EHEC O104:H4 in Germany 2011:
Large outbreak of bloody diarrhea and haemolytic uraemic syndrome by
Shiga toxin-producing E.coli via contaminated food

Reinhard Burger
( for the HUS investigation team)

Institute of Medicine, Forum on Microbial Threats
Robert Koch Institute units involved

- Department Infectious Disease Epidemiology
  Prof. Gerard Krause and many colleagues
  (e.g. Dr. Frank, Dr. Werber, Prof. Stark, Dr. Buchholz)
- Department Infectious Diseases
  Prof. Martin Mielke, Dr. Angelika Fruth
- RKI-Consultant Laboratory for HUS / EHEC
  Prof. Helge Karch, Münster
## Epidemic profile (final report, Sept. 9, 2011)

<table>
<thead>
<tr>
<th>Outbreak cases</th>
<th>N</th>
<th>Age (median)</th>
<th>% Female</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHEC</td>
<td>2.987</td>
<td>46 years</td>
<td>58%</td>
<td>18 (0,6%)</td>
</tr>
<tr>
<td>HUS</td>
<td>855</td>
<td>42 years</td>
<td>68%</td>
<td>35 (4,1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;20%</td>
<td>&gt;♀</td>
</tr>
</tbody>
</table>

**Total** 3.842 53

### Comparison with previous years

- **EHEC:** ~1,000 illnesses/ year  
  median age: 5 years
- **HUS:** 65 illnesses/ year  
  median age: 2 years
Incidence of HUS by likely county of infection (cases / 100,000)
**EHEC/HUS: International situation**
( July 11, 2011 )

<table>
<thead>
<tr>
<th>Country</th>
<th>EHEC</th>
<th>HUS</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden</td>
<td>35</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Denmark</td>
<td>15</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>France</td>
<td>10</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Single cases in 12 additional European countries in US ( 2/4/1 ) and Canada ( 1/0/0 )
Time line of epidemiological studies
(according to date of initiation)

Source: Robert Koch Institute, 20.6.2011

Several cohort studies
2nd online-survey on food consumption
1st online-survey on food consumption
3rd explorative interviews
2nd explorative interviews
4th case-control study
3rd case-control study
2nd case-control study
1st case-control study

Onset of diarrhea

First call from local health authority
Invitation from HH
First days: course of events I

- Thursday 19.05:
  - Call from local Public Health Department: invitation from Hamburg
  - RKI informs BfR (Federal Institute for Risk Assessment) and BMG (Federal Ministry of Health)

- Friday 20.05:
  - 1st team goes to Hamburg
  - Discussion of the situation with local public health authorities, first patient interviews

- Saturday 21.05:
  - First qualitative evidence on the role of vegetables is passed on to Food Safety Authorities
  - 1st case-control study

Source: Robert Koch Institute, 20.6.2011
**First days: course of events II**

- **Sunday 22.05:**
  - Analysis of the 1st case-control study
  - *First European Early Warning EWRS*+ information submitted to the WHO
  - Warning to local public health authorities
  - Interview with dpa (German Press Agency), possible role for uncooked vegetables

- **Monday 23.05:**
  - Activation of the Situation Room
  - Information on website
  - Preparations for 2nd case-control study

- **Tuesday 24.05:**
  - Press conference
  - *First official IHR notification*
  - Start of 2nd case-control study
First days: course of events III

Wednesday 25.05:
- Identification of the pathogen by RKI
- Interdisciplinary teleconference organised by the RKI
- Teleconference with state public health authorities
- Ministerial press conference
- Press conference BfR + RKI: results of the 2nd case-control study,
  Advice on food consumption:
  No raw tomato, cucumber, salad IN Northern Germany

Thursday 26.05:
- Information on website in English
- Epidemiological Bulletin
- Article in „Eurosurveillance“

Source: Robert Koch Institute, 20.6.2011
Cohort studies in disease clusters

- Over 30 cohorts investigated since June 1, 2011 to identify the vehicle of infection and further cases, e.g.
  - Cohort studies of travel groups (in cooperation with foreign authorities)
  - Cluster analysis of different restaurant-associated outbreaks
  - Analysis of billing data of guests at an affected canteen; results published on June 3, 2011 (press release RKI-BfR)
  - „Recipe-based restaurant cohort study“

Source: Robert Koch Institute, 20.6.2011
Recipe-based Restaurant Cohort Study

Goal: independent of patient memory
- 10 Cohorts (total 168) identified
- Dinner in same restaurant (May 12-16)
- 18% with bloody diarrhea or EHEC/HUS (31) within 14 days
- Questioned: which meals ordered (photographs as reminder)
- Booking/billing and group photos used for confirmation
- Chef of restaurant interviewed for detailed ingredients of dish

Relative risk of disease (RR):
14.2 times higher (univariate analysis) compared to people not served sprouts – All 31 patients had sprouts!

It is the sprouts!
### Cohort study 4: Univariable risk factor analysis

**Table 3. Relative Risk of Infection Associated with Sprouts and Other Raw Food Items in Univariable Analysis.**

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Total Subjects Evaluated</th>
<th>Subjects Exposed (Percent of Cohort)</th>
<th>Cases among Subjects Exposed (Attack Rate)</th>
<th>Subjects Not Exposed (Percent of Cohort)</th>
<th>Cases among Subjects Not Exposed (Attack Rate)</th>
<th>Relative Risk (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprouts</td>
<td>152</td>
<td>115 (76)</td>
<td>31 (27)</td>
<td>37 (24)</td>
<td>0</td>
<td>14.23 (2.55–∞)</td>
<td>0.001</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>152</td>
<td>50 (33)</td>
<td>14 (28)</td>
<td>102 (67)</td>
<td>17 (17)</td>
<td>1.68 (0.77–3.62)</td>
<td>0.18</td>
</tr>
<tr>
<td>Cucumbers</td>
<td>152</td>
<td>50 (33)</td>
<td>14 (28)</td>
<td>102 (67)</td>
<td>17 (17)</td>
<td>1.68 (0.77–3.62)</td>
<td>0.18</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>152</td>
<td>45 (30)</td>
<td>13 (29)</td>
<td>107 (70)</td>
<td>18 (17)</td>
<td>1.72 (0.77–3.71)</td>
<td>0.17</td>
</tr>
<tr>
<td>Radicchio</td>
<td>152</td>
<td>45 (30)</td>
<td>13 (29)</td>
<td>107 (70)</td>
<td>18 (17)</td>
<td>1.72 (0.77–3.71)</td>
<td>0.17</td>
</tr>
<tr>
<td>Lettuce</td>
<td>152</td>
<td>45 (30)</td>
<td>13 (29)</td>
<td>107 (70)</td>
<td>18 (17)</td>
<td>1.72 (0.77–3.71)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Cohort study 4: Methodology

- Guests: which meal(s) ordered/eaten?
- Chef: which ingredient in each menu item?
- Merging of the two databases
How sprouts were served...

(1) On top of the main dish

(2) As part of the side salad
Forward-/Backward-Tracing Strategy:
Links of clusters to distribution from one single farm
Fed. Inst. for Risk Assessment (Food Safety), July 5, 2011
Delivery channels of fenugreek seeds explain German and French EHEC outbreak

- horticultural farm in Lower Saxony
- retail in France
- seed producer
- intermediaries (colours: European States)
- outbreak clusters
- sprout delivery
- seed delivery

Status 12th July 2011
Common link between German and French outbreak

Fenugreek seeds (Trigonella foenum-graecum)
(including seed mixes and homegrown, risk of cross-contamination)

(Source Galster's Kräuter, Fürth)
## Comparable outbreaks

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Deaths</td>
<td>3-11</td>
<td>2</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Time to detection</td>
<td>&gt; 7 weeks</td>
<td>~ 4 weeks</td>
<td>~ 2 weeks</td>
</tr>
<tr>
<td>Time from detection to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>identification of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total duration</td>
<td>~ 12 weeks</td>
<td>~ 16 weeks</td>
<td>➥6 weeks</td>
</tr>
<tr>
<td>No</td>
<td>&gt; 12000</td>
<td>1500</td>
<td>&gt; 4000</td>
</tr>
</tbody>
</table>
Microbiological characterisation of EHEC O104:H4
Severe outbreak in Germany May 2011

EHEC O104:H4

- Rare EHEC serotype ( May 24 )
- Not described in animals previously
- Only rarely in humans ( total: 7 )

- Germany 2001 HUSEC041 ( Karch )
- Korea 2006
- Georgia 2009
- Finland 2010
Microbiological characterisation of EHEC O104:H4

Virulence markers

- Shigatoxin 1: - (negative)
- Shigatoxin 2 (vtx2a): + (positive)
- Intimin (eae): - (negative)
- Enterohemolysin (hly): - (negative)

Tests by PCR available: May 23, 2011, tests of two outbreaks isolates

Variant vtx2a of Shigatoxin 2: May 25, 2011, (Karch and Natl. Ref. Center RKI)
Microbiological characterisation of EHEC O104:H4

Bielaszewska et al, Lancet, June 2011

Virulence characteristics of enteroaggregative E. coli (1)

- Typical EAEC virulence plasmid with adhesion fimbriae type AAF/I
- First time described in EHEC
- Any other known EAEC or STEC/EAEC O104:H4 had AAF/III fimbriae
- Sequence data:
  Strong homology to an Enteroaggregative E. coli (55989)

Virulence combination two different pathogens!
Microbiological characterisation of EHEC O104:H4

- **ESBL resistance phenotype**
  - Unusual for intestinal E. coli
  - Allows use of corresponding selective media for targeted search
  - ESBL-plate with multiplex PCR screening for genes stx1 or alternatively stx2
  - stx2
  - rfbO0104
  - eae
  - fliCH4
Proposed scheme of the origin of the new *E. coli* pathotype

( Brzuszkiewicz, E et al, Arch. Microbiol., Doi 10.1007, June 2011 )
Bacteriological screening of sprouts and seeds and of production site

ALL samples negative!!

Exception: One single box with sprouts from incriminated producer, opened, in household with EHEC cases
Incriminated Fenugreek seed lot

- 15,000 kg
- Farm received another lot same day (same distributor)
- Lot distributed to 70 different companies (54 in Germany; 16 in 11 European countries)
- Still in supply chain?
- In households ("self sprouters")?
- Long survival of *E. coli* on dried seeds (years)
EHEC O104 : H4

**Incubation time:** 8 days (Median)

- Max.: 18 days !
- 75 %: 10 days

**Shedding time:** Up to 20 weeks ! !

- Longer ??
- Carrier status ?
Several secondary infections

- to household partners
- in hospitals
- via food distribution
- Laboratory infections

 Raised awareness!

End of outbreak:
announced: July 26, 2011