Controlling non-host specific *Salmonella* in poultry using vaccination and feed additives

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Non-host specific *Salmonella*: what is the problem?

- Zoonosis: transmission to humans
- One of the most important foodborne pathogens worldwide
- Animals carry the bacteria, do not get ill
EU prevalence!
Human *Salmonella* prevalence EU - 2006

![Graph showing the prevalence of *Salmonella* infections by month and serovar.

- **S. Enteritidis** (orange line with square markers)
- **S. Typhimurium** (black line with circle markers)
- **Other serovars** (blue line with square markers)

The graph illustrates the number of cases per 100,000 population from January to December 2006, with peaks observed in August and September.
Human *Salmonella* prevalence EU - 2006

<table>
<thead>
<tr>
<th>Serovar</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteritidis</td>
<td>90,362</td>
<td>62.5</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>18,685</td>
<td>12.9</td>
</tr>
<tr>
<td>Infantis</td>
<td>1,246</td>
<td>0.9</td>
</tr>
<tr>
<td>Virchow</td>
<td>1,056</td>
<td>0.7</td>
</tr>
<tr>
<td>Newport</td>
<td>730</td>
<td>0.5</td>
</tr>
<tr>
<td>Hadar</td>
<td>713</td>
<td>0.5</td>
</tr>
<tr>
<td>Stanley</td>
<td>522</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Derby</td>
<td>477</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Agona</td>
<td>367</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Kentucky</td>
<td>357</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

Cases per 100,000 population
2005 – data from WHO

ENTERITIDIS worldwide pandemic!
Where is Enteritidis coming from?
Where is Enteritidis coming from?
Outbreaks of *Salmonella enterica* serotype Enteritidis infection in the United States, with confirmed vehicle, by egg status, 1985–2003 (Braden, 2006)
Laying hens

- High *Salmonella* prevalence
- Enteritidis most prevalent serotype
- Transmission to eggs: specifically *Enteritidis*!
Salmonella Enteritidis flock prevalence
Salmonella Enteritidis predominantly contaminates eggs!!!
Laying hen flocks

FOCUS should be on controlling *Salmonella* serotype ENTERITIDIS

of 17 November 2003
on the control of salmonella and other specified food-borne zoonotic agents

D. Specific requirements concerning flocks of laying hens

1. With effect from 72 months after entry into force of this Regulation, eggs must not be used for direct human consumption (as table eggs) unless they originate from a commercial flock of laying hens subject to a national programme established under Article 5 and not under official restriction.

2. Eggs originating from flocks with unknown health status, that are suspected of being infected or from infected flocks may be used for human consumption only if treated in a manner that guarantees the elimination of all salmonella serotypes with public health significance in accordance with Community legislation on food hygiene.

3. When birds from infected flocks are slaughtered or destroyed, steps must be taken to reduce the risk of spreading zoonoses as far as possible. Slaughtering must be carried out in accordance with Community legislation on food hygiene. Products derived from such birds may be placed on the market for human consumption in accordance with Community legislation on food hygiene and, once applicable, part E. If not destined for human consumption, such products must be used or disposed of in accordance with Regulation (EC) No 1774/2002.
Routes of egg contamination

• Outer shell contamination

Fecal contamination!

REDUCE SHEDDING!
Routes of egg contamination

- Internal egg contamination
  - Eggshell and membrane penetration
  - Reproductive tract colonization
Internal egg contamination

Reproductive tract colonization

Depending on colonization place: incorporation in egg white, yolk or albumen

PREVENT SYSTEMIC SPREAD!
Prevention/control in layers

- 1 serotype to focus on
- Reduce shedding
- Decrease systemic spread

VACCINATION !!!
Vaccination of laying hens

Vaccination

active immunisation

live vaccines

inactivated vaccines

passive immunisation

maternal transferred antibodies

antibody containing egg powder
What is the ideal vaccine strain?

- a high degree of protection against systemic and intestinal infection
- against a variety of important serovars (serogroups)
- adequate attenuation for poultry, other animal species, humans and the environment as well as animal welfare issues
- the vaccines should not affect growth of the animal
- vaccine strains should not be resistant to antibiotics
What is the ideal vaccine strain?

- Vaccines should be easy to administer and need to have markers facilitating the differentiation from *Salmonella* wild-type strains.

- Application of vaccines should not interfere with *Salmonella* detection methods.

- Humoral antibody response after vaccination should be distinguishably from a *Salmonella* wild-type response to allow the use of serological detection methods.
Inactivated vaccines

- a vaccine made from an infectious agent that has been inactivated or killed in some way
- parenteral administration to the single animal
- induction of (mostly) high antibody titres (detectable in blood and yolk)
- correlation between antibody titre and „level of protection“ ?
Inactivated vaccines

• administration of *Salmonella*-inactivated vaccines to laying hens during production is possible
  
  – Not allowed in registration files
  – Drop in egg production

• Safe with regard to spread of vaccine strain
EXAMPLE: SALENVAC

- An inactivated *S. enteritidis* phage type 4 vaccine
- Reduces both horizontal and vertical transmission of *S. enteritidis*.
- Grown under conditions of iron restriction, resulting in an improved immune response of chickens to natural infection with *S. enteritidis*.
- Aluminium Hydroxide Gel as an adjuvant: safe for birds and safe for operators in case of accidental self-injection
- A proven tool for the elimination of *S. enteritidis* in both poultry meat and eggs

Nobilis Salenvac represents an advance in vaccine technology. One vaccine, developed for both breeders and layers with an excellent safety profile and proven efficacy.
Example: Nobilis Salenvac

• **Description**
  – Inactivated vaccine poultry vaccine
  – Contains formalin killed cells of *Salmonella* Enteritidis phage type 4
  – Aluminium hydroxide gel as adjuvant

• **Indication**
  – Induces active and passive immunity against *S. Enteritidis* in layers and breeders
  – Aimed at reduction of *Salmonella* in eggs and poultry meat

• **Administration**
  – Intramuscular injection into the leg or breast muscle
  – The breast muscle is recommended for broiler breeders to avoid the risk of leg injuries
Example: Nobilis Salenvac

- **Vaccination schedule**
  - **Normal vaccination schedule:**
    Twice 1 single dose of 0.5ml
    Age: 10-12 weeks first, 14-18 weeks second administration
  - **High risk of early infection:**
    Triple dose
    Age: 0.1 ml at day-old, 0.5 ml 4 weeks, booster dose 14-18 weeks
Example: Nobilis Salenvac

- Protection
  - Protection develops after 2nd vaccination
  - Full protection about 2 weeks after completion of regime
  - Last vaccination should therefore be done at least 2 weeks prior to transfer, because infection is likely to occur during or directly after transfer.
Some experimental data

• Woodward data !
• Clifton-Hadley data !
Example: autologous vaccines

Isolate a local strain of *Salmonella* spp. from a poultry house or animals

+ Kill culture by heat, formalin, etc. and add adjuvants

produce a specific inactivated vaccine for a particular poultry farm
Live vaccines

- oral administration via drinking water (natural route of „infection“)

- induction of a local intestinal humoral and cellular immune response, production of secretory IgA

- weaker serological antibody response

- because of the excretion of live *Salmonella* vaccine strains after administration, no use during egg production
Example: Nobilis SG 9R
Example: Nobilis SG 9R

• **Description**
  – Live attenuated vaccine
  – Based on S. Gallinarum strain 9R (rough strain)

• **Indication**
  – Active immunisation of layers against S. Gallinarum and S. Enteritidis

• **Administration and vaccination schedule**
  – Subcutaneous in the neck at week 6 and week 14-16
Example: AviPro Salmonella VacE

FOR LONG-LASTING PROTECTION

- High immunogenicity – imitates natural route of infection
- Drinking water: easy and cost-effective alternative to injections
- Long-lasting protection
- Does not survive in the environment
- Does not interfere with monitoring programmes and management practices

(*) Due to differences in registration and licensing processes for each different country, the product may not be under the new name in certain areas.
Example: AviPro *Salmonella* VacE

- **Description**
  - Live attenuated *Salmonella* vaccine
  - Contains metabolic drift mutant bacteria of *S. Enteritidis Sm24/Rif12/SSq*

- **Indication**
  - Long-lasting protection in layers

- **Administration**
  - Oral via drinking water
Example: AviPro Salmonella VacE

• Vaccination schedule
  – At day-old, 4 weeks, 16 weeks

• Protection
  – Protection develops a few days post-vaccination
  – Protection throughout the production cycle (at least until 60 weeks of age)
Oral immunisation of laying hens with the live vaccine strains of TAD Salmonella vac® E and TAD Salmonella vac® T reduces internal egg contamination with Salmonella Enteritidis

Inne Gantois *, Richard Ducatelle, Leen Timbermont, Filip Boyen, Lotte Bohez, Freddy Haesebrouck, Frank Pasmans, Filip van Immerseel
Some experimental data

- 4 groups of animals:
  - Non-vaccinated
  - AviPro Salmonella VacE day 1, week 4 and 16
  - AviPro Salmonella VacT day 1, week 4 and 16
  - AviPro Salmonella VacE/T day 1, week 4 and 16

- Challenge infection intravenous with $10^{exp7}$ cfu of a Salmonella Enteritidis field strain

- Sampling eggs for 3 weeks
- Euthanasia 3 weeks post-infection: bacteriological analysis of internal organs
Some experimental data

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen</th>
<th>Oviduct</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23/29(^a) (0(^b) A</td>
<td>15/29 (1) A</td>
<td>23/29 (14) A</td>
</tr>
<tr>
<td>TAD Salmonella vac(^E)</td>
<td>12/28 (0) B</td>
<td>6/28 (1) B</td>
<td>16/28 (12) AB</td>
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<tr>
<td>TAD Salmonella vac(^T)</td>
<td>15/30 (0) B</td>
<td>4/30 (0) B</td>
<td>17/30 (9) AB</td>
</tr>
<tr>
<td>TAD Salmonella vac(^E)/TAD</td>
<td>9/30 (0) B</td>
<td>4/30 (0) B</td>
<td>11/30 (7) B</td>
</tr>
<tr>
<td>Salmonella vac(^T)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reduction of internal organ colonization, including reproductive tissues, even after iv infection!
Some experimental data

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15/35 A</td>
<td>7/35 A</td>
<td>6/35 A</td>
</tr>
<tr>
<td>TAD Salmonella vac® E/TAD Salmonella vac® T</td>
<td>1/35 B</td>
<td>0/35 B</td>
<td>0/35 B</td>
</tr>
</tbody>
</table>

Values with different letters are statistically significant different from each other within the same column ($P < 0.05$).

* Number of positive batches/total number of batches.

* Statistically different from the control animals at $P < 0.1$.

Reduced egg contamination!
EU legislation

COMMISSION REGULATION (EC) No 1177/2006

of 1 August 2006

implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella in poultry

1. Live salmonella vaccines shall not be used in the framework of national control programmes where the manufacturer does not provide an appropriate method to distinguish bacteriologically wild-type strains of salmonella from vaccine strains.
EU legislation

COMMISSION REGULATION (EC) No 1177/2006

of 1 August 2006

implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella in poultry

2. Live salmonella vaccines shall not be used in the framework of national control programmes in laying hens during production unless the safety of the use has been demonstrated and they are authorised for such purpose in accordance with Directive 2001/82/EC.
EU legislation

COMMISSION REGULATION (EC) No 1177/2006

of 1 August 2006

implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella in poultry

3. Vaccination programmes against Salmonella enteritidis reducing the shedding and contamination of eggs, shall be applied at least during rearing to all laying hens at the latest from 1 January 2008 on in Member States as long as they did not demonstrated a prevalence below 10 % based on the results of the baseline study in accordance with Article 1 of Commission Decision 2004/665/EC or based on the monitoring to follow up the Community target, set in accordance with Article 4(1) of Regulation (EC) No 2160/2003.
Salmonella Enteritidis flock prevalence

VACCINATION!!
Salmonella food poisoning in humans in Belgium

Start vaccination of laying hens

The following univariate analysis was carried out on this subset of data of the clean dataset (15,484 samples out of 2,212 holdings).

The design-based analysis takes account of the 8 countries and 2,212 holdings.

<table>
<thead>
<tr>
<th></th>
<th>Number of samples</th>
<th>S. Enteritidis positive samples</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>flocks</td>
<td>13,979</td>
<td>1,799 (12.9)</td>
</tr>
<tr>
<td>unvaccinated against SE</td>
<td></td>
<td>[11.8 - 13.9]</td>
</tr>
<tr>
<td>flock vaccinated against SE</td>
<td>1,505</td>
<td>60 (4.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[1.9 - 6.1]</td>
</tr>
</tbody>
</table>

The S. Enteritidis proportion positive sample is higher in flocks unvaccinated against SE, compared to flocks vaccinated against SE.
Broilers

- A variety of serotypes
- Also Enteritidis and Typhimurium
E. Specific requirement concerning fresh meat

1. With effect from 84 months after entry into force of this Regulation, fresh poultry meat from animals listed in Annex I may not be placed on the market for human consumption unless it meets the following criterion:

   'Salmonella: absence in 25 grams'

2. Within 72 months of entry into force of this Regulation, detailed rules for this criterion will be laid down in accordance with the procedure referred to in Article 14(2). These will specify, in particular, sampling schemes and analytical methods.

3. The criterion laid down in paragraph 1 does not apply to fresh poultry meat destined for industrial heat treatment or another treatment to eliminate salmonella in accordance with Community legislation on food hygiene.
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>% Pos</td>
<td>N</td>
<td>% Pos</td>
<td>N</td>
</tr>
<tr>
<td><strong>At slaughter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>6432</td>
<td>9.4</td>
<td>228</td>
<td>5.7</td>
<td>-</td>
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<tr>
<td>Denmark</td>
<td>775</td>
<td>1.9</td>
<td>1,174</td>
<td>2.3</td>
<td>1,472</td>
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<tr>
<td>Estonia</td>
<td>52</td>
<td>0</td>
<td>56</td>
<td>8.9</td>
<td>62</td>
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<tr>
<td>Latvia</td>
<td>1,081</td>
<td>6.9</td>
<td>39</td>
<td>5.1</td>
<td>70</td>
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<tr>
<td>Spain</td>
<td>93</td>
<td>15</td>
<td>203</td>
<td>13.8</td>
<td>151</td>
</tr>
<tr>
<td>Sweden</td>
<td>3,369</td>
<td>0.1</td>
<td>3,506</td>
<td>0</td>
<td>3,730</td>
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<tr>
<td>Norway</td>
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<td>&lt;0.1</td>
<td>6,056</td>
<td>&lt;0.1</td>
<td>7,239</td>
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<tr>
<td><strong>At retail</strong></td>
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<td></td>
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<tr>
<td>Belgium</td>
<td>40</td>
<td>7.5</td>
<td>46</td>
<td>2.2</td>
<td>126</td>
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<tr>
<td>Greece</td>
<td>-</td>
<td>-</td>
<td>33</td>
<td>18.2</td>
<td>25</td>
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<tr>
<td>Latvia</td>
<td>-</td>
<td>-</td>
<td>96</td>
<td>11.5</td>
<td>345</td>
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<tr>
<td>Spain</td>
<td>294</td>
<td>3.4</td>
<td>400</td>
<td>3.8</td>
<td>495</td>
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<tr>
<td>Sweden</td>
<td>-</td>
<td>-</td>
<td>196</td>
<td>4.1</td>
<td>197</td>
</tr>
</tbody>
</table>
Salmonella prevalence in broiler flocks

EU mean
Serotypes data EFSA 2006

Broilers

- S. Enteritidis
- S. Hadar
- S. Infantis
- S. Livingstone
- S. Montevideo
- S. Blockley
- S. Havana
- S. Kentucky
- S. Virchow
- S. Paratyphi B var. Java
- S. Corvallis
- S. Heidelberg
- S. Typhimurium
- S. Agona
- S. Gallinarum
- S. Indiana
- S. Senftenberg
- S. Mbandaka
- Other serotypes
COMMISSION REGULATION (EC) No 646/2007

of 12 June 2007


(Text with EEA relevance)

1. The Community target, as referred to in Article 4(1) of Regulation (EC) No 2160/2003, for the reduction of *Salmonella enteritidis* and *Salmonella typhimurium* in broilers (Community target) shall be a reduction of the maximum percentage of flocks of broilers remaining positive of *Salmonella enteritidis* and *Salmonella typhimurium* to 1 % or less by 31 December 2011.
Reduce prevalence!!

Prevalence *Salmonella* (Enteritidis + Typhimurium)
Controlling *Salmonella* on meat

- Good cooking principles
- Decontamination meat
- Slaugterhouse hygiene
- Primary production measures: prevent entry of *Salmonella* to slaugterhouse
Controlling *Salmonella* in primary production

- **Difficulties:**
  - Young slaughter age
  - Susceptibility immediate post-hatch
  - Variation of serotypes
  - Housing type
Controlling *Salmonella* in primary production

- Difficulties:
  - Young slaughter age
  - Susceptibility immediate post-hatch
  - Variation of serotypes
  - Housing type

Classical vaccination not useful!
PRIMARY PRODUCTION MEASURES

- Feed additives
- Vaccination
- Biosecurity
- Hygiene
- Disinfection
- Monitoring
- Management
- People
- Rodents
- Litter
- Air
- Food
- Insects
- Transport
- Water


Feed additives for use in broilers

- Antibiotics
- Competitive exclusion products
- Probiotics
- Prebiotics
- Short and medium-chain fatty acids
- Plant extracts - botanicals
of 22 September 2003
on additives for use in animal nutrition

Phasing out

1. With a view to a decision on the phasing out of the use of coccidiostats and histomonostats as feed additives by 31 December 2012, the Commission shall submit to the European Parliament and the Council before 1 January 2008 a report on the use of these substances as feed additives and available alternatives, accompanied, where appropriate, by legislative proposals.

2. By way of derogation from Article 10 and without prejudice to Article 13, antibiotics, other than coccidiostats and histomonostats, may be marketed and used as feed additives only until 31 December 2005; as from 1 January 2006, those substances shall be deleted from the Register.
Antibiotics for *Salmonella* control

**COMMISSION REGULATION (EC) No 1177/2006**

of 1 August 2006

implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella in poultry

**Use of antimicrobials**

1. Antimicrobials shall not be used as a specific method to control salmonella in poultry.

Reasons: antibiotic resistance, induction of carrier animals
Table AB SA 4. Antimicrobial resistance in *Salmonella* spp. from broiler meat, 2006

<table>
<thead>
<tr>
<th>Country</th>
<th>Monitoring program</th>
<th>N</th>
<th>Ampicillin</th>
<th>Cefotaxime</th>
<th>Chloramphenicol</th>
<th>Ciprofloxacin</th>
<th>Gentamicin</th>
<th>Nalidixic acid</th>
<th>Streptomycin</th>
<th>Sulfonamide</th>
<th>Tetracycline</th>
<th>Trimethoprim</th>
<th>Fully sensitive to &gt;4 antimicrobials</th>
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<tbody>
<tr>
<td>Austria</td>
<td>-</td>
<td>100</td>
<td>6.0</td>
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<td>0</td>
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<td>48.1</td>
<td>1.9</td>
<td>81.5</td>
<td>75.9</td>
<td>-</td>
<td>66.7</td>
<td>54</td>
<td>3.7</td>
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<tr>
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<td>10.3</td>
<td>0</td>
<td>13.2</td>
<td>10.3</td>
<td>26.5</td>
<td>17.7</td>
<td>23.5</td>
<td>1.5</td>
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<td>Total, N</td>
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<td>720</td>
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<td>1</td>
<td>7</td>
<td>33</td>
<td>1</td>
<td>366</td>
<td>304</td>
<td>122</td>
<td>323</td>
<td>175</td>
<td>187</td>
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<tr>
<td>Total, %</td>
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<td>1.0</td>
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<td>0.1</td>
<td>50.8</td>
<td>42.2</td>
<td>16.9</td>
<td>44.9</td>
<td>24.3</td>
<td>26.0</td>
<td>10.8</td>
</tr>
</tbody>
</table>

EFSA, 2006
Competitive exclusion (CE) products

- freeze-dried products derived from healthy, pathogen-free birds

- It prevents colonization of the gastro-intestinal tract of poultry by pathogenic (harmful) bacteria

- occupying all of the ecological niches in the gut of the birds and so denying pathogenic bacteria space to establish themselves, SCFA production, …
CE products - application

- Day-old chicks
- Poultry after treatment with antibiotics, creating an imbalance in the normal gut flora
- Birds that are stressed due to relocation, laying or moulting.
Example: Aviguard

- White free flowing powder that is easily dispersible in water
- Storage temperature 2°C to 8°C
- Contains over 200 normal avian gut flora micro-organisms
- Shelf life from manufacture is 18 months
- The product is packaged in foil laminate sachets sufficient for 2000, 5000 or 10,000 chicks
- Product can be applied by spray treatment or drinking water application
Example: Aviguard

Effect of Aviguard on the intestinal colonisation of day-old chicks by Salmonella Enteritidis Ref. 1

- Control
- Aviguard

% positive birds

- Day 10: 67%
- Day 21: 40%
- Day 43: 47%

Aviguard given at day-old
Challenge: 5 founder chicks / 50 birds, inoculated with 5.10⁴ S. Enteritidis PT4 at day 2
Example: Aviguard

Treatment with Baytril and Aviguard of 32 commercial broiler breeder flocks infected with S. Enteritis. (1.1.1994 - 1.4.1995, NL) Ref. 3

Before treatment

After 1 treatment success rate 72%

After 2 treatments success rate 93%

- Salmonella Enteritidis positive
- Salmonella Enteritidis negative
- Flocks not retreated due to age of the flock
Competitive exclusion (CE) products

• Disadvantage:
  – Microbiota undefined
    • Possibility of pathogens in products
    • Possibility of antimicrobial resistance gene transmission

→ Not very commonly used !!
Probiotics

- Live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance
- Defined products
- On the market:
  - Mainly lactobacilli
In vivo characterization of *Lactobacillus johnsonii* FL9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry

R.M. La Ragione¹, A. Narbad², M.J. Gasson² and M.J. Woodward¹

¹Department of Bacterial Diseases, Veterinary Laboratories Agency (Weybridge), Addlestone, Surrey, UK, and ²Food Safety Science Division, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK

Protection much less pronounced compared to CE products!

Trying multi-species defined products??
Prebiotics

• non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health
• Mono-, di-, oligo- or polysaccharides
• Natural or synthetic
Prebiotic examples: mannose / MOS

Type I fimbriae
Prebiotic examples: mannose / MOS

Mannose will block adhesion!
Prebiotic examples: mannose / MOS

Figure 2. *Faecal* Salmonella enteritidis counts from three groups of 20 chicks fed either the control diet or supplemented diets containing 0.1% β-mannose or 0.1% β1-4 mannobiase. Chicks were inoculated orally with $2 \times 10^7$ cfu SE/ml and shedding monitored up to 19 d post infection (d.p.i.). Mean SE log$_{10}$ CFU/g ± S.E.M. (n = 5). *a*Significantly different from control. *b*Significantly different from Mannose (MAN) (P ≤ 0.05, Tukey-Kramer Test, Graphpad Instat 3™).
Prebiotic examples: mannose / MOS

| Days post-infection | Liver | | | | | | Caecal contents | | | |
|---------------------|-------|---|---|---|---|---|---|---|---|---|---|---|---|
|                     | 7     | 14 | 23 | | | | 7     | 14 | 23 | | | | |
| Liver               |       |   |   | | | |       |   |   | | | | |
| MAN                 | <0.01 (0/5)<sup>a</sup> | 1.15±0.66 (2/5)<sup>a</sup> | 2.46±0.05 (5/5)<sup>a</sup> | | | | 7.09±0.82 (5/5) | 3.73±1.21 (2/5) | 2.89±1.10 (2/5) | | | | |
| MNB                 | 1.64±0.71 (2/5)<sup>a</sup> | 1.61±0.48 (2/5)<sup>a</sup> | 1.76±0.54 (5/5)<sup>a</sup> | | | | 5.39±0.71 (5/5) | 4.77±0.55 (5/5) | <0.01 (0/5)<sup>a</sup> | | | | |
| Control             | 4.89±0.29 (5/5) | 5.90±0.21 (5/5) | 6.71±0.51 (5/5) | | | | 5.85±0.75 (5/5) | 4.99±0.69 (3/5) | 6.34±1.98 (3/5) | | | | |

Table 2. The effect of dietary β1–4 mannanbiose or D-mannose provided for two weeks after hatching on the numbers of Salmonella enteritidis in the liver and caecal contents of broilers infected at 15 d of age<sup>1</sup>
Prebiotic examples: many more …

- Beta-glucans: would induce immunity
- FOS (fructo-oligosaccharides), inulin: bifidogenic
- GOS
- Guar gum
- Many, many more …

- Working action? Benefit?
Acidic compounds

• Short-chain fatty acids (SCFA)
  – Formic, acetic, propionic, butyric acid

• Medium-chain fatty acids (MCFA)
  – Caproic, carpic, caprylic acid

• Combination of acids
  – Example: butyric and capric acid
SCFA to control *Salmonella*

- Drinking water additives:
  - Effects limited to water itself and crop

- Feed additives:
  - Effects can be long-lasting dependent on formulation (powder, coating)

\[\text{Resorption} \quad \rightarrow \quad \text{Gradual release} \quad \rightarrow \quad \text{Effect in crop} \quad \rightarrow \quad \text{Effect in whole GI tract}\]
In vivo trial

Five groups of 20 chickens:

- CTRL
- FORMIC
- ACETIC
- PROPIONIC
- BUTYRIC

Infection at day 5 with $5 \times 10^3$ cfu S. Enteritidis
Euthanasia at day 8

Bacteriological analysis of caeca, liver and spleen: titration on BGA
Caecal colonization

CTRL FOR ACE BUT PROP

mean log cfu/g

CTRL FOR ACE BUT PROP
### Number of animals colonizing the caeca at day 8 of life

<table>
<thead>
<tr>
<th></th>
<th>CTRL (n=20)</th>
<th>ACE (n=20)</th>
<th>PROP (n=20)</th>
<th>BUT (n=20)</th>
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</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td>0*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$x &lt; 10^2 \text{ cfu/g}$</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td><strong>11</strong></td>
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<tr>
<td>$10^2 &lt; x &lt; 10^3 \text{ cfu/g}$</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>$10^3 &lt; x &lt; 10^4 \text{ cfu/g}$</td>
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<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$10^4 &lt; x &lt; 10^5 \text{ cfu/g}$</td>
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<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>$10^5 &lt; x &lt; 10^6 \text{ cfu/g}$</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>$10^6 &lt; x &lt; 10^7 \text{ cfu/g}$</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>More than 10^7 \text{ cfu/g}</strong></td>
<td>1</td>
<td><strong>13</strong></td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Number of animals
In vivo trial – butyric acid

Five groups of 20 chickens:

CTRL              POWDER                  COATED                  COMBI

butyric acid 0.63g/kg

Infection at day 5 with $5 \times 10^3$ cfu S. Enteritidis

Euthanasia at day 8

Bacteriological analysis of caeca, liver and spleen: titration on BGA
Colonization

Caecal colonization

number of animals (n = 25)

- Low
- Intermediate
- High

CTRL
POWDER
COATED
COMBI
Number of animals colonizing the caeca at day 8 of life

<table>
<thead>
<tr>
<th></th>
<th>CTRL (n=25)</th>
<th>POWDER (n=25)</th>
<th>COATED (n=25)</th>
<th>COMBI (n=25)</th>
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</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0*</td>
<td>0</td>
<td>2</td>
<td>1</td>
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<tr>
<td>$x &lt; 10^2$ cfu/g</td>
<td>6</td>
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<td>12</td>
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<tr>
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<td>1</td>
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<td>$10^4 &lt; x &lt; 10^5$ cfu/g</td>
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<td>1</td>
<td>0</td>
<td>6</td>
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<td>$10^5 &lt; x &lt; 10^6$ cfu/g</td>
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<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<td>More than $10^6$ cfu/g</td>
<td><strong>10</strong></td>
<td>12</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

* Number of animals
Broiler experiment – seeder model

Two groups of 40 chickens:

CTRL  POWDER  COATED  COMBI

butyric acid 0.63g/kg
Infection of 15 animals at day 5 with $5 \times 10^3$ cfu S. Enteritidis
Weekly cloacal swabs
Euthanasia at day 42
Bacteriological analysis of caeca, liver and spleen: titration on BGA
Broiler experiment – seeder model

excretion

% pos

CTRL

BUT

age

0 6 12 18 24 30 36 42
Microencapsulated Short-Chain Fatty Acids in Feed Modify Colonization and Invasion Early After Infection with *Salmonella* Enteritidis in Young Chickens

F. Van Immerseel,† V. Fievez,† J. de Buck,* F. Pasmans,* A. Martel,* F. Haesebrouck,* and R. Ducatelle*

Supplementation of Coated Butyric Acid in the Feed Reduces Colonization and Shedding of *Salmonella* in Poultry


Butyrate Specifically Down-Regulates *Salmonella* Pathogenicity Island 1 Gene Expression

I. Gantois,† R. Ducatelle,† F. Pasmans,† F. Haesebrouck,† I. Hautefort,‡ A. Thompson,‡ J. C. Hinton,‡ and F. Van Immerseel†
Butyric acid

• Also ‘fuel’ for epithelial cells
  – Longer villi
  – More absorptive surface
  – Faster repair mucosal damage

Better performance
Medium-chain fatty acids

Medium-Chain Fatty Acids Decrease Colonization and Invasion through hilA Suppression Shortly after Infection of Chickens with Salmonella enterica Serovar Enteritidis


Figure 1. Number of chicks with log cfu of Salmonella/g spleen as mentioned in the legend 3 days post infection with S. enteritidis 76Sa88
Vaccination of broilers?

- Passive immunisation – maternal antibodies

- Active vaccination?
  - Colonization - inhibition
Vaccination of broilers?

- Laying hens: live and killed vaccines available
  one serotype predominating (Enteritidis)
- Broilers: some specific problems with vaccination:
  - Young slaughter age (6 weeks)
  - Immature immune system
  - The induction of a specific antibody response takes about 2 weeks
  - Protective mucosal immunity at the intestinal level is difficult to achieve

Requirements:

A) Confer resistance in the immediate post-hatch period
B) Maintain long-term protective effects
C) Protect against multiple serotypes

Classical vaccination not applicable!
Colonization-inhibition (CI)

• Oral administration of *Salmonella* organisms to newly hatched chickens confers, within 24hrs of inoculation, a high degree of resistance against challenge infection with another Salmonella strain of the same serotype
Wild type strain

Caecal colonization

Wild type strain (inoculated day of hatch)

- Log cfu/g cecum vs. age (days)

0 5 10 15 20 25 30

0 2 4 6 8 10
Wild type strain as ‘CI’ strain

Wild type strain
Wild type ‘CI’ strain (76Sa88) (inoculated day of hatch)
Wild type challenge strain (147) (inoculated 24h later)

Caecal colonization
$\Delta hilA$ strain as ‘CI’ strain in seeder model

Wild type strain

$\Delta hilA$ strain as ‘CI’ strain
(inoculated day of hatch)

Wild type challenge strain (147)
(inoculated 24h later)
$\Delta hila$ strain as ‘CI’ strain in seeder model

Wild type strain

$\Delta hila$ strain as ‘CI’ strain
(inoculated day of hatch)

Wild type challenge strain (147)
(inoculated 24h later)

Conclusion: Reduced shedding up to slaughter age in seeder bird model !!
Colonization-inhibition using live attenuated vaccine strains

• Still at an experimental scale
• Applicable for broilers provided that:
  – Vaccine strains are safe
  – Vaccine strains are cleared at slaughter age

Still need additional attenuations!
Salmonella enterica serovar Enteritidis colonization of the chicken caecum requires the HilA regulatory protein

Lotte Bohez\textsuperscript{a,*}, Richard Ducatelle\textsuperscript{a}, Frank Pasmans\textsuperscript{a}, Nadine Botteldoorn\textsuperscript{b}, Freddy Haesebrouck\textsuperscript{a}, Filip Van Immerseel\textsuperscript{a}

Long-term colonisation–inhibition studies to protect broilers against colonisation with Salmonella Enteritidis, using Salmonella Pathogenicity Island 1 and 2 mutants

Lotte Bohez\textsuperscript{a,*}, Richard Ducatelle, Frank Pasmans, Freddy Haesebrouck, Filip Van Immerseel

The effect of oral administration of a homologous hilA mutant strain on the long-term colonization and transmission of Salmonella Enteritidis in broiler chickens

Lotte Bohez\textsuperscript{a,*}, Jeroen Dewulf\textsuperscript{b}, Richard Ducatelle\textsuperscript{a}, Frank Pasmans\textsuperscript{a}, Freddy Haesebrouck\textsuperscript{a}, Filip Van Immerseel\textsuperscript{a}
Conclusion for broiler feed additives

• Will not completely eliminate *Salmonella*

• Have to be used in an overall control plan
  – Hygiene, biosecurity, management, ….

• Can contribute to decreased shedding and colonization
Overall conclusion

- Tools are available to decrease shedding, colonization, meat and egg contamination

- Multiple control strategies should be used in combination to be effective
Thanks for your attention!