The Future of the One Health Approach: From Tracing Foodborne Pathogens and Spoilers to Mobile Genetic Elements and from Farm to Fork via the Environment

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One Health

• **One Health** is "the collaborative effort of multiple disciplines — working locally, nationally, and globally — to attain optimal health for people, animals and the environment". (USA, 2008)

• One Health’ (OH) is an integrated approach to health that focuses on the interactions between animals, humans and their diverse environments. It encourages collaborations, synergies and cross-fertilisation of all professional sectors and actors in general whose activities may have an impact on health. (EU, 2013)
History

- On Airs, Waters, and Places". Hippocrates (c. 460 BCE – c. 370 BCE)
- Zoonosis. Rudolf Virchow (1821–1902)
- "One Medicine“. Calvin W. Schwabe (1927–2006)
- "One Health“. the Washington Post (2003)
- One Health Platform:
  - 1st International One Health Congress, 2011 Melbourne , Australia
  - One Health journal (2015)
One Health

• Rigid way of thinking
• Holistic view
  – Flow/transfer of pathogens, commensals/indicators and genes of concern
  – Use of appropriate monitoring technology & mechanistic studies (WGS)
  – evidence based control strategies
  – Consequences of interventions in the whole agrifood chain
One Health

Modified from Chee-Sanford (2008)
Tracing foodborne zoonotic pathogens

Modified from Chee-Sanford (2008)
Tracing zoonotic foodborne pathogens in the animal food production chain

*Salmonella* on pig carcasses: positive pigs and cross contamination in the slaughterhouse

Botteldoorn et al. Journal of Applied Microbiology 2003

Tracing foodborne zoonotic pathogens

Modified from Chee-Sanford (2008)
Effect of intervention measures

Phage Typing of *Salmonella* Enteritidis Isolated from Layers and Humans in Belgium from 2000–2010, A Period in which Vaccination of Laying Hens was Introduced

Layer hen isolates

Period 1; 2000–2004: before the implementation of vaccination
Period 2; 2005–2006: voluntary vaccination
Period 3; 2007–2010: mandatory vaccination

Dewaele et al. 2013 Zoonoses and Public Health

Human isolates

Bertrand et al. 2015. WIV-ISP
Multiresistant *Salmonella* Typhimurium

12 pig farms in Flanders (Belgium): 137 S. Typhimurium isolates

Rasschaert et al. 2012, J. Food Protection 75: 859-866
MDR *Salmonella* Typhimurium DT104 WGS Scottish isolates

Bayesian maximum clade credibility phylogenetic tree and most probable ancestral state reconstruction of host population for *S. Typhimurium* DT104 in Scotland.
135 human isolates 113 animal isolates 1990-2011
A. E. Mather et al. Science 2013;341:1514-1517
Distinguishable epidemics of MDR S. Typhimurium DT104 in animal & human hosts

- Large proportion of transmission within each host population, but relatively few animal-to-human or human-to-animal transmissions
- Larger genetic diversity (AMR) in human isolates
- Majority of human infections in Scotland unlikely to be sourced from the local animal population
- Hypothesis:
  - Imported food, foreign travel, environmental reservoirs as significant sources for *Salmonella* and AMR in humans in Scotland
WGS of MRSA CC398

Use of tetracycline and cephalosporins in food animal production

Use of zinc (& other metals) in animal feed formulations

MSSA CC398 Livestock (pigs)

MSSA CC398 humans

Tetracycline resistance

Methicillin resistance

SCCmec cassette

Decreased capacity for human colonization, transmission and virulence

Partial SCC mec excision

MRSA CC398 Livestock (pigs)

Co-selection

Loss of human associated Immunomodulatory genes (prophage ϕSa3)

One Health

Modified from Chee-Sanford (2008)
Antibiotic resistance

Transfer of resistance genes

Livestock
- ABR Genetic Selection
- ABR Horizontal Gene Transfer
- Gut Commensals
- ABR Gene
- Zoonotic pathogens
- Antibiotic resistance

Human
- ABR Genetic Selection
- ABR Horizontal Gene Transfer
- Gut Commensals
- ABR Gene Vertical Inheritance

Soil
- Bacteria
- Groundwater
- Surface Water

Plants

Manure

Waste Holding Lagoon/Pit

Land application
- Load
- Bacteria

Modified from Chee-Sanford (2008)
Acquired antimicrobial resistance: two ways of spreading

**TWO WAYS TO SPREAD**
Vertical and horizontal transfer mechanisms can spread resistance to antibiotics in bacteria.

**Vertical transfer**
- Chromosome
- Plasmid

The chromosomes and plasmid are replicated during cell division and both offspring cells get complete copies.

**Horizontal transfer**
- Plasmid is copied and transferred to recipient cell.

Plasmid provides recipient cell with antibiotic resistance.

Cannon B. 2014, Nature 509: S6-S8
Mobile genetic elements tool box

Integrative Conjugative/mobilisable elements (ICE)

Genomic islands

For example Salmonella Genomic Island 1 (SGI 1): 43 kb chromosomal region

Cambray et al. Mobile DNA 2011 2:6

Genetic map of Erythromycin Resistance-Conferring Plasmid pRSB105, Isolated from a Sewage Treatment Plant
Extended spectrum β-lactamase (ESBL) diversity at broiler farms

295 ceftiofur-resistant *E. coli* isolates from 5 Belgian broiler farms

<table>
<thead>
<tr>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM-52</td>
<td>TEM-52</td>
<td>CMY-2</td>
<td>TEM-52</td>
<td>TEM-52</td>
</tr>
<tr>
<td>CMY-2</td>
<td>CTX-M-1</td>
<td>CMY-2</td>
<td>CTX-M-1</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>CMY-2</td>
<td></td>
</tr>
</tbody>
</table>

Smet et al. 2008
ESBL-gene transfer from commensal *E. coli* to human *E. coli* gut flora

- *In vitro* simulation of human colon (continuous fermentation system)
- Inoculation with a $\text{bla}_{\text{TEM-52}}$ plasmid carrying *E. coli* poultry strain
- Appearance of human *E. coli* transconjugants

**Graph:**
- CFU/ml
- E. coli (human)
- B1-54
- transconjugants
- Inoculation of ESBL-donor strain
- Cefotaxime administration

Resistome/mobilome (WGS) = the collection of transferable resistance genes in an ecosystem:

Example: simulated pig cecum with 4 mg/l of doxycycline

DNA extraction

Plasmid isolation

Whole Genome Shotgun Sequencing

Mapping against a resistance gene database

De Mulder T. (unpublished results)
Heat resistance & mobile genetic elements

**Bacillus subtilis** (Berendsen et al. 2016)

**Salmonella Senftenberg** ATCC 43845
TLPQC (transmissible loci for protein quality control)
Nguyen et al. 2017
From farm to fork via the environment

Modified from Chee-Sanford (2008)
Antibiotic residues in swine manure

antibiotic residue concentrations (ppb; μg/kg) recovered from swine manure samples from the manure pit at different Belgian farms

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Farm 1a</th>
<th>Farm 1b</th>
<th>Farm 2a</th>
<th>Farm 2b</th>
<th>Farm 3a</th>
<th>Farm 3b</th>
<th>Farm 4</th>
<th>Farm 5</th>
<th>Farm 6</th>
<th>Farm 7</th>
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</thead>
<tbody>
<tr>
<td>Sulfadiazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>762</td>
<td>3881</td>
<td>23</td>
<td>21</td>
<td>&lt;1</td>
<td>217</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>&lt;1</td>
<td>6</td>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>8</td>
<td>73</td>
<td>131</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>44</td>
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<tr>
<td>Colistin B</td>
<td>626</td>
<td>&gt;20000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1434</td>
</tr>
<tr>
<td>Colistin A</td>
<td>1319</td>
<td>&gt;20000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1982</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>1335</td>
<td>&gt;20000</td>
<td>&gt;20000</td>
<td></td>
<td>1221</td>
<td>227</td>
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<td></td>
<td>1399</td>
<td>&gt;20000</td>
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<tr>
<td>Tylosin A</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

Manure treatment

Antibiotic residues (µg/kg)

Manure slurry

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sulfadiazine</th>
<th>Doxycycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadiazine</td>
<td>4.61</td>
<td>231.11</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>195.57</td>
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<tr>
<td>Sulfadiazine</td>
<td>8.68</td>
<td>7744.58</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>9.09</td>
<td></td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0</td>
<td>327.6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>66</td>
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<tr>
<td>Sulfadiazine</td>
<td>4.4</td>
<td>196.5</td>
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<tr>
<td>Doxycycline</td>
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</table>

Watery fraction

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sulfadiazine</th>
<th>Doxycycline</th>
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</thead>
<tbody>
<tr>
<td>Sulfadiazine</td>
<td>7.98</td>
<td>139.37</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>8.11</td>
<td>298.71</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>10.3</td>
<td>2540.81</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0</td>
<td>397.9</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0</td>
<td>135.2</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0</td>
<td>124.6</td>
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</tbody>
</table>

Solid fraction

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sulfadiazine</th>
<th>Doxycycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim</td>
<td>5</td>
<td>663.21</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0</td>
<td>1658.3</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0</td>
<td>79.03</td>
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<tr>
<td>Sulfadiazine</td>
<td>2.3</td>
<td>14496.80</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Trimethoprim</td>
<td>1.3</td>
<td>665.7</td>
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<tr>
<td>Sulfadiazine</td>
<td>NA</td>
<td>1456.6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>2.9</td>
<td>764.1</td>
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<td>Sulfadiazine</td>
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<td>2191.1</td>
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<tr>
<td>Doxycycline</td>
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Biological treatment

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sulfadiazine</th>
<th>Doxycycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim</td>
<td>1.81</td>
<td>37.48</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>1.95</td>
<td>17.39</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>2.26</td>
<td>25.65</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>5</td>
<td>364.6</td>
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<tr>
<td>Sulfadiazine</td>
<td>3.4</td>
<td>292.6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>2.9</td>
<td>13.5</td>
</tr>
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</table>

Lagoon
## Manure treatment

### Zoonotic pathogens

<table>
<thead>
<tr>
<th>Manure slurry</th>
<th>Watery fraction</th>
<th>Biological treatment</th>
<th>Lagoon</th>
</tr>
</thead>
</table>

### Table:

<table>
<thead>
<tr>
<th>Salmonella (CFU/g)</th>
<th>Campylobacter coli (CFU/g)</th>
<th>E.coli (log CFU/g)</th>
<th>Salmonella (kve/g)</th>
<th>Campylobacter coli (kve/g)</th>
<th>E.coli (log kve/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-10^2</td>
<td>-</td>
<td>4,09</td>
<td>-</td>
<td>-</td>
<td>0-1*</td>
</tr>
<tr>
<td>10-10^2</td>
<td>-</td>
<td>3,74</td>
<td>-</td>
<td>-</td>
<td>0-1*</td>
</tr>
<tr>
<td>1-10</td>
<td>-</td>
<td>4,93</td>
<td>-</td>
<td>-</td>
<td>-1.0*</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>3,85</td>
<td>-</td>
<td>-</td>
<td>0-1*</td>
</tr>
<tr>
<td>0.1-1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2-3*</td>
</tr>
</tbody>
</table>

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P2-32: Tina Van den Meersche
Tracing spoilers in the agrifood chain

Contamination routes of spores of *Bacillus cereus* (black arrows) and *Clostridium tyrobutyricum* (grey arrows) in the dairy production chain. The importance of contamination sources and routes is indicated by the size of arrow.

Conclusions

• Tracing of pathogens/spoilers = **One Health/Quality**
• Tracking of antibiotic resistance and other relevant genes and of residues
• Outbreaks vs. epidemics
  – Flaws in agrifood chain
  – Bidirectional flow of zoonotic pathogens
  – Importance of other sources such as environment, imported food, travels
• Sanitary environmental regulations
• Control measures based on evidence not on belief/assumptions
Thanks to:

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- Geertrui Rasschaert
- Els Van Coillie
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- Els Daeseleire
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- Ellen Lambrecht
- Nadine Botteldoorn (WIV)
- Isabelle Dewaele (Poulpharm)
- Annemieke Smet (UA)
- Thijs De Mulder
- Tina Van den Meersche
- Helder Maertens
- Sharon Maes
- Laura Peeters (CODA)
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