Salmonella: Epidemics, Reservoirs, and Resistance

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Salmonella

Two species (more than 2500 different antigenic types):

- **Salmonella enterica**
  - I. enterica
  - II. salamae
  - III. Arizona
    - IIIa. arizonae
    - IIIb. diarizona
  - IV. houtenae
  - VI. Indica

- **Salmonella bongori** *(previously sub-species V)*

Widely distributed

- Zoonoses

- Most serotypes that infect mammals are found in subspecies I
Disease

- Wide spectrum of clinical illness
- Life-threatening systemic disease (enteric fever)
- Non-invasive salmonella usually characterised by self-limiting gastroenteritis of varying severity
- Major cause of food-borne infection
Laboratory reporting of *Salmonella* in England and Wales 1981 – 2009
Laboratory reporting of *Salmonella* in England and Wales 1981 – 2009

All salmonella Reports England and Wales 1981-2009
Laboratory reporting of Salmonella in England and Wales 1981 – 2009
Laboratory reporting of *Salmonella* in England and Wales 1981 – 2009

All *Salmonella* Reports by primary group, England and Wales 1981-2009

- Enteritidis
- Typhimurium
- Others
Salmonella Enteritidis

- SE Non PT4
- SE PT 4

Layers vaccinated

Broilers vaccinated
Salmonella Enteritidis
Illness due to *Salmonella* Enteritidis PT4

**Salmonella** in eggs - a thing of the past?
Salmonella Enteritids – Emerging phage types
FSA, EU and HPA multi-disciplinary R&D

- Laboratory Investigations
- Outbreak Investigations
- Epidemiological Investigations
- Traceback Investigations
- Egg Contamination Investigations

Advice to:
- FSA
- DEFRA
- Industry
- EU
- Spain

Other outputs:
- Reports
- Peer-reviewed publications
Salmonella Enteritidis PT 14b (NxCpl), 2000 - 2009

S. Enteritidis 14b 2000-09
Salmonella Enteritidis PT 14b (NxCpl), 2000 - 2009

S. Enteritidis 14b 2000-09

Graph showing the number of reports of fully sensitive and NxCpl strains of Salmonella Enteritidis PT 14b from 2000 to 2009. The x-axis represents the year and month of the specimen, and the y-axis represents the number of reports. The graph uses blue for fully sensitive and red for NxCpl.
Salmonella Enteritidis PT 8 and duck eggs, 2010

England, Wales, Northern Ireland, Ireland 2010: >70 cases, 1 death*

Republic of Ireland, 2010

Outbreak (6 cases +)**

* Health Protection Report 17 September 2010

** Disease Surveillance Report of HPSC, Ireland, 11(5), May 2010
Salmonella Typhimurium
Other salmonellas – Top 10 most frequently identified

England & Wales, 1981 - 2009
Other salmonellas – Top 10 most frequently identified

England & Wales, 1981 - 2009
Food animal reservoirs of *Salmonella*
Salmonella in foods
S. Typhimurium DT191A: case locations, 2008
S. Typhimurium DT 191A, tet'$

Epidemiological investigations

8 of 10 cases investigated kept a reptile in the house, mostly a snake.
‘Pinkies’!

Thanks to:

Katy Hawker
Chris Lane

For slides and information
S. Typhimurium DT 191A- infection pathways
S. Typhimurium DT 191A- infection pathways
S. Typhimurium DT 191A- infection pathways

Salmonella

Mouse
S. Typhimurium DT 191A- infection pathways

- Salmonella
- Mouse
  - Snake/reptile
  - Reptile handler
  - Environment
S. Typhimurium DT 191A- infection pathways
S. Typhimurium DT 191A- infection pathways

Salmonella

Mouse

Snake/reptile

Environment

Reptile handler

Baby/child
Leaflet

Produced by HPA, DoH and Defra

“Reducing the risks of salmonella infection from reptiles”

Available on the HPA website
Areas of current concern

- Increasing resistance to Critically Important Antimicrobials (CIAs)
  - Quinolones
  - 3/4 generation cephalosporins

- Emergence of new drug (multi)-resistant epidemic clones of Salmonella 1,4,[5],12:i:-

Quinolone resistance – isolates from humans, 10 MS, 2002-08

2002: < 2%
2008: >14%

Serovars:

Typhimurium:
2002: <1%
2008: 2 – 6 %

Enteritidis:
2002: 6 - 10%
2008: 10 – 30%

[Highest incidence: S. Virchow: 2006: > 50%]

Quinolone resistance – *Salmonella* isolates from animals. EU Member States, 2005-06.

<table>
<thead>
<tr>
<th>Serovars not specified</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle:</td>
<td>2005 (5 MS)</td>
<td>0 – 20%</td>
</tr>
<tr>
<td>Pigs:</td>
<td>2006 (8 MS)</td>
<td>0 – 26%</td>
</tr>
<tr>
<td>Turkeys:</td>
<td>2005 (4 MS)</td>
<td>10 - 84%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S. Enteritidis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry (hens)</td>
<td>2006 (14 MS)</td>
<td>0 - 95%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S. Java</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry (hens)</td>
<td>Netherlands, 2006:</td>
<td>50%</td>
</tr>
</tbody>
</table>

Source: EFSA, 2007, 2008; MARAN, 2008; ECDC, EFSA, EMA, SCENIHR, 2009
Cephalosporin resistance – Salmonella isolates from humans, 10 EU MS, 2002-08

<table>
<thead>
<tr>
<th>Year</th>
<th>Typhimurium</th>
<th>Enteritidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>0.2%</td>
<td>&gt;0.1%</td>
</tr>
<tr>
<td>2005</td>
<td>0.6%</td>
<td>0.1%</td>
</tr>
<tr>
<td>2008</td>
<td>0.9%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

[Highest incidence: S. Java (Netherlands): 2008 ->15%]

<table>
<thead>
<tr>
<th>Serotype</th>
<th>CTX-M</th>
<th>Country</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paratyphi B Java</td>
<td>1, 2</td>
<td>The Netherlands</td>
<td>Poultry</td>
</tr>
<tr>
<td>Virchow</td>
<td>2</td>
<td>Belgium, France</td>
<td>Human, Poultry</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Turkey</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Belgium, France, Spain, UK*</td>
<td>Human, Poultry</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Spain</td>
<td>Human</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>5</td>
<td>Greece</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Greece</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>UK</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>France, UK</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>UK</td>
<td>Human</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>14</td>
<td>Spain</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>UK</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>17/18</td>
<td>UK</td>
<td>Human</td>
</tr>
<tr>
<td>Mbandaka</td>
<td>3</td>
<td>Poland</td>
<td>Human</td>
</tr>
<tr>
<td>Oranienburg</td>
<td>3</td>
<td>Poland</td>
<td>Human</td>
</tr>
<tr>
<td>Bovismorbificans</td>
<td>1</td>
<td>Italy</td>
<td>Human</td>
</tr>
<tr>
<td>Stanley</td>
<td>17/18</td>
<td>UK*</td>
<td>Human</td>
</tr>
<tr>
<td>Kentucky</td>
<td>15</td>
<td>France*</td>
<td>Human</td>
</tr>
<tr>
<td>Anatum</td>
<td>15</td>
<td>UK*</td>
<td>Human</td>
</tr>
</tbody>
</table>
Conclusions

Quinolones:
- substantive increases
- *Salmonella* Enteritidis
- EU-wide
- poultry-associated

3rd generation cephalosporins:
- small increase
- mainly *S. Typhimurium*, but also in other serovars
- *S. Java* in Netherlands (poultry-associated)
- foreign travel-associated
### Organisms

**Antigenic structure:** 1,4,[5],12:i:1,2

**Phage type / antimicrobial resistance / plasmids**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antigenic Structure</th>
<th>Plasmids/Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT 29</td>
<td>ASSuT (Fu)</td>
<td>Plasmids</td>
</tr>
<tr>
<td>DT204/193/204c</td>
<td>CSSuT, ACKSSuT, ACGKSSuT, ACKSSuTTm</td>
<td>Plasmids</td>
</tr>
<tr>
<td>DT104</td>
<td>ACSSuT</td>
<td>Chromosome</td>
</tr>
<tr>
<td></td>
<td>ACSSuTTm, ACSSuTCP_L</td>
<td>Chromosome, plasmid(s)</td>
</tr>
</tbody>
</table>
Multiple antimicrobial resistance in S. Typhimurium DT104 from humans in England and Wales 1981 – 2009: Resistance types
Arrangement of genes encoding pentaresistance (~ 13 kb)

CLASS I INTEGRON

S | C | T | A | Su

aadA2 | cmlA | tetG | bla_{CARB-2} | sul1

**Reported as:**
1,4,[5],12:i:-  
4, [5], 12:i: -  
4, 12: -  
4, [5], 12: -  
4, : -  
untypable

**Resistance:** ASSuT (+G, Tm)

**Numerous human infections**

**Animal / food association:**
Pigs, pork  
(also, cattle, poultry)
PCR reactions of *Salmonella* serovars

Multiplex PCR to support the serological identification of the antigens.

Lane 1: S. Lagos
Lane 2: S. Agama
Lane 3: S. Agama
Lane 4: S. 4,[5],12:i:-
Lane 5: S. Typhimurium
Lane 6: Negative control

Source: Antonia Ricci, unpublished; EFSA, 2010
Molecular recognition:

18kb-acquired \textit{thrW} island of monophasic S. Typhimurium DT193

Common to most isolates

G/C content different from LT2

Data from Wolfgang Rabsch, Wernigerode, Germany)
7 primer multiplex PCR for identification of monophasic S. Typhimurium

Data: Wolfgang Rabsch, Werningerode, Germany
Geographic distribution of monophasic *Salmonella* strains in Germany 2008

Nine large outbreaks

- Diffuse distribution
- Complex typing
- Dominant type: DT193, R-type ASSuT
STYMXB.0131-pattern of monophasic *Salmonella* 4,5,12:i:- strains from the outbreak, Germany
(R. Prager, RKI, Wernigerode)

<table>
<thead>
<tr>
<th>Lane</th>
<th>Source</th>
<th>Resistance pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 6, 11</td>
<td>S. Braenderup</td>
<td>ASSuT</td>
</tr>
<tr>
<td>2 - 3</td>
<td>human</td>
<td>ASSuT</td>
</tr>
<tr>
<td>4</td>
<td>feed</td>
<td>ASSuT</td>
</tr>
<tr>
<td>5</td>
<td>cattle</td>
<td>ASSuT</td>
</tr>
<tr>
<td>7 - 10</td>
<td>human</td>
<td>ASSuT</td>
</tr>
</tbody>
</table>
Detection of DT193 ASSuT with 18kb-acquired thrW island STYMXB.0131 in France 2008

May 2010:
90 cases
Dried pork sausage

[Eurosurveillance June 2010]
Regional distribution of monophasic DT193 ASSuT in Italy

[11 strains DT193 carry the 18 kb island]
Geographical distribution of cases with monophasic Salmonella 4,5,12:i:- ASSuT and S. Typhimurium in Austria, 2008

monophase Salmonellen und S. Typhimurium DT193, ASSuT in Österreich, 2008
Monophagic *Salmonella* 4,5,12:i:- with at least ASSuT from humans in Austria (data C. Kornschober, Graz)
Luxembourg, March 2006

- Health Authorities observed *Salmonella* 04,5,12 :i:- DT193 outbreak
- 24 hospitalized patients, one death
- Unique PFGE *XbaI* and ASSuT resistance pattern

[Joel Mossong et al. 2007 *Eurosurveillance* 12, E11]
Joël Mossong, Luxembourg

XbaI-pattern

Human and Vet. samples

2006 – 2009

Eurosurv. 12(6)2007

STYMXB. 0131

human and vet. isolates, 2006

human, 2008

pork, 2008

human, 2008

human, 2009
Joël Mossong, Luxembourg
XbaI-pattern
Human and Vet.-samples
2006 – 2009
Eurosurg. 12(6) 2007

STYMXB. 0131

human and vet. isolates, 2006
human, 2008
pork, 2008
human, 2008
human, 2009
pork, 2008
Joël Mossong, Luxembourg
XbaI-pattern
Human and Vet.-samples
2006 – 2009
Eurosurrv.12(6)2007

STYMXB. 0131

pork, 2008
human and vet. isolates, 2006
human, 2008
human, 2008
pork, 2008
human, 2009
MLVA O3-11-09-00-211 (pig)
MLVA Human 4 x 03-13-09-00-211 all cases
March and May 2009

Six strains with 18 kb island
Lanes 7, 8, 9, 11, 12 and 13 are all 4,5,12:i-; i.e. monophasic S. Typhimurium isolates, i.e. mutation in phase 2 flagellar antigen whereby H:2 is not expressed.

Lanes 2, 12 & 13 contain isolates from swine (matches with human isolates).

Lane 7 & 8 = STYMXB.0131(outbreak profile).
England and Wales

Full serotyping not routinely undertaken at Reference Laboratory

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

2005: 47
2009: 151 (increase of 321%)

2010: 376 isolates of S. Typhimurium DT 193, of which 270 (72%) lack fljB and hin – i.e., Monophasic
Med-Vet-Net study 2009-10

- 7 participating labs.

- Requested:
  - $\leq 10$ isolates of S. 4,[5],12:i:-, R-type ASSuT from humans, pigs or pig meat isolated 2006-2008.
  - $\leq 10$ isolates of S. 4,[5],12:i:-, other R-types.

- 122 S. 4,[5],12:i:- isolates.
  - 116 confirmed as S. 4,[5],12:i:- by HPA *Salmonella* Reference Unit.
  - 72% R-type ASSuT +/- other resistances.

- Screening:
  - Phage typing.
  - Breakpoint resistance typing.
  - PFGE.
  - MLVA.
  - MLST.
  - PCR for resistance genes.
  - Deletion genotyping.
Phage typing

- 16 different phage types identified.

- Most common phage types: DT193 (44%), DT120 (23%) and RDNC (9%).

- Some association between phage type and country of origin.

- Spanish S. 4,[5],12:i:- were phage type U302.
Molecular subtyping - PFGE

• 36 banding profiles identified.

• 65% represented by one of five profiles:

Some associations between PFGE, PT and country:
- STYMXB.0131 and STYMXB.0022 – DT193; other 3 patterns – DT120.
- STYMXB.0079 – Italy, STYMXB.0010 – Poland, STYMXB.0131 – Netherlands.

STYMXB.0131 associated with outbreak in Luxembourg in 2006.

Molecular subtyping - MLVA

• MLVA identified 45 profiles.

• 5 profiles accounted for 43% isolates:
  - 3-11-9-NA-211, N=12
  - 3-12-9-NA-211, N=13
  - 3-13-10-NA-211, N=12
  - 3-14-9-NA-211, N=7
  - 3-13-9-NA-211, N=6

• Previously identified among STm R-type ASSuT DT193 and DT120 from humans, pigs and cattle.
Summary: Monophasic salmonellas – an emerging problem in Europe?

Antigenic structure: 1,4, [5], 12:i: -
Antibiogram: ASSuT
Phage types: 193, 120, U302
PFGE profile (s): STYMXB.0131, STYMXB.0083
VNTR profile (s): 3-11-9-NA-211, 3-12-9-NA-211
Antimicrobial resistance: Chromosomal island (not SGI-1)
Resistance genes: \( \text{bla}_{\text{TEM}}, \text{strA}, \text{sul2}, \text{tet}(B) \)
Infections in at least 10 EU countries

Source: Hopkins et al Euro Surveilance 2010,
Lucarrelli et al J Clin Microbiol 2010,
Occurrence of S. Typhimurium DT9, DT204, DT104 and monophasic DT193 strains from humans
(Germany, 1966 – August 2009)

Many thanks to Wolfgang Rabesch, RKI, Wernigerode, Germany for slide and information
Occurrence of S. Typhimurium DT9, DT204, DT104 and monophasic DT193 strains from humans
(Germany, 1966 – August 2009)

Many thanks to Wolfgang Rabsch, RKI, Wernigerode, Germany for slide and information
EFSA recommendations (2010)

‘Identification of monophasic S. 1,4,[5],12:i:-’

‘Ideally, all isolates of putative Salmonella should be fully serotyped and full antigenic formula reported’

‘If full antigenic formula is not available but a phage type consistent with S. Typhimurium lacking phase two flagellar antigens has been confirmed, and lack of the second phase flagellar antigen has been verified by PCR, then the term ‘monophasic S. Typhimurium’ is recommended for reporting purposes in the current situation.'
European Community Network of Excellence, MEDVETNET

For funding of multicentre study on monophasic *Salmonella* in Europe
Thanks to

All colleagues in the HPA Gastrointestinal Pathogens departments (laboratory and epidemiology)

Wolfgang Rabsch, Werningerode, Germany,

with data from
Christian Kornschober, Graz, Austria
Mia Torpdahl, Copenhagen, Denmark
Francoise-Xavier Weill, Paris, France
Simon Le Hello, Paris, France
Martin Cormican, Galway, Ireland
Nial De Lappe, Galway, Ireland
Ida Luzzi, Rome, Italy
Claudia Lucarelli, Rome, Italy
Joël Mosson, Luxembourg, Luxembourg
Max Heck, Bilthoven, The Netherlands
Kim van der Zwaluw, Bilthoven, The Netherlands

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Kim van der Zwaluw, Bilthoven, The Netherlands
Chris Lane, HPA, Cfl, London, UK

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