OIE Seminar:

Monitoring and Intervention Strategies for *Salmonella* in Poultry - with Korean Experience

Min-Su Kang  DVM, MS, PhD
Avian Disease Division, QIA, Korea (ROK)
Salmonella infections in poultry

- Pullorum Dis./Fowl Typhoid
  - Only avian species
  - Nonmotile / host-adapted
    - *Salmonella* Pullorum
    - *Salmonella* Gallinarum
  - A single serotype

- Paratyphoid Infections
  - Animals and humans
  - Motile / nonhost-adapted
    - *Salmonella* Enteritidis
    - *Salmonella* Typhimurium, etc.
  - Food-borne pathogens
Cycle of *Salmonella* infection

- Importation of poultry and poultry products
  - Breeding flocks
    - Lateral spread
    - Vertical transmission:
      - Contamination of eggshell
      - Contamination of egg via ovaries
    - Horizontal transmission:
      - Lateral spread among day-old stock
      - Delivery vehicle, personnel, etc.
  - Hatchery
  - Broiler and egg production flocks
    - Lateral spread
  - Processing plant
    - Carcass cross-contamination
    - Contaminated waste and wild birds

*(Poultry Meat Science. 1999, p. 259)*
Vertical and horizontal transmission of *Salmonella* - within integrated broiler production system

(J. Vet. Sci. 2007, 8:155–161)
On farm control of *Salmonella* in poultry

**Gallinarum-Pullorum**
- Chicks and poult's from *Salmonella*-free sources
- Biosecurity practices - all in all out, feed, wildlife
- Serological tests (bacteriological confirmation)
- Elimination of positive chickens or flocks
- Vaccination/treatment

**Other serotypes**
- Chicks and poult's from *Salmonella*-free sources
- Biosecurity practices - all in all out, feed, wildlife
- Bacteriological examination (and serological tests)
- Elimination of positive chickens or flocks
- Competitive exclusion/acid/vaccination/treatment

**Intervention**

**Monitoring**

*e.g., commercial flocks.*

#*e.g., breeder flocks in some developed countries.*
Salmonella monitoring programs

Monitoring for *Salmonella* in poultry

- Carried out at all stages of the production cycle
- Mostly mandatory in breeding flocks and hatcheries
- Regularly conducted by sampling of poultry flocks

National control programs for *Salmonella* in poultry

- EU (Regulation (EC) No. 2160/2003): Enteritidis, Hadar, Infantis, Typhimurium, and Virchow in chicken breeding flocks; Enteritidis and Typhimurium in laying hens, broilers and turkeys
- US (NPIP - voluntary): Gallinarum, Pullorum, and Enteritidis in chicken breeding flocks; Gallinarum and Pullorum in other breeders
- Sampling => testing => reporting => measures (if positive findings)
## Salmonella monitoring in EU

### Chicken breeder flocks

<table>
<thead>
<tr>
<th>Flock</th>
<th>Sampling time</th>
<th>Sampling place</th>
<th>Sample material</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rearing</strong></td>
<td>Day old</td>
<td>Hatchery</td>
<td>meconium from 250 chicks (\Rightarrow) 1 pool (Sweden)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Holding</td>
<td>5 chick boxes per delivery: box liners or swab samples (&gt;1m² in total) (\Rightarrow) 1 pool (Denmark)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 chick box liner or hatcher tray liner per 500 chicks/hatchery (~10 liners) and ~60 dead birds/hatchery (\Rightarrow) 1 pool (UK)</td>
</tr>
<tr>
<td></td>
<td>4 weeks old</td>
<td>Holding</td>
<td>2 pairs of boot swabs (\Rightarrow) 1 pool (Sweden/Demark)</td>
</tr>
<tr>
<td></td>
<td>2 weeks before being moved</td>
<td>Holding</td>
<td>2 pairs of boot swabs (\Rightarrow) 1 pool (Sweden/Demark)</td>
</tr>
</tbody>
</table>
Boot swab

- absorptive paper/fabric overboots
- moistened with 0.8% NaCl, 0.1% peptone water, or sterile water
- produce representative samples for all parts of the house

(http://www.worldpoultry.net; defra)
# Salmonella monitoring in EU

## Chicken breeder flocks

<table>
<thead>
<tr>
<th>Flock</th>
<th>Sampling time</th>
<th>Sampling place</th>
<th>Sample material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Every 2 weeks</td>
<td>Hatchery or Hatchery</td>
<td>Hatcher basket liners from 5 baskets (&gt;1m² in total) or 10g of broken egg shells from each of 25 hatcher baskets ⇒ reduced to 25g ⇒ 1 pool (Denmark)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Holding</td>
<td>5 pairs of boot swabs* ⇒ 2 pools (Sweden)</td>
</tr>
<tr>
<td></td>
<td>[Official sampling]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Every 16 weeks</td>
<td>Hatchery and Hatchery</td>
<td>Same as above</td>
</tr>
<tr>
<td></td>
<td>0-4 weeks after moving, 8-0 weeks before slaughter, once in between</td>
<td>Holding</td>
<td>Same as above</td>
</tr>
</tbody>
</table>

*Feces: 300 samples/ ≥1,000 chicks, ≥1 gram/sample ⇒ 2 pools, or 2 × 150g mixed feces (cage flocks)*
## Salmonella monitoring in EU

### Layer and broiler flocks

<table>
<thead>
<tr>
<th>Flock</th>
<th>Sampling time</th>
<th>Sampling place</th>
<th>Sample material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layers – rearing</td>
<td>Day old</td>
<td>Holding</td>
<td>10 samples of crates and 20 dead birds (Denmark)</td>
</tr>
<tr>
<td></td>
<td>3 weeks old</td>
<td>Holding</td>
<td>5 pairs of boot swabs ⇒ 1 pool (Denmark)</td>
</tr>
<tr>
<td></td>
<td>2 weeks before being moved*</td>
<td>Holding</td>
<td>2 pairs of boot swabs ⇒ 1 pool, or 150g fecal samples in cage birds (Sweden); or 5 pairs of boot swabs ⇒ 1 pool and 60 blood samples (Denmark)</td>
</tr>
<tr>
<td>Layers – laying</td>
<td>Every 15 weeks (Sweden) or every 9 weeks (Denmark)*</td>
<td>Holding</td>
<td>2 pairs of boot swabs ⇒ 1 pool (Sweden/Demark)</td>
</tr>
<tr>
<td>Broilers</td>
<td>1-2 weeks before slaughter*</td>
<td>Holding</td>
<td>2 pairs of boot swabs ⇒ 1 pool (Sweden)</td>
</tr>
</tbody>
</table>

*One of the samples are taken by official veterinarians in each holding.
Salmonella monitoring in US

National Poultry Improvement Plan (NPIP)

US Pullorum-Typhoid Clean
- Official Pullorum-Typhoid blood tests
- 300 birds per flock at 4 months of age

US Salmonella Enteritidis (SE) Clean
- Official blood tests
- 300 non-vaccinated birds per flock at 4 months of age
- Official bacterial examinations
  - 4 manure drag swabs per house
  - 2 nest/egg belt samples once in the lay house
- Egg-type: 1st sample at 2 to 4 weeks of age, every 30 days
- Meat-type: 1st sample at 4 months of age, every 30 days
Salmonella monitoring in US

Egg quality assurance program (EQAP) Principle: HACCP

Best Management Practices
- Chicks from NPIP SE Clean breeders
- Cleaning and disinfection of houses between flocks
- Biosecurity, rodent control, egg sanitation, etc.

Education/Training

Record keeping

Verification
# Salmonella monitoring in US

**PEQAP – sample collection**

<table>
<thead>
<tr>
<th>Verification test</th>
<th>Required by programs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick box paper testing</td>
<td>PA, MD, NY</td>
</tr>
<tr>
<td>Pullet manure testing</td>
<td>PA, MD, NY</td>
</tr>
<tr>
<td>Young layer manure testing (30 weeks)</td>
<td>PA, MD</td>
</tr>
<tr>
<td>Mid-lay manure testing (45 weeks)</td>
<td>PA, MD</td>
</tr>
<tr>
<td>Post-molt manure testing</td>
<td>PA, MD, NY</td>
</tr>
<tr>
<td>Pre-moveout testing (within 8 weeks of moveout)</td>
<td>NY, OH, SC, UEP</td>
</tr>
</tbody>
</table>

* Eggs – required if positive manure samples
Chick paper swab and manure drag swab

- sterile gauze pads with skim milk
  - 1 pad (5 chick papers) per 5,000 chicks

- sterile gauze pads with skim milk
  - 2 pads per row/bank of cages

(http://extension.psu.edu)
Salmonella detection percentage from all sample types

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total</th>
<th>Integrator A</th>
<th>Integrator B</th>
<th>Integrator C</th>
<th>Integrator D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper pads</td>
<td>50.8</td>
<td>32.5</td>
<td>47.4</td>
<td>26.6</td>
<td>96</td>
</tr>
<tr>
<td>Feces</td>
<td>6.6</td>
<td>0.8</td>
<td>10.3</td>
<td>9.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Water line</td>
<td>1.4</td>
<td>0</td>
<td>1.3</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>Water cup</td>
<td>1.9</td>
<td>0.5</td>
<td>1.3</td>
<td>2.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Litter</td>
<td>10.5</td>
<td>1.6</td>
<td>15.4</td>
<td>15</td>
<td>9.4</td>
</tr>
<tr>
<td>Feed hopper</td>
<td>2.3</td>
<td>1.6</td>
<td>3.9</td>
<td>0</td>
<td>3.2</td>
</tr>
<tr>
<td>Feeder</td>
<td>2.3</td>
<td>0</td>
<td>3.9</td>
<td>0</td>
<td>4.8</td>
</tr>
<tr>
<td>Drag swab</td>
<td>14.2</td>
<td>2.1</td>
<td>21.1</td>
<td>16.7</td>
<td>15.6</td>
</tr>
<tr>
<td>Wall swab</td>
<td>3.4</td>
<td>3.1</td>
<td>2.6</td>
<td>7.8</td>
<td>0</td>
</tr>
<tr>
<td>Fan swab</td>
<td>3.4</td>
<td>1.6</td>
<td>1.3</td>
<td>7.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Mouse samples</td>
<td>6.1</td>
<td>12.5</td>
<td>0</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td>Wild-bird feces</td>
<td>6.6</td>
<td>6.1</td>
<td>14.3</td>
<td>4.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Animal feces</td>
<td>3</td>
<td>3.8</td>
<td>0</td>
<td>0</td>
<td>2.6</td>
</tr>
<tr>
<td>Insects</td>
<td>2.8</td>
<td>6.1</td>
<td>0.8</td>
<td>4.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Dirt, near entrance</td>
<td>6.1</td>
<td>6.3</td>
<td>13.5</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>Standing water</td>
<td>5.1</td>
<td>4.8</td>
<td>4.2</td>
<td>8.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Boot swab</td>
<td>12</td>
<td>14.3</td>
<td>16.7</td>
<td>11.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Fly strip</td>
<td>18.7</td>
<td>25</td>
<td>5.3</td>
<td>29.6</td>
<td>17.1</td>
</tr>
<tr>
<td>Cecal droppings</td>
<td>4.4</td>
<td>1</td>
<td>9.2</td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
<td>Total</td>
<td>9.8</td>
<td>5.2</td>
<td>10.8</td>
<td>9.7</td>
<td>13.4</td>
</tr>
</tbody>
</table>

(Bailey et al. 2001, J. Food Prot.)
Examination of samples (e.g.)

**Feces and environmental samples (boot swabs, etc)**

<table>
<thead>
<tr>
<th>Pre-enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 1:10 ratio</td>
</tr>
<tr>
<td>- 25g sample + 225ml buffered peptone water (BPW), 37°C, 18h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Selective-enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 0.1ml culture + 10ml Rappaport-Vassiliadis (RV) broth</td>
</tr>
<tr>
<td>- or 1ml culture + 10ml tetrathionate broth</td>
</tr>
<tr>
<td>- or 0.1ml/3drops + modified semi-solid Rappaport-Vassiliadis (MSRV) agar</td>
</tr>
<tr>
<td>- 42°C, 24-48h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Selective plating</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Rambach agar, XLD agar, etc. (two different media), 37°C, 24h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Biochemical tests and serotyping, polymerase chain reaction (PCR) etc.</td>
</tr>
</tbody>
</table>
Salmonella intervention measures

EU

Sweden and Denmark
- No Salmonella in breeder flocks
- Salmonella +ve in <3% of layer and broiler flocks in Denmark (2003)
- Active surveillance to monitor Salmonella at all times at all levels
- Eradicate all positive breeders (and commercial chickens)
- Eliminate Salmonella from all feed

UK and other countries
- Salmonella +ve in 5.1% of breeding flocks in EU (19/25 countries, 2004)
- Salmonella surveillance programs
- Slaughter Enteritidis or Typhimurium +ve breeder flocks (UK)
- Vaccination program (against Enteritidis and Typhimurium)
**Salmonella intervention measures**

**US**

- **Breeders, layers, and broilers**
  - Use of *Salmonella*-free chicks
  - Competitive exclusion treatments
  - Vaccination program (breeders)
  - Control of feed ingredients
  - Biosecurity (rodent, insect, footbaths, workers, etc.)
  - *Salmonella* +ve in ~12% of broilers (2006)
  - *Salmonella* Enteritidis +ve in 2% of layer flocks (Ohio, 2011)

- **Hatchery**
  - Hatch eggs from *Salmonella*-free breeder flocks
  - Cleaning and disinfection (incubator, hatching cabinets & eggs)
Korean situation?

The poultry industry in Korea (2012)

- **Chickens:** 9.6 million breeders (330 farms), 61 million layers (1,320 farms), and 68.5 million broilers (1,590 farms)
- **Ducks:** 1 million breeders (120 farms) and 11 million ducks (870 farms)

Salmonella status in poultry in Korea

- **Gallinarum-Pullorum:** <3% of chicken breeder flocks (serologically +), ~80 cases / year (Gallinarum; commercial layer and broiler chickens)
- **Salmonella sp.:** ~13% of layer chicken flocks and ~28% of broiler chicken flocks between 2009 and 2010 (bacteriologically +)
- Predominant serotypes: Enteritidis (chickens) and Typhimurium (ducks)
Salmonella monitoring programs in Korea

**National control program - statutory**

- **Gallinarum-Pullorum:** serological monitoring of chicken breeding flocks at 120 days of age under government-backed control schemes
- **Enteritidis:** bacteriological examination of table eggs from layer farms raising 10,000 or more chickens every year

**Salmonella monitoring - voluntary**

- **Poultry farm HACCP (since 2008):** breeders (15%), layers (32%), broilers (23%), and ducks (8%) are monitored for Enteritidis
- Periodical monitoring of birds, houses, eggs, feed, etc.
Salmonella Enteritidis testing in HACCP (e.g.)

**Breeders and hatcheries**
- **Day old**: chick box liners (meconium) and dead or weak birds
- **Thereafter**: blood, cloacal swabs, litter, dust, etc.; every 3-5 weeks
- **Hatchery**: fluff, eggshells, dead birds, etc.; every month (*Salmonella* )

**Layers**
- **Day old**: chick box liners (meconium) and dead or weak birds
- **Laying hens**: blood samples; 4 times/year

**Broilers and ducks**
- **Day old**: chick box liners (meconium) and dead or weak birds
- **Thereafter**: feces, litter, dust etc.; twice a year
**Salmonella** intervention measures in Korea

**Chicken breeders**

- **Gallinarum-Pullorum**: reportable diseases, statutory slaughtering of positive flocks
- **Enteritidis and other serotypes**: sanitation measures, medication, competitive exclusion, acid treatment, etc.

**Layers, broilers, and ducks**

- **Gallinarum-Pullorum**: vaccination, medication, bacteriophage treatment, etc. (mainly in layers)
- **Other Salmonella serotypes**: sanitation measures, medication, competitive exclusion, acid treatment, etc.
Conclusions – a Korean perspective

- The most effective way to control *Salmonella* in the poultry food system from farm to retail (table) is likely to control *Salmonella* on the farm.

- Statutory national control programs for *Salmonella* (e.g., Enteritidis and Typhimurium) at least in breeding flocks will significantly improve *Salmonella* status on the farm.

- Expanded application of farm HACCP will facilitate the reduction of *Salmonella* contamination in poultry and enhance poultry production food safety.
Thank you for your attention!

Questions?