Prevalence of *Salmonella* and *Campylobacter spp.* following the discontinued use of antimicrobial growth promoters in broilers and swine in Denmark

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AGP usage in Denmark

- 1970’s: AGPs widely used in food production
- 1970’s: EU directive restricting use
- May 1995: Avoparcin banned in Denmark
- Jan 1998: Virginiamycin banned in Denmark
AGP usage in Denmark

- February 1998, Danish cattle and broiler industries voted to stop all use of AGP’s
- Pig industry withdrew use of all AGP’s in pigs >35 kg
- Remaining use of AGP’s in pigs phased out during 1999

![Graph showing AGP usage in Denmark from 1990 to 2000](image)
Producer Concerns

- Decreased productivity
- Increased morbidity and mortality
- Increased therapeutic consumption of antimicrobials
- Increase in *Salmonella* infected herds and contaminated meat
  → Pathogen Load
### Pathogen Load Studies

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Study</th>
<th>Antibiotics/Combinations</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>Evangelisti et al. (1975)</td>
<td>S. Typhimurium</td>
<td>Oxytetracycline</td>
</tr>
<tr>
<td></td>
<td>Holmberg et al. (1984)</td>
<td>S. Infantis</td>
<td>Avoparcin, Monesin</td>
</tr>
<tr>
<td></td>
<td>Bolder et al. (1999)</td>
<td>S. Enteritidis</td>
<td>Flavophospholipol, Salinomycin</td>
</tr>
<tr>
<td>Swine</td>
<td>Girard et al. (1976)</td>
<td>S. Typhimurium</td>
<td>Oxytetracycline + Neomycin</td>
</tr>
<tr>
<td></td>
<td>Williams et al. (1978)</td>
<td>S. Typhimurium (resistant/sensitive)</td>
<td>Chlortetracycline (sensitive)</td>
</tr>
<tr>
<td></td>
<td>Ebner/Matthew (2000)</td>
<td>S. Typhimurium</td>
<td>Apramycin/Oxytetracycline</td>
</tr>
</tbody>
</table>

Objective

• To examine the effect of discontinued use of antimicrobial growth promoters on pathogen load in Danish food production animals
  – *Salmonella* in broilers and swine
  – *Campylobacter* in broilers
Surveillance and control programs in Denmark

- Feed compounds
  - *Salmonella* in feeding stuff
- Primary production
  - *Salmonella* and *Campylobacter* in broilers
  - *Salmonella* in layers
  - *Salmonella* in slaughter pigs
  - BSE in cattle
- Slaughterhouses
  - *Salmonella* in pork and beef
  - *Salmonella* in broilers
- Retail level
  - *Salmonella*, *Campylobacter*, *Yersinia enterocolitica*, and *E. coli* O157 in food

Total No. of control samples
> 3 million/year
Sample collection

Broiler flocks

• *Salmonella:*
  – AM- sock samples 3 weeks before slaughter
  – PM- neck skin samples at slaughter

• *Campylobacter:*
  – Cloacal swab samples of 10 birds per flock at slaughter

Swineherds

• *Salmonella:*
  – Serological test of meat juice samples
  – Monthly slaughterhouse samples
Analysis

Broilers: AM
- 1/95
- 12/97

Broilers: PM
- 1/96
- 10/97
- 1/99
- 10/00
- 1/99
- 12/00
- 1/00
- 12/01

Broilers: Campylobacter
- 1/96
- 12/97
- 1/99
- 12/00
- 1/00
- 12/01

Swine
- 1/96
- 12/97

Pork
- 1/97
- 12/97
- 1/00
- 12/00

• Excluded: 1998 (broilers); 1998 and 1999 (swine)
• A t-test for comparisons of means
Salmonella in Broilers

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>14.4 (3.7-33.6)</td>
<td>2.4 (0.2-5.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PM</td>
<td>17.0 (8.1-38.8)</td>
<td>4.9 (0.7-24.9)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Percent positive flocks

AM and PM data shown in the chart.
Campylobacter in Broilers

Before
35.3 (11.4-64.8)

After
40.8 (18.0-77.0)

Excluded

Percent positive flocks

Jan Jul Jan Jul Jan Jul Jan Jul Jan Jul Jan Jul

0.2470
Salmonella in Swine and Pork

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td>5.0 (4.2-6.2)</td>
<td>3.3 (2.5-4.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pork</td>
<td>1.1 (0.5-1.8)</td>
<td>0.8 (0.4-1.5)</td>
<td>0.0290</td>
</tr>
</tbody>
</table>
Limitations

• Short time periods = small sample size
• Focuses primarily on *Salmonella spp.*
• Looks at combined effect of all antibiotics
• Does not account for other factors that might explain decreasing trend
Conclusion

• Increase in pathogen load?  **NO!**
  – Decreased levels *Salmonella* in broilers and swine
  – No change in levels *Campylobacter* in broilers

• Can decreases be explained by withdrawal of AGPs?  **???
  – Likely due to control programs, but role of growth promoters cannot be discounted

• Is additional research needed?  **Maybe**
More Information

www.vetinst.dk
Thank you
### Salmonella surveillance 1999

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Authority</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed stuffs</td>
<td>7,000</td>
<td>PD</td>
</tr>
<tr>
<td>Herds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Poultry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>central rearing</td>
<td>160,000</td>
<td>VFA</td>
</tr>
<tr>
<td>parent stock</td>
<td>480,000</td>
<td>VFA</td>
</tr>
<tr>
<td>hatcheries</td>
<td>10,000</td>
<td>VFA</td>
</tr>
<tr>
<td>layers</td>
<td>250,000</td>
<td>VFA</td>
</tr>
<tr>
<td>broilers</td>
<td>250,000</td>
<td>VFA</td>
</tr>
<tr>
<td>- Pigs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>breeders</td>
<td>36,000</td>
<td>Private</td>
</tr>
<tr>
<td>slaughter</td>
<td>800,000</td>
<td>VFA</td>
</tr>
</tbody>
</table>
## Salmonella surveillance 1999

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Authority</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Broilers</td>
<td>200,000</td>
<td>VFA</td>
</tr>
<tr>
<td>- Pork</td>
<td>30,000</td>
<td>VFA</td>
</tr>
<tr>
<td>- Beef</td>
<td>3,000</td>
<td>VFA</td>
</tr>
<tr>
<td>Manufacturing</td>
<td>20,000</td>
<td>VFA</td>
</tr>
<tr>
<td>and retail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humans</td>
<td>120,000</td>
<td>Min. Health</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,375,000</strong></td>
<td></td>
</tr>
</tbody>
</table>
### Salmonella sampling program for poultry, 2000

<table>
<thead>
<tr>
<th>Age/time</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central rearing</td>
<td>Day old 10 crates + 20 chicks&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>(broiler and table-egg)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; week 40 chicks</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week 2 pairs sock samples</td>
</tr>
<tr>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt; week 60 faecal samples&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>8&lt;sup&gt;th&lt;/sup&gt; week 2 pairs sock samples</td>
</tr>
<tr>
<td></td>
<td>2 weeks before movement 60 faecal samples +</td>
</tr>
<tr>
<td></td>
<td>60 blood samples&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breeders (hatching-egg</td>
<td>Every 2nd week 50 chickens or meconium</td>
</tr>
<tr>
<td>production)</td>
<td>from 250 chickens&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Every week 2 pairs sock samples</td>
</tr>
<tr>
<td>Hatchery</td>
<td>After each hatching Wet dust</td>
</tr>
</tbody>
</table>

<sup>1</sup> Requirements of the EU Zoonosis Directive (92/117/EEC)
## Salmonella sampling program for poultry, 2000

<table>
<thead>
<tr>
<th>Age/time</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rearing flocks</strong> (table-egg)</td>
<td></td>
</tr>
<tr>
<td>Day old</td>
<td>10 crates + 20 chicks₁</td>
</tr>
<tr>
<td>3rd week</td>
<td>10 sock samples or 300 faecal samples</td>
</tr>
<tr>
<td>12th week</td>
<td>10 sock samples or 300 faecal + 60 blood</td>
</tr>
<tr>
<td><strong>Table egg production</strong></td>
<td></td>
</tr>
<tr>
<td>Every 9th week for egg packing center</td>
<td>2 pairs sock samples or faecal + egg samples</td>
</tr>
<tr>
<td>Every 6 mos. for sale at barnyard</td>
<td>2 pairs sock samples or faecal + egg samples</td>
</tr>
<tr>
<td><strong>Broilers</strong></td>
<td></td>
</tr>
<tr>
<td>3 weeks prior to slaughter</td>
<td>5 pairs sock-samples</td>
</tr>
<tr>
<td>At slaughter</td>
<td>5 pooled samples of 10 neck skin samples per flock</td>
</tr>
</tbody>
</table>
Salmonella control in broilers and table-egg producers

- Flocks testing positive for Salmonella under routine exam placed on suspicion of infection and re-tested
- If second set of samples positive, infected breeder and rearing flocks slaughtered and eggs to heat treatment
- More frequent (4 week) testing of non-infected layer flocks
- Cleaning/disinfection of houses prior to introduction of new flocks
**Salmonella control of Danish slaughter pig herds**

- Continuous testing of all herds producing >100 finishers per year
- Serological exam of 8-60 samples of meat juice per herd quarterly
- Diagnostic method: mix-ELISA technique, based on LPS-antigen factors (O:1,4,5,6,7,12)
- Based on the proportion of sero-reactors each herd is assigned to one of three status levels
  - Level 1: No or few sero-reacters, no intervention required
  - Level 2: Higher proportion of sero-reacters, owner seek advice
  - Level 3: High proportion sero-reacter, owner seek advice and slaughter under special hygienic precautions
Campylobacter control in poultry

- Initiated in 1998 (broilers, hens, ducks) and 1999 (turkeys)
- Ten birds from each flock examined by cloacal swabs at slaughter
- 1998-99: special study Campylobacter prevalence in broilers from different production categories
Surveillance of foodborne zoonoses in Denmark

DANIZO

Veterinary practice
Private Laboratories
Slaughter plants
Regional Food Control Laboratories
Regional hospital laboratories
General practice

Diagnostic submission

Danish Veterinary Laboratory

Danish Veterinary and Food Administration

Statens Serum Institut

Danish Zoonosis Centre

Samples
Data
Isolates
Data
Samples
Data
 Samples
Data
 Samples
Data
 Samples
Data
 Samples
Data
 Samples
Data
 Samples
Data
 Samples
Data
Surveillance of antimicrobial resistance Denmark

**DANMAP**

- **Veterinary practice**
  - Samples
  - Diagnostic submission
  - Isolates

- **Private Laboratories**

- **Slaughter plants**

- **Regional Food Control Laboratories**

- **Regional hospital laboratories**

- **General practice**
  - Samples

- **Danish Veterinary Laboratory**
  - Samples
  - Isolates
  - Data

- **Danish Veterinary and Food Administration**
  - Isolates
  - Data

- **Statens Serum Institut**
  - Samples
  - Isolates / data
  - Data

- **Danish Zoonosis Centre**
  - Data

**Food animals**

**Foods**

**Humans**
## Pathogen Load Studies-Broilers*

<table>
<thead>
<tr>
<th>Study</th>
<th>Pathogen(s)</th>
<th>Antimicrobial(s)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evangelisti et al. (1975)</td>
<td><em>S.</em> Typhimurium</td>
<td>Oxytetracycline</td>
<td>&lt;</td>
</tr>
<tr>
<td>Gustafson et al. (1981)</td>
<td><em>S.</em> Typhimurium</td>
<td>Avoparcin</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virginiamycin w/ monesin</td>
<td>No effect</td>
</tr>
<tr>
<td>Abou Youssef et al. (1982)</td>
<td><em>S.</em> Typhimurium</td>
<td>Virginiamycin</td>
<td>No effect</td>
</tr>
<tr>
<td>Holmberg et al. (1984)</td>
<td><em>S.</em> Infantis</td>
<td>Avoparcin</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monesin</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoparcin + Monesin</td>
<td>&gt;</td>
</tr>
<tr>
<td>Hinton et al. (1986)</td>
<td><em>Salmonella</em></td>
<td>Monesin sodium</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Furazolidone</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penicillin</td>
<td>&gt;</td>
</tr>
<tr>
<td>Barrow et al. (1989)</td>
<td><em>S.</em> Typhimurium</td>
<td>Avoparcin</td>
<td>&gt;</td>
</tr>
<tr>
<td></td>
<td>Other <em>Salmonella</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolder et al. (1999)</td>
<td><em>S.</em> Enteritidis</td>
<td>Flavophospholipol Salinomycin</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td><em>C.</em> jejuni</td>
<td></td>
<td>No effect</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Pathogen</th>
<th>Antimicrobial(s)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridges et al. (1952)</td>
<td>Total bacteria, enterobacteriaceae</td>
<td>Penicillin, Streptomycin</td>
<td>&gt; No effect</td>
</tr>
<tr>
<td>Evangelisti et al. (1975)</td>
<td>S. Typhimurium</td>
<td>Oxytetracycline</td>
<td>No effect</td>
</tr>
<tr>
<td>DeGeeter et al. (1976)</td>
<td>S. Typhimurium</td>
<td>Lincomycin</td>
<td>No effect</td>
</tr>
<tr>
<td>Girard et al. (1976)</td>
<td>S. Typhimurium</td>
<td>Oxytetracycline + Neomycin</td>
<td>&lt;</td>
</tr>
<tr>
<td>Williams et al. (1978)</td>
<td>S. Typhimurium (resistant/sensitive)</td>
<td>Chlortetracycline</td>
<td>&gt; resistant &lt; sensitive</td>
</tr>
<tr>
<td>Jacks et al. (1988)</td>
<td>S. Typhimurium</td>
<td>Efrotomycin</td>
<td>No effect</td>
</tr>
<tr>
<td>Ebner/Matthew (2000)</td>
<td>S. Typhimurium</td>
<td>Ceftiofur sodium/oxytetracycline, Apramycin/oxytetracycline, Carbadox/oxytetracycline</td>
<td>No effect &lt; No effect</td>
</tr>
</tbody>
</table>