Vibrio spp. in recreational waters in the Netherlands

Ciska Schets
rationale

- *Vibrio* infections in Europe in 2006
  - Germany, Sweden: Baltic Sea
  - Denmark
  - The Netherlands: Oosterschelde

- unknown *Vibrio* status of Dutch bathing waters
  - ca. 700 official bathing sites
    > ca. 87% inland waters
    > ca. 13% coastal waters, mainly North Sea
  - biweekly monitoring for *E. coli* and enterococci from May 1st to October 1st

- climate change
climate change

● global warming
  – increase of atmospheric temperature
  – increase of water temperature

● enhanced growth of indigenous bacteria that multiply in the environment at elevated temperatures

● enhanced survival and growth opportunities for emerging species

● increased number of health conditions
  – higher concentrations
  – more exposure
  – other species
aim

● to quantify and type potentially human pathogenic *Vibrio* species in marine and fresh bathing waters
● to relate *Vibrio* numbers to environmental conditions
  – water temperature
  – salinity
● to relate *Vibrio* numbers to requirements in European bathing water legislation
● active surveillance for patients with *Vibrio* infections related to contact with recreational water
set up *Vibrio* monitoring

- funding for 5-year monitoring of Dutch bathing waters
  - starting in 2009
  - capture year-to-year weather variation
  - starting point for climate change study
- 6 official bathing sites
  - sampled during bathing season
- enumeration of total *Vibrio* spp.
- identification of *Vibrio* spp. present
- evaluation of detection method
sampling sites

- North Sea
  - Bergen aan Zee (2009-2012)
  - Katwijk aan Zee (2009-2012)
- Oosterschelde
  - St. Maartensdijk (2009-2012)
- Binnenschelde
  - Bergen op Zoom (2010-2012)
- IJsselmeer
  - Enkhuizen (2009)
- Wadden Sea
  - Harlingen (2011-2012)
Vibrio detection and identification

- ABPW enrichment - TCBS culture
- 10 characteristic colonies per sample
  - oxidase
  - O129 sensitivity
  - API 20E (adjusted for Vibrio spp.)
    - V. cholerae
      - O1/O139 antigen agglutination test
      - PCR for toxR and ctxA genes
    - V. parahaemolyticus
      - PCR for gyrB, tdh and trh genes
## Detection Method Evaluation

<table>
<thead>
<tr>
<th>Enrichment Condition</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>ABPW w/o NaCl, 36°C, 18-20 h</td>
<td>Enhance growth of <em>V. cholerae</em></td>
</tr>
<tr>
<td>36°C, 6-8 h</td>
<td>Cefas protocol</td>
</tr>
<tr>
<td>36°C, 18-20 h</td>
<td>Extended incubation time</td>
</tr>
<tr>
<td>41.5°C, 18-20 h</td>
<td>ISO/TS 21872-1</td>
</tr>
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</table>

### Culture Medium

<table>
<thead>
<tr>
<th>Culture Medium</th>
<th>Colonies Description</th>
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<tbody>
<tr>
<td>TCBS</td>
<td>Green/blue/yellow colonies</td>
</tr>
<tr>
<td>ChromID Vibrio (VID)</td>
<td>Blue-green/pink/colourless colonies</td>
</tr>
</tbody>
</table>
patient surveillance

● clinical microbiology labs in sampling areas (2009)
  - screened all submitted ear and wound swabs for *Vibrio* spp.
    › culture on TCBS
    › contact patient in case of positive sample
    › questionnaire about swimming in surface water

● local GP’s in Oosterschelde, St. Maartensdijk area (2010-2011)
  - 5 campsites and recreational areas
  - all patients with ear and wound conditions
    › swab samples to RIVM
    › questionnaires administered by
      Public Health Service Zeeland
results enrichment procedure

- **ABPW w/o NaCl, 36±2 C, 18-20 h**
  - no enhanced detection of *V. cholerae*
  - not applied after 2009
- **36±2 C, 6-8 h**
  - low incubation temperature: more *V. parahaemolyticus*
  - short incubation time: lower total *Vibrio* numbers
- **36±2 C, 18-20 h**
  - higher total *Vibrio* numbers
  - no increase of non-*Vibrio* spp.
- **41.5±1 C, 18-20 h**
  - more *V. cholerae*
- from 2011
  - all samples in ABPW at 36 C and 41.5 C, 18-20 h
total *Vibrio* numbers – 5 sites, 1 year
total *Vibrio* numbers – 1 site, 3 years

Oosterschelde TCBS, ABPW 41.5, 18-20 h, 2009-2011
**Vibrio** species, all sites, all years

<table>
<thead>
<tr>
<th>Location</th>
<th>Salinity (range)</th>
</tr>
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<tbody>
<tr>
<td>Oosterschelde</td>
<td>2.8-3.1%</td>
</tr>
<tr>
<td>Katwijk aan Zee</td>
<td>3.4-3.5%</td>
</tr>
<tr>
<td>Bergen aan Zee</td>
<td>3.4-3.5%</td>
</tr>
<tr>
<td>IJsselmeer</td>
<td>0.007-0.01%</td>
</tr>
<tr>
<td>Binnenschelde</td>
<td>0.04-0.08%</td>
</tr>
<tr>
<td>Waddenzee</td>
<td>1.8-2.5%</td>
</tr>
</tbody>
</table>

Pathogenic *Vibrio* spp. in Northern European Waters | May 31st - June 1st, 2012, Koblenz | Ciska Schets
patients

- 1 patient in 2009
  - went swimming in Binnenschelde
  - injury and wound infection
    - *V. cholerae* non-O1/O139 cultured from wound and water

- 2 patients from St. Maartensdijk surveillance in 2010
  - 1 attended swimming classes in indoor pool
    - *V. alginolyticus* + other bacteria cultured from ear swab
  - 1 went swimming in Oosterschelde
    - *V. alginolyticus* cultured from ear swab

- 3 patients from St. Maartensdijk surveillance in 2011
  - no *Vibrio* cultured from wound and ear swabs
PFGE

- comparison of all 2009 *V. cholerae* non-O1/O139 isolates
  - lanes 1–9, 11–13, 20:13 Binnenschelde isolates
  - lane 10: Lambda Ladder PFG marker
  - lanes 14, 21: 2 patient isolates
  - lanes 16–19, 22–25: 8 IJsselmeer isolates
  - lane 15: 1 Oosterschelde isolate
- many different profiles
- no identical profiles from patient and water
- larger clonal heterogeneity among isolates from Binnenschelde
faecal indicators

- classification of bathing sites according to EU bathing water directive 2006/7/EC
- 2009-2011 data
- variable correlation *Vibrio* numbers – faecal indicator numbers

<table>
<thead>
<tr>
<th>bathing site</th>
<th>classification</th>
<th>E.coli</th>
<th>enterococci</th>
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<tbody>
<tr>
<td>Katwijk aan Zee</td>
<td>good</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>Bergen aan Zee</td>
<td>excellent</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>Binnenschelde</td>
<td>poor</td>
<td>&gt;500*</td>
<td>&gt;185*</td>
</tr>
<tr>
<td>Oosterschelde</td>
<td>excellent</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>Waddenzee</td>
<td>sufficient</td>
<td>500*</td>
<td>185*</td>
</tr>
<tr>
<td>IJsselmeer</td>
<td>good</td>
<td>250</td>
<td>200</td>
</tr>
</tbody>
</table>
summarize

- pathogenic vibrios detected in recreational water in the Netherlands
- levels not very high (median 4-383 MPN/L; max $10^5$ MPN/L)
  - compare Italy ($10^7$-$10^8$ MPN/L Masini et al 2007)
- more samples positive at higher water temperature
  - no clear quantitative relation
    - water temperatures not very high
    - summers of 2009-2011 moderate
- *V. alginolyticus* most isolated species
  - compare US (*V. vulnificus* Yoder et al 2008)
- limited number of patients
  - may be underestimation
    - vibriosis not notifiable disease
current and future work

- continue monitoring in 2012 and 2013
- in 2012 ad hoc patient surveillance
  - triggered by warm summer and high water temperatures
  - contact GP’s, public health services and clinical microbiology labs
- MALDI-TOF of stored isolates
  - with Gunnar Gerdts – VibrioNet
  - at drinking water company lab
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