The future of *Salmonella* vaccines in a geographically diverse and changing epidemiological environment, with emphasis on poultry

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A brief introduction on the global *Salmonella* problem

- Host-specific serotypes
  - Systemic spread to bloodstream
  - Cause septicemia, severe disease
  - Specific serotypes affect specific hosts

Examples: *Salmonella* Cholerasuis, Gallinarum, Dublin, ...

**SYSTEMIC DISEASE!**
A brief introduction on the global *Salmonella* problem

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Examples: *Salmonella* Cholerasuis, Gallinarum, Dublin, ...

SYSTEMIC DISEASE!

- Broad host-range serotypes
  - Intestinal colonization is most important
  - Either asymptomatic or causing diarrhea
  - Spread between different animal species, and humans

Examples: *Salmonella* Enteritidis, Typhimurium, many others ...

FOOD POISONING!
Food sources of human outbreaks of *Salmonella* (EU 2017)

- Eggs/egg products: 36.8%
- Bakery products: 16.7%
- Mixed food: 12.6%
- Meat products: 8.2%
- Pig meat: 4.5%
- Broiler meat: 2.2%
- Others: 11.1%

Serotypes involved in human *Salmonella* cases (EU 2017)

- Enteritidis: 49.1%
- Typhimurium: 13.4%
- Monophasic Typhimurium: 8%
- Infantis: 2.5%
- Newport: 2.5%
- Many serotypes, all below 1%
Link between serotypes and animals/food sources

% of isolates from that specific serotype derived from a feed source

Distribution of serotypes in animal species and products is key for control plans
### Current vaccines and their protection

<table>
<thead>
<tr>
<th></th>
<th><strong>Live vaccines</strong></th>
<th><strong>Inactivated vaccines</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immune mechanism</strong></td>
<td>Cell-mediated immunity, mucosal immunity, IgA, antibodies</td>
<td>Antibodies</td>
</tr>
<tr>
<td><strong>Administration method</strong></td>
<td>Drinking water, spray</td>
<td>Injection</td>
</tr>
<tr>
<td><strong>Type</strong></td>
<td>Chemical mutagenesis, metabolic drift mutants, undefined mutants</td>
<td>Bacterins</td>
</tr>
<tr>
<td><strong>Safety issues</strong></td>
<td>Potential persistence, spread, reversion to virulence</td>
<td>No isolation possible</td>
</tr>
<tr>
<td><strong>Animal species</strong></td>
<td>layers, broilers, pigs</td>
<td>breeder poultry, cattle</td>
</tr>
<tr>
<td>Other aspects:</td>
<td>adjuvant requirement, cost, duration of immunity, markers (DIVA), multivalent use, etc ...</td>
<td></td>
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</tbody>
</table>
Host-specific serotypes (example *Salmonella* Gallinarum in poultry)

Current vaccines and their protection

- Decreased mortality
- Less diseased animals
- Lower organ colonization levels
- No complete elimination

Lee, Mo and Kang, 2005
Current vaccines and their protection

Food poisoning serotypes (example *Salmonella* Enteritidis in poultry)

**Lower gut colonization levels, shedding**
- *Lower organ colonization levels*
- *Less egg contamination*
- *No complete elimination*

**Week 6** (day 42)
- Challenge by Oral gavage in the crop
  - *S. Enteritidis* $10^9$ cfu

**Week 7** (day 49)
- Euthanasia for Organ Sampling (caecum, spleen and liver)

**Week 8** (day 56)
- Vaccination

**Week 24**
- Intravenous challenge
  - *S. Enteritidis* $10^{7}$ cfu

**Week 27**
- Organ sampling

% positive egg batches

CTRL | VAC
--- | ---

Eckhaut et al., 2018
Challenges in *Salmonella* control, vaccine design and use
Challenge 1. Geographical differences in serotype distribution (example of poultry)

Worldwide Epidemiology of *Salmonella* Serovars in Animal-Based Foods: a Meta-analysis

AEM, 2019
Challenge 2. New emerging *Salmonella* serotypes

Emergence of a Clonal Lineage of Multidrug-Resistant ESBL-Producing *Salmonella* Infantis Transmitted from Broilers and Broiler Meat to Humans in Italy between 2011 and 2014

Alessia Franco 1, Pimilopa Leekitcharoenphon 2, Fabiola Feltrin 1, Patricia Alba 1, Gessica Cordaro 1, Manuela Iureasca 2, Rita Toi 1, Mario D’Incau 1, Monica Staffoian 1, Elisabetta Di Giannatale 2, René S. Hendrikson 3, Antonio Battisti 1

A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent *Salmonella enterica* serovar infantis strain

Gilli Avrin,1,2 Katherine Tsyba,1,2 Natalie Steck,1,2 Malik Salmon-Diouf,3,4 Antje Cornelius,1,4 Galia Rahav,1,2 Guntram A. Grassl1,4 and Ghod Gali-Mor1,2 (1) Institute of Genomic Research, Hebrew University-Hadassah Medical Center, Jerusalem, Israel; (2) Department of Microbiology, Hadassah Hebrew University Hospital, Jerusalem, Israel; (3) Department of Veterinary Medicine, University of Femin, Bangladesh; (4) Department of Food Science, University of Femin, Bangladesh)

**Table 1:** Incidence of *Salmonella enterica* serovars in animal and food samples

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<thead>
<tr>
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<th>Serotype</th>
<th>Food</th>
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<td>2615 (13)</td>
<td>594 (13)</td>
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<tr>
<td>S. Enteritidis</td>
<td>752 (16)</td>
<td>136 (12)</td>
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<tr>
<td>S. Livingstone</td>
<td>459 (6)</td>
<td>38 (4)</td>
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<tr>
<td>S. Cerro</td>
<td>420 (3)</td>
<td>0 (1)</td>
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<td>363 (7)</td>
<td>15 (3)</td>
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<td>S. Typhimurium</td>
<td>298 (16)</td>
<td>43 (9)</td>
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<tr>
<td>S. Ohio</td>
<td>41 (3)</td>
<td>54 (2)</td>
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<tr>
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<td>34 (2)</td>
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Percentage (%)

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Percentage (%)
Challenge 3. Critical periods of increased sensitivity, age

- Young animals
- Short life span (eg. broilers)
- Limits in duration of protection for laying hens
- Stress periods (lay, molting)
- Interference with feed additives and antimicrobials
Challenge 4. Interference with monitoring programs

• Bacteriological testing:
  • Limited shedding
  • Low environmental survival
  • Differentiation on culture media

• Serological testing:
  • Differentiation of vaccinated from infected animals (DIVA)
Questions for future vaccines

• Challenge 1. Geographical differences in serotype distribution
• Challenge 2. New emerging *Salmonella* serotypes
  
  Are current vaccines cross-protective? Against which serogroups or serotypes? Broad cross-protection? Level of cross-protection? Do we need serotype-specific vaccines?

• Challenge 3. Critical periods of increased sensitivity, age
  
  Can we have immediate protection from day 1 of life? Can we get good data on dynamics of protection in time? Can we boost protection at susceptible periods?

• Challenge 4. Interference with monitoring programs
  
  Can we introduce markers to differentiate bacteriologically and serologically?
Cross-protection between serotypes

• Challenge 1. Geographical differences in serotype distribution
• Challenge 2. New emerging *Salmonella* serotypes

**Graph:***
- **X-axis:** Log 10 cfu Salmonella / g
- **Y-axis:** Day
- **Legend:**
  - CTRL
  - VAC
- **Data Points:**
  - INFANTIS
  - ENTERITIDIS
- **Statistical Significance:** ***

**Timeline:**
- **Week 1:** Day 1 Vaccination
- **Week 6:** Day 42 Challenge by Oral gavage in the crop SE+SI 10^9 cfu
- **Week 7:** Day 49 Vaccination
- **Week 8:** Day 56 Euthanasia for Organ Sampling (caecum, spleen and liver)

**Cloacal Swabs:**
- Cloacal Swabs (ALL neg.)
- Cloacal Swabs (ALL-Vac.)
- Cloacal Swabs (ALL-Chal.)

**Cross-protection of a *Salmonella* Enteritidis/Typhimurium vaccine against Infantis**

How broad is cross-protection? Unclear

Eeckhaut et al., 2018
Early protection

- Challenge 3. Critical periods of increased sensitivity, age

Day 1
Vaccination
10^8 cfu

Day 2
Oral
challenge
S. Enteritidis
5.10^3 cfu

Day 7
Organ
colony sampling

Log CFU/g caecum

Control
Live Enteritidis vaccine

1000-fold reduction in caecal colonization at day 7 post-infection

Colonization-inhibition (serotype-specific effect)
Genetically modified organisms (GMO)

- Guaranteed safety for poultry and mammals, based on pathogenesis
- Marker genes (e.g., LPS, fli for serology; biochemical pathways for bacteriology)
- Risk to revert ~ zero (multiple gene deletions)
- Introduce markers that change phenotype (cfrr isolation)
- Consumer acceptance? Regulatory issues?
Example: Choice of mutations based on pathogenesis

- **Oral uptake**
- **Intestinal colonization**
  - Oral uptake
  - Caecal colonization
- **Systemic spread**
  - ΔhilA
  - ΔssrA
  - ΔtolC
- **Egg contamination**
  - ΔhilA
  - ΔssrA
  - ΔtolC
Example 1. A *Salmonella* Enteritidis ΔhilA ΔssrA ΔfliG mutant

- + not pathogenic in rodent models
- + contains a serological marker
- + does not grow on MSRV

Safety confirmed
Example 1. A *Salmonella* Enteritidis ΔhilA Δ ssrA ΔfliG mutant

Day 1
Spray vaccination
10⁸ cfu

Day 7
Day 21
Day 42
sampling
sampling
sampling

Day 2
Oral challenge
*S. Enteritidis*
5.10⁸ cfu

**Early and persistent protection in broilers**

De Cort et al., 2015
Example: Choice of mutations based on pathogenesis

Intestinal colonization

Oral uptake

Caecal colonization

\( \Delta \text{hilA} \)

Systemic spread

\( \Delta \text{ssrA} \)

Egg contamination

\( \Delta \text{tolC} \)
Example 2. A *Salmonella* Enteritidis Δ*tolC* mutant

- No egg white survival at body temperature, no transmission
- + defects in resistance in environment
- + defects in resistance against host responses

No isolation of vaccine strain

Day 1
Oral Vaccination

Week 6
Oral Vaccination

Week 16
Oral Vaccination

Week 24
Iv challenge with *Salmonella* Enteritidis

Week 27
Organ Sampling

Raspoet et al., 2019
Example 2. A *Salmonella* Enteritidis ΔtolC mutant

**Graphs:**
- Bars showing percentage of positive samples for Caeca, Spleen, Uterus, Ovary, and Oviduct.
  - Controls vs. DtolC mutants.
  - Data from Kilroy et al., 2016.

**Timeline:**
- Day 1: Oral Vaccination
- Week 6: Oral Vaccination
- Week 16: Oral Vaccination
- Week 24: Iv challenge with *Salmonella* Enteritidis
- Week 27: Organ Sampling

**Legend:**
- Blue: Control
- Gray: DtolC

**Graphs:**
- Bar graph showing percentage of positive egg batches for week 1 and week 2.
  - Controls vs. DtolC.
  - Data from Kilroy et al., 2016.
Example 2. A *Salmonella* Gallinarum Δ*tolC* mutant

- Day 33: 10^6 cfu Vaccination
- Day 65: 10^7 cfu challenge
- Day 72: weight

**Statistical Analysis**

- ****: Significant difference
- *: Moderate difference

Graph showing body weight (g) with different groups:
- PBS intramuscular
- Commercial vaccine
- TolC orally
- TolC intramuscular
- TolC subcutaneously
Challenge 5. Regulatory aspects

- GMOs
- Transfer mutations to other serotypes/strains for rapid vaccine production
- ...

Challenge 6. Evaluating safety and efficacy under field conditions

• Expectations and misconceptions
  • Flock still positive = vaccine did not work?

• Was biosecurity optimal?
• What about sources of *Salmonella*? Infection pressure?

Can work because less gut colonization, shedding, organ spread ... but only combined with good biosecurity measures
What can we expect in the future?

• *Salmonella* will remain a problem because of
  
  • Globalization and trade
  • Intensification of poultry production
  • Antibiotic resistance?
  • The asymptomatic nature of the infection (food poisoning strains)
  • Specific virulence traits
  • New serotypes, strains

Monitoring and control is essential and cannot be weakened!
What can we expect in the future?

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Monitoring and control is essential and cannot be weakened!

Technologically, platforms for serotype, strain or flock-specific vaccines are easy to set up.