Reduction of verotoxigenic *Escherichia coli* in fermented sausages

Askild Holck
Nofima AS, Norway
Contents

• Food borne outbreaks from fermented sausages
• The Norwegian outbreak from sausage in 2006
• Reduction of *E. coli* by changing production parameters
• Reduction of *E. coli* by post-processing treatments
## Reported food-borne outbreaks in Europe, 2009

<table>
<thead>
<tr>
<th>Organism/agent</th>
<th>Outbreaks</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>1502</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>1722</td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td>1043</td>
<td>Calicivirus, Hepatitis A + others</td>
</tr>
<tr>
<td>Bacterial toxins</td>
<td>558</td>
<td><em>Bacillus, Clostridium, Staphylococcus</em></td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>333</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>214</td>
<td>Mushroom toxins, marine biotoxins, histamine, mycotoxins + others</td>
</tr>
<tr>
<td>Pathogenic <em>E. coli</em></td>
<td>75</td>
<td>228 cases, 62 hospitalisations</td>
</tr>
<tr>
<td>Other bacterial</td>
<td>52</td>
<td><em>Brucella, Listeria, Shigella, Vibrio, Yersinia</em></td>
</tr>
<tr>
<td>Parasites</td>
<td>51</td>
<td><em>Trichinella</em> + others</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5550</strong></td>
<td>~49,000 cases, ~4,400 hospitalisations, 46 deaths</td>
</tr>
</tbody>
</table>
Characteristics of EHEC

- EHEC is a subgroup of VTEC
- Verotoxins (shiga-like toxins) similar to those in *Shigella dysenteriae*, Stx1 and/or Stx2
- *eae* intimin gene for attachment
- Bloody diarrhea
- Haemolytic anemia, acute renal failure (uremia) and low platelet count (thrombocytopenia)
- Low infectious dose (5-2000 bacteria)
- *E. coli O157:*H7
- (O26, O45, O103, O111, O121, O145)
- Meat, poultry, ground beef, fermented sausages, dairy products, apple, juice/cider, potatoes, lettuce, sprouts, mayonnaise, drinking water

Pedestal formation upon binding to gut
Recent EHEC outbreak from bean sprouts

- May 21st: Germany reports large outbreak
- *E. coli* O104:H4
- May 1st - June 28th: 3091 STEC cases, 838 HUS cases, 47 deaths
- May 24th: France reports outbreak
- By June 28th: 16 cases, 8 HUS cases, 0 deaths
- Outbreaks most likely linked to fenugreek seeds imported from Egypt
- By July 5th: 4178 cases, 48 deaths

*EFSA, 2011*
Cost of food-borne outbreaks

- Tragedy for the sick and relatives
- Costly work to identify source
- Costly recalls of foods
- Costly destruction of food (e.g. Spanish cucumbers)
- Loss of confidence
- Loss of market shares
### Fermented sausage food outbreaks (not EHEC)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Product</th>
<th>Cases</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>Salami sticks</td>
<td>101</td>
<td>England</td>
<td>1989</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>Fermented pork</td>
<td>17</td>
<td>The Netherlands</td>
<td>1986</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>Salami</td>
<td>83</td>
<td>Italy</td>
<td>1998</td>
</tr>
<tr>
<td><em>C. botulinum</em></td>
<td>Ferm. beaver tail and paw</td>
<td>14</td>
<td>USA (Alaska)</td>
<td>2001</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>Salami</td>
<td>-</td>
<td>Poland</td>
<td>1998</td>
</tr>
<tr>
<td><em>Salmonella and Staphylococcus</em></td>
<td>Dry fermented sausage</td>
<td>-</td>
<td>The Netherlands</td>
<td>1987</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>Salami</td>
<td>-</td>
<td>USA (Philadelphia)</td>
<td>1989</td>
</tr>
<tr>
<td><em>Salmonella Kedougou</em></td>
<td>Salami</td>
<td>62</td>
<td>Norway</td>
<td>2006</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>Minisalami</td>
<td>-</td>
<td>Italy</td>
<td>2005</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>Salami</td>
<td>-</td>
<td>Spain</td>
<td>2007</td>
</tr>
</tbody>
</table>

Adapted from Moore, J.E. 2004
# Fermented sausage EHEC outbreaks

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Serotype</th>
<th>Cases*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1994</td>
<td>O157:H7</td>
<td>20/--/-</td>
<td>Salami</td>
</tr>
<tr>
<td>Australia</td>
<td>1995</td>
<td>O111:NM</td>
<td>53/23/1</td>
<td>Mettwurst</td>
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<tr>
<td>Germany</td>
<td>1995-96</td>
<td>O157:H-</td>
<td>300-600/28/-</td>
<td>Teewurst, Mortadella</td>
</tr>
<tr>
<td>Canada</td>
<td>1998</td>
<td>O157:H7</td>
<td>39/2/0</td>
<td>Genoa salami</td>
</tr>
<tr>
<td>Canada</td>
<td>1999</td>
<td>O157:H7</td>
<td>143/5/-</td>
<td>Hungarian-style salami</td>
</tr>
<tr>
<td>Sweden</td>
<td>2002</td>
<td>O157:H7</td>
<td>38/12/-</td>
<td>Cold smoked sausage</td>
</tr>
<tr>
<td><strong>Norway</strong></td>
<td>2006</td>
<td>O103:H25</td>
<td>17/10/1</td>
<td>Morr</td>
</tr>
<tr>
<td>Denmark</td>
<td>2007</td>
<td>O26</td>
<td>-</td>
<td>Dry fermented sausage (organic)</td>
</tr>
<tr>
<td>Italy</td>
<td>2007</td>
<td>O157</td>
<td>-</td>
<td>Dry fermented pork salami</td>
</tr>
</tbody>
</table>

*Reported cases/HUS/died

*Holck et al, Meat Science 2011*
**E. coli O103 food outbreak in Norway in 2006**

- 17 reported cases
- 10 hospitalised with HUS
- 1 death
- Found in 3 types of “morr” sausages
- Found in “fenalår” (dry cured meat from sheep)
- Traced to mutton diaphragm and mutton hearts

- pH=4.8-5.0, $a_w<0.90$
- Production time: approx. 21 days
- 3.5 tons produced Des. 2005 to March 2006
- Cost >12 mill. Euro in recalls and tracing
Regulations and recommendations

- The US Food Safety and Inspection Service requires a 5 log reduction in products containing beef.
- Canada: 5 log reduction recommended.
- Australia: 3 log reduction required.
- EU food safety through
  - (EC) No. 852/2004 Hygiene of Foodstuffs
  - (EC) No. 853/2004 Hygiene rules for food of animal origin
  - (EC) No. 854/2004 Organisation of official controls
  - (EC) No. 882/2004 Official controls for compliance
- Norway: voluntary adherence to specific guidelines
Fermented sausages

Main hurdles:
Low pH
Lactic acid
Low $a_w$
NaNO$_2$

- Lactic acid bacteria
- pH
- Water activity

Nissen, H. and Holck, A. Food Microbiol. 1998
Safe sausages: Interventions at different stages

Pre-process

Slaughter strategies
Quality of meat, ingredients etc.

Process

Recipe and process parameters:
salt, nitrite, fermentation temp., $a_w$ etc.

Post-process

• Storage
• Heat treatment
• High pressure etc.
Microbiological quality of raw materials

- Cattle main VTEC source
- *E. coli* O157 on hides 4.5% - 56% depending on country
- Prevalence retail meat (beef) 0-9%
- Prevalence meat for fermented sausage (beef) 0.12%
- Prevalence meat (mutton) up to 3%

- Reduction of contamination of raw meat => reduction of hazard in sausage
- Producers should develop guidelines for acceptable presence of total *E. coli*
- *E. coli* also from spices

See references in Holck et al Meat Science, 2011
Important considerations when changing production parameters

- Microbial safety must be ensured
- Sensory changes must be acceptable
- Changes must be feasible and simple to implement in industrial production
Importance of process parameters

- **NaCl, NaNO$_2$, glucose, fat, fermentation temperature, strain, sausage type**

Exp. 1
- 7 variables
- 80 sausages
- First screening
- Plackett-Burman design
- 4 replicates
- Omit variables
- Adjust levels

Exp. 2
- 6 variables
- 102 sausages
- Advanced screening
- Fractional factorial design, resolution IV
- 3 replicates
- Optimise recipe

Exp. 3
- 3 variables
- 48 sausages
- Validation
- Full factorial design
- 4 replicates

- **NaCl, NaNO$_2$, glucose, fat, casing diameter, sausage type**

- 3 defined recipes, 2 sausage types, 2 temperatures

*Heir et al. Int. J. Food Microbiol. 2010*
Sausage production
**E. coli** reductions from process parameter Exp. #2: fermentation at 20ºC

### Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exp. 2 parameters&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>NaCl (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>NaNO₂ (ppm)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90</td>
</tr>
<tr>
<td>Glucose (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35</td>
</tr>
<tr>
<td>Fat (%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.5;12.2</td>
</tr>
<tr>
<td>Casing diameter (mm)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43</td>
</tr>
</tbody>
</table>

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*Heir et al. Int. J. Food Microbiol. 2010*
**E. coli** reductions from process parameter Exp. #3

![Graph showing E. coli reduction with varying recipe and fermenting temperature](image)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exp. 3 recipes^\text{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td>NaCl (%)^\text{b}</td>
<td>3.6</td>
</tr>
<tr>
<td>NaNO₂ (ppm)^\text{b}</td>
<td>100</td>
</tr>
<tr>
<td>Glucose (%)^\text{b}</td>
<td>0.5</td>
</tr>
<tr>
<td>Fat (%)^\text{c}</td>
<td>14.6; 14.2</td>
</tr>
<tr>
<td>Casing diameter (mm)^\text{d}</td>
<td>43; 70</td>
</tr>
</tbody>
</table>

*Heir et al. Int. J. Food Microbiol. 2010*
Sensory evaluation of sausages with standard, moderate and high salt recipe

Preference test, 20 persons asked: "How much do you like or dislike this sausage?" Scale: 1-7

- Only small differences in preference between recipes
- Morr: Moderate and High recipe optimal in JAR test
- Salami: High recipe slightly too salty in JAR test

Fresh sausages (day 27), stored 3 months, 4°C
Different starter cultures give similar reduction in *E. coli*

- 13 starter cultures tested
- Fermenting salami batter
- Fermentations in 50 ml tubes

No differences in *E. coli* reductions were detected after 14 days.
Conclusions process parameter experiments

• Each parameter contributes only to a small change in reduction
• High NaCl, NaNO$_2$, glucose and fermentation temp. give higher $E. coli$ reduction
• Some synergy between NaCl and NaNO$_2$
• Some combinations inhibited the starter culture (high NaCl, low fermentation temp.)
• Only effect of NaNO$_2$ at >100 ppm
• $E. coli$ reduction increased from 1.5 log (“standard”) to 3 log (“high salt” recipe)
• Only small differences in sensory preference with different recipes
• Different starter cultures give similar reduction in $E. coli$
E. coli reduction by post-processing treatments

- Storage
- Freezing/thawing
- Heat treatment
- High pressure processing

Not tested:
Gamma irradiation
X-ray
Starter cultures with specific inhibiting properties
Additions (e.g., allyl isothiocyanate)
Reduction of *E. coli* after storage of standard Salami and Morr

Standard sausages stored at 4, 16 and 20°C for 1 and 2 months

![Boxplot of Reduction of *E. coli* (log)](image)

- 50 % of samples within box, 99.7 % within line region, * = outliers
- Temperature and time both contribute to *E. coli* reduction
- Relatively large variation within each treatment
- No changes in sensory properties after storage for 2 months
Reduction of *E. coli* after storage of experimental Salami and Morr at 20°C.

**Graph:**
- **Y-axis:** Reduction of *E. coli* (log)
- **X-axis:** Recipe
- **Legend:**
  - Orange: H (High salt)
  - Green: M (Moderate salt)
  - Blue: S (Standard recipe)

**Legend:**
- **Type:** Morr, Salami
- **Storage:** 1 Month, 2 Months

**Observations:**
- Sausages with different recipes, H: High salt, M: Moderate salt, S: standard recipe
- High and moderate salt recipes give higher reductions when storing at 20°C
- Little extra reduction when storing at 4°C
Reduction of *E. coli* by freezing and thawing

- Sausages vacuum packed, frozen and thawed (1x or 4x) and stored for 6 weeks (4°C, 20°C).
- < 1 log reduction by freezing
- Only small extra effects of several cycles of freezing-thawing
Reduction of *E. coli* by heat treatment

- Health Canada: time-temp combinations to obtain 5 log reduction
- 43 °C, up to 24 h
- Average of 11 food outbreak strains
- Heat treatment gives 4 extra log reduction in addition to fermentation reduction
Sensory evaluation of heat treated Morr sausage

• 3 different heat treatments
• Scored for overall acceptability (preference), redness, salt, fattiness, texture
• 270 respondents (total)
• Scale: 1 - 7
• Little difference in scores for both salami and morr
• Similar results after 2 months storage at 4°C

Heat treatments:
1: Control (no heat)
2: 32°C, 6 days
4: 43°C, 24 h
5: 43°C+ 53°C, 1h+ 6h
Heat treated Norwegian salami

No heat 43°C 4 days 43°C, 2h 27°C, 8h No heat
52°C, 7h 38°C, 24h 43°C, 24h Rancid (?)

Slight smearing of fat particles observed
Summary of some post-processing treatments

Production condition

E. coli reduction (log)

- Standard process
- Modified process
- Storage 4°C, 1 month
- Storage 20°C, 1 month
- Freezing/thawing 1x
- Heat treatment 43°C, 24 h
High Pressure Processing of fermented sausages

- Packaged products are placed in steel cylinder.
- Water pump creates pressure of 4000 - 6000 atm.
- Bacteria die from cell membrane damage when pressure is released

NC Hyperbaric, Spain
Reduction of *E. coli* in HPP sausages

- Salami and Morr treated 3x6000 atm or 1x6000 atm.
- High salt and standard recipe tested
- 2.7 to 3.3 log reduction of *E. coli*
- Little effect of HPP on lactic acid bacteria

*Omer et al. Meat Sci. 2010*
Sensory analysis of HPP sausages

- Sensory preference by pair-wise comparisons
- 8 sausage producers as panelists
- 6 types of sausage tested
- No differences in sensory properties after HPP treatment

A: Moose sausage
B: Jubel salami
C: Spicy salami
D: Sognemorr
E: Special salami
F: Stabbur sausage

Omer et al Meat Sci. 2010
## E. coli strain characterisation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serogroup/ type</th>
<th>stx profile</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O157:H7</td>
<td>1, 2</td>
<td>Outbreak USA, 1982</td>
</tr>
<tr>
<td>2</td>
<td>O157:H7</td>
<td>2</td>
<td>Outbreak Sweden, 2002</td>
</tr>
<tr>
<td>3</td>
<td>O157:H-</td>
<td>2</td>
<td>Outbreak Norway, 2009</td>
</tr>
<tr>
<td>4</td>
<td>O145</td>
<td>1</td>
<td>Sporadic (Human, clinical)</td>
</tr>
<tr>
<td>5</td>
<td>O111:H-</td>
<td>1, 2</td>
<td>Outbreak Australia, 1995</td>
</tr>
<tr>
<td>6</td>
<td>O103:H25</td>
<td>2</td>
<td>Outbreak Norway, 2006 (Human, clinical)</td>
</tr>
<tr>
<td>7</td>
<td>O103:H25</td>
<td>neg</td>
<td>Outbreak Norway, 2006 (sausage)</td>
</tr>
<tr>
<td>8</td>
<td>O103:H?</td>
<td>1</td>
<td>Sporadic (Human, clinical)</td>
</tr>
<tr>
<td>9</td>
<td>O26</td>
<td>1</td>
<td>Sporadic (Human, clinical)</td>
</tr>
<tr>
<td>10</td>
<td>OR:H-</td>
<td>neg</td>
<td>Apathogenic</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>neg</td>
<td>Lab strain (C-600) containing Cm(^R) phage</td>
</tr>
</tbody>
</table>
Reduction of 11 different *E. coli* strains in standard salami

- Two sausage productions, 3 replicates from each
- Reductions vary from 1.3 to 2.4 log
- Strains with different letters show different reduction
## Summary post-production measures for 11 *E. coli* strains

<table>
<thead>
<tr>
<th>Condition</th>
<th>Duration</th>
<th>Average <em>E. coli</em> reduction (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating (43 °C)</td>
<td>24 h</td>
<td>4.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Freezing*</td>
<td>1 month, 20 °C</td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Storage</td>
<td>2 months, 20 °C</td>
<td>3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Freezing*</td>
<td>1 month, 16 °C</td>
<td>3.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heating (43 °C)</td>
<td>6 h</td>
<td>2.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Storage</td>
<td>2 months, 16 °C</td>
<td>2.4&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Storage</td>
<td>1 month, 20 °C</td>
<td>2.2&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heating (43 °C)</td>
<td>3 h</td>
<td>1.9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Storage</td>
<td>1 month, 16 °C</td>
<td>1.5&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Freezing*</td>
<td>1 month, 4 °C</td>
<td>1.3&lt;sup&gt;fg&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heating (43 °C)</td>
<td>1.5 h</td>
<td>1.2&lt;sup&gt;fg&lt;/sup&gt;</td>
</tr>
<tr>
<td>Storage</td>
<td>2 months, 4 °C</td>
<td>1.2&lt;sup&gt;gh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Freezing*</td>
<td>24 h</td>
<td>1.0&lt;sup&gt;gh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Storage</td>
<td>1 month, 4 °C</td>
<td>0.8&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Freezing: 24h, -18°C
Possible implementation in the food industry

<table>
<thead>
<tr>
<th>Measure</th>
<th>Advantage</th>
<th>Disadvantage</th>
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<tbody>
<tr>
<td>Change recipe/fermentation</td>
<td>Avoiding process changes</td>
<td>Limited reduction</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td>Sensory changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Robustness</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>Robust</td>
<td>Sensory changes (?)</td>
</tr>
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<td>Logistics changes</td>
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<tr>
<td>High Pressure processing</td>
<td>Rapid</td>
<td>Costly entrance</td>
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<td>Logistics changes</td>
</tr>
<tr>
<td>Storage</td>
<td>Simple</td>
<td>Robustness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Storage costs</td>
</tr>
<tr>
<td></td>
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<td>Reduced shelf-life in food stores</td>
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<tr>
<td>Freezing/thawing</td>
<td>Simple</td>
<td>Limited reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Robustness</td>
</tr>
<tr>
<td></td>
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<td>Logistics changes</td>
</tr>
</tbody>
</table>
Conclusions

• Only limited improvements in VTEC reductions (1-2 log) can be obtained by optimisation of recipe and process parameters

• Effective post-processing strategies for reduction of VTEC in sausages include:
  – Mild heat treatments
  – Storage at elevated temperatures (16-20 °C)
  – High pressure processing (600 MPa)
  – (Freezing and thawing)

• Treatments do not negatively affect sensory quality
• Animalia (Meat research institute, Norway)
  – Mohamed Omer Abdella
  – Ole Alvseiike
  – Miguel Prieto (Univ. Léon, Spain)
• Norwegian Veterinary College
  – Per Einar Granum
  – Trine L’Abee-Lund
• Nofima
  – Ahmed Abdelghani
  – Lars Axelsson
  – Birgitta Baardsen
  – Janina Berg
  – Even Heir
  – Askild Holck
  – Tone Mari Rode
  – Anette Wold Åsli
  – Martin Høy
  – Ingrid Måge

Financing