Foodborne viruses: the European perspective

Fabienne Loisy-Hamon, Benoît Lebeau
1. What is the situation?
2. How to prevent the risk?
3. What should be done?
What is the situation?

2007 EFSA DATA 2010

OUTBREAKS

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What is the situation?

October 2012, Germany: SODEXO (catering company), CHINESE STRAWBERRIES

>11,400 contaminated children
   Origin of products: China
   Causative agent: norovirus

Origin of contamination: TO BE DETERMINED

IMMEDIATE CONSEQUENCES:
   Trademark impact
   Legal proceedings
   Financial compensation
   Audit of suppliers
   Needs of analyses on a large panel of matrices with urgent reply
1. What is the situation?
2. How to prevent the risk?
3. What should be done?
EUROPEAN STANDARDS AND REGULATIONS

COMMISSION REGULATION (EC) No 2073/2005: Whereas (2) & (27)

(2) Foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health.

(27) In particular, criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently. There is a need for development of reliable methods for other microbial hazards too, e.g. Vibrio parahaemolyticus.

CEN TG275/WG6/TAG4: Dr F. LOISY is one of the Expert Members
Develops a CEN standard for detection of norovirus and hepatitis A in foodstuffs, including bivalve molluscs/ fruits and vegetable/ bottled water- publication no later than 2012
EUROPEAN STANDARDS AND REGULATIONS

EFSA report - Scientific Opinion
Foodborne viruses : occurrence and control
Published 2011 July 14th
Main Sections

- Hazard characterization
- Exposure assessment: persistence, effect of treatments/food processing, diagnostic tests, occurrence data in food
- Risk characterization
- Control option: bivalve molluscs, fresh produces, products of animal origin, ready to eat, vaccination
- Microbiological criteria and microbiological testing option
EUROPEAN STANDARDS AND REGULATIONS

EFSA report - Scientific Opinion
Foodborne viruses : occurrence and control
Published 2011 July 14th

EFSA report - Scientific Opinion
Norovirus in Oysters : methods, limits and control options
Published 2012 January 17th
Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food
INTERNAL INDUSTRIAL GUIDELINES

• FOOD INDUSTRIES - MULTINATIONALS

THEY IMPLEMENT THEIR OWN INTERNAL GUIDELINES TO PREVENT VIRAL RISKS
METHODS FOR FOODBORNE VIRUS DETECTION
CEN/TC275/WG6/TAG4

- CEN is ISO equivalent body
- CEN/TC 275– Food analysis – Horizontal methods
- WG 6 – Microbial contamination
- TAG 4 – Detection of viruses in food
- Technical advisory group comprised of European food and water virology experts, 30 participants from 13 countries
- Tasked by CEN in 2004 with development of a standard method for the detection of viruses in foodstuffs
- Publication anticipated 2012
Framework for method

• Horizontal method (all foodstuffs included)

• Viruses of primary focus:
  - Norovirus
  - Hepatitis A virus

• Matrices of primary focus:
  - Hard surfaces
  - Salad crops
  - Soft fruits
  - Bivalve shellfish
  - Bottled water
Sample pre-processing:

- Hard surfaces – swabbing followed by elution into sample buffer

- Soft fruit/salad vegetables – elution with agitation & PEG/NaCl precipitation

- Bivalve shellfish – treatment of digestive gland with Proteinase K

- Bottled water – adsorption/elution using positively charged membranes & concentration by ultrafiltration
Viral RNA extraction:

- Boom technology (virus capsid disruption with chaotropic reagents, adsorption of RNA to silica particles)

- Use of magnetic silica technology preferred to centrifugation based protocol

- Nuclisens miniMAG magnetic reagents (BioMerieux) selected for inclusion in “bench protocols” and in any validation assay

- extraction of 500 µL sample concentrate; elution into 100 µL buffer using guanidinium thiocyanate and silica
RT-PCR:

- one-step TaqMan (“hydrolysis probe”) RT-PCR for all targets

- primers and probes “must be published in a peer-reviewed journal and be verified for use against a broad range of strains of target virus”

- Norovirus primers must target junction of ORF1/2

- HAV primers must target 5’ NCR
Controls:

- provide test results in a repeatable, reproducible manner
- Complex methodology necessitates several classes of controls
- EXTRACTION EFFICIENCY: viral process control (mengo virus vMC0)
- RT-PCR AMPLIFICATION EFFICIENCY (internal positive control)
- QUANTIFICATION (plasmid RNA for standard curve)
- RT-PCR POSITIVE CONTROLS
- NEGATIVE CONTROLS (for process and RT-PCR)
CEN method performance criteria (ceeram evaluation)

artificial contamination with NoV GI, NoV GII and HAV
2 levels of contamination: $5 \times 10^3$ or $5 \times 10^2$ genome copies

experiments and analyses in triplicate using CEN method
CEN method performance criteria (ceeram evaluation)

virus recovery efficiencies (mengo virus):

<table>
<thead>
<tr>
<th>matrix</th>
<th>% of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>oyster (DT)</td>
<td>5.4</td>
</tr>
<tr>
<td>raspberries</td>
<td>4.9</td>
</tr>
<tr>
<td>salad</td>
<td>8.8</td>
</tr>
<tr>
<td>green pepper</td>
<td>46.4</td>
</tr>
<tr>
<td></td>
<td>(hard surface)</td>
</tr>
</tbody>
</table>

1% accepted by CEN method
CEN method performance criteria (ceeram evaluation)

limit of detection (LOD):

<table>
<thead>
<tr>
<th>matrix</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>oyster (DT)</td>
<td></td>
</tr>
<tr>
<td>raspberries</td>
<td>$5 \times 10^2$ genome copies/ 2g of DT, 25 g or 100 cm$^2$</td>
</tr>
<tr>
<td>salad</td>
<td></td>
</tr>
<tr>
<td>green pepper (hard surface)</td>
<td></td>
</tr>
</tbody>
</table>

adaptations required for a routine analyses
## Optimisation for routine analyses

<table>
<thead>
<tr>
<th>modifications of CEN methods</th>
<th>impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>optimisation of virus concentration</td>
<td>extraction of the totality of samples submitted for analyses = results more representative of sampling</td>
</tr>
<tr>
<td>optimisation of clarification phases</td>
<td>less inhibition effect</td>
</tr>
<tr>
<td>optimisation of viral capsid lysis</td>
<td>higher extraction efficiency</td>
</tr>
<tr>
<td>development, optimisation validation and production of ceeramTools detection kits for RT-PCR detection</td>
<td>higher reliability of the results</td>
</tr>
</tbody>
</table>

### Determination of LOD in comparison with CEN protocols
## Optimisation for routine analyses

<table>
<thead>
<tr>
<th>Matrices</th>
<th>Extraction efficiency (%)</th>
<th>LOD (genome copies/ 2g of DT, 25g, 1 L or 100 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oysters (2g DT)</td>
<td>5.4 11</td>
<td>500 100</td>
</tr>
<tr>
<td>Raspberries (25g)</td>
<td>4.9 8.8</td>
<td>500 100</td>
</tr>
<tr>
<td>Salad (25 g)</td>
<td>8.8 44.7</td>
<td>500 100</td>
</tr>
<tr>
<td>Green pepper (surface, 100 cm²)</td>
<td>46.4 100</td>
<td>500 50</td>
</tr>
<tr>
<td>Bottled water</td>
<td>5.2 14</td>
<td>500 100</td>
</tr>
</tbody>
</table>
Standardization of the detection

- **ceeramTools® MOLECULAR DETECTION SYSTEM**
- Fast
- Robust
- Sensitive
- 100% specificity
- 95% amplification efficiency
- Multi-Platforms
- Amplification conditions standardized
- Conforms with TAG4
- Multi-matrices
- Multiplex
- External Control MengoVirus
- DLU 1 year
Validation on a large panel of matrices:

- shellfish (oysters, mussels, cockles, clams, scallops…)
- Fruits (fresh, frozen, dried, coulis, purée…)
- vegetables (salads, tomatoes, carrots, …)
- ready-to-eat, complex foods
- Herbs and spices
- Surfaces
1. What is the situation?
2. How to prevent the risk?
3. What should be done?
What should be done?

AN INTEGRATED APPROACH

- Risk assessment of matrices
- Evaluation of countries of origin
- Risk measurement (analysis)
- Analytical Surveillance Plan
- Determination of food process impact
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Thank you