery
Summary (1)

- Prevalence of human salmonellosis cases has fallen continuously in the EU since the introduction of the NCPs (-30.7% since 2008)
- SE and ST cases have fallen, while the number of monophasic ST cases and S. Infantis cases is rising
- Differences in health systems and reporting/confirmation of cases make it difficult to estimate an EU-wide under-reporting factor
- Countries with the lowest notification rate (i.e. cases/100,000) have the highest hospitalization rates
Summary (2)

- EU – wide prevalence for breeding and laying chickens has been below the set targets since 2010
- EU – wide prevalence for broiler chickens has been below the set target since 2009
- Individual member states are still above the set targets in one or more production sectors
- Vaccination, improved biosecurity, hygiene, cleaning and disinfection and rodent/wildlife control all contributed to the lower prevalence in the chicken industry
• But.....

• Vigilance is required to detect new and emerging salmonelllas and multi-drug resistant salmonelllas early
Acknowledgments

• Dr. Rob Davies, AHVLA Weybridge
• Lesley Larkin, AHVLA / VA to Defra, London
• Public Health England (PHE)

• The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012
Thank you for your attention