Detection and survival of enteric viruses in water

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General context: Fecal pollution of water

Parasites: Giardia, Cryptosporidium...

Bacteria: Salmonella...

Enteric viruses pathogenic for human

http://www.vetparasitology.ugent.be/page1/page1.html

http://upload.wikimedia.org/wikipedia/commons/e/ee/Salmonella_typhimurium.png

http://www.worsleyschool.net/science/files/virus/page.html

http://tpeeaupotable.ifrance.com/maphoto/egout1.jpg
The problem was identified more than 70 years ago…

Infected human

High viral excretion in stools: Up to $10^{10}$ / g

Low infective dose: 10-100 infectious units

Human

Virus inactivation/removal

4 log reduction

1/10000 = risk
The main targets:

- **Norovirus**

- Hepatitis viruses (A and E)

- Rotavirus/Astroivirus/Adenovirus/Enterovirus/Aichivirus…

- Other emerging viruses (SRAS, H5N1…)

20-30 nm ssRNA

70-80 nm dsRNA or DNA

http://www.worsleyschool.net/science/files/virus/page.html

http://virology-online.com/viruses/Diarrhoea5.htm

http://pathmicro.med.sc.edu/mhunt/RNA-HO.htm
<table>
<thead>
<tr>
<th>Virus</th>
<th>Type of sample</th>
<th>Collection site</th>
<th>Concentration</th>
<th>% Positive samples</th>
<th>Quantification method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Sewage (raw)</td>
<td>Spain</td>
<td>4–7 GC logs/100 ml</td>
<td>100%</td>
<td>qPCR</td>
<td>Bofill-Mas et al. (2006)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Sewage (secondary effluent)</td>
<td>Spain</td>
<td>3 GC logs/100 ml</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Biosolids</td>
<td>Spain</td>
<td>4–7 GC logs/100 g</td>
<td>100%</td>
<td></td>
<td>Albinana-Gimenez et al. (2009b)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>River water</td>
<td>Spain</td>
<td>1–3 GC logs/l</td>
<td></td>
<td></td>
<td>Calgua et al. (2008)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Sewage (raw)</td>
<td>USA</td>
<td>4–5 GC logs/100 ml</td>
<td>90%</td>
<td>qPCR</td>
<td>Fong et al. (2010)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Sewage (tertiary effluent)</td>
<td>USA</td>
<td>3–4 GC logs/100 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus 40, 41</td>
<td>River water</td>
<td>Japan</td>
<td>3–5 GC logs/l</td>
<td>61%</td>
<td>qPCR</td>
<td>Haramoto et al. (in press)</td>
</tr>
<tr>
<td>JC Polyomavirus</td>
<td>Sewage (raw)</td>
<td>Spain</td>
<td>5 GC logs/100 ml</td>
<td>100%</td>
<td>qPCR</td>
<td>Bofill-Mas et al. (2006)</td>
</tr>
<tr>
<td>JC Polyomavirus</td>
<td>Biosolids</td>
<td>Spain</td>
<td>3–5 GC logs/100 g</td>
<td>100%</td>
<td></td>
<td>Albinana-Gimenez et al. (2009b)</td>
</tr>
<tr>
<td>JC Polyomavirus</td>
<td>River water</td>
<td>Spain</td>
<td>0–3 GC logs/l</td>
<td>90%</td>
<td></td>
<td></td>
</tr>
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<td>JC Polyomavirus</td>
<td>Sewage (raw)</td>
<td>Brazil</td>
<td>4–7 GC logs/100 ml</td>
<td>96%</td>
<td>qPCR</td>
<td>Fumian et al. (in press)</td>
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<tr>
<td>JC Polyomavirus</td>
<td>Sewage (secondary effluent)</td>
<td>Brazil</td>
<td>4–5 GC logs/100 ml</td>
<td>39%</td>
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<td></td>
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<td>River water</td>
<td>Japan</td>
<td>2–3 GC logs/l</td>
<td>11%</td>
<td>qPCR</td>
<td>Haramoto et al. (in press)</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>Sewage (raw)</td>
<td>France</td>
<td>5–7 GC logs/100 ml</td>
<td>100%</td>
<td>qRT-PCR</td>
<td>Le Cann et al. (2004)</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Sewage (raw)</td>
<td>France</td>
<td>7 GC logs/100 ml</td>
<td></td>
<td>qPCR</td>
<td>Schvoor et al. (2001)</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>Sewage (raw)</td>
<td>Spain</td>
<td>4 GC logs/100 ml</td>
<td></td>
<td>qPCR</td>
<td>Rodriguez-Manzano et al. (2010)</td>
</tr>
<tr>
<td>Hepatitis E virus</td>
<td>Sewage (raw)</td>
<td>Spain</td>
<td>3 GC logs/100 ml</td>
<td></td>
<td>qPCR</td>
<td>Rodriguez-Manzano et al. (2010)</td>
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<tr>
<td>Norovirus</td>
<td>Sewage (raw)</td>
<td>United Kingdom</td>
<td>6 GC logs/100 ml</td>
<td></td>
<td>qPCR</td>
<td>Laverick et al. (2004)</td>
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<td>GII</td>
<td>Sewage (effluent)</td>
<td>Brazil</td>
<td>2–3 GC logs/l</td>
<td></td>
<td>qPCR</td>
<td>Victoria et al. (in press)</td>
</tr>
<tr>
<td>GI</td>
<td>Sewage (raw)</td>
<td>Brazil</td>
<td>2 GC logs/l</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some criteria for the universal ideal indicator:

- Present at the same time as the pathogenic organisms (fecal pollution) and more abundant if possible. Absent in unpolluted water.

- No multiplication

- Easily detectable with simple method

- Not pathogenic

- More resistant than the corresponding pathogen in environment but also with respect to water treatment
Fecal bacteria indicators

1881: total cultivable bacteria...

Regulation for drinking water, bathing water, oysters...
Limits of fecal bacteria as viral indicator:

Survival lower than pathogens in environment: virus et parasites

Resistance to treatment lower than pathogens

Different behavior: soil migration, filtration...

E. coli and enterococci are indicators of:
Fecal pollution which do not take into account virus survival in environment and virus behavior. They cannot be used as model for estimating virus treatment efficiency.
**Recent fecal pollution**

- **E. coli**
- **Enterococci**

- Presence or High level
- Absence or Low level

- Probability of the presence of infectious viruses:
  - + + + +
  - + + +
  - + +
  - +
  - +/-
  - -

- Treatment or survival in environment

- How may we discriminate such situations?
Some examples: Detection of pathogenic viruses in tapwater without the presence of fecal indicators.

In South Africa

Grabow et al. (2004): 11-16% positive samples for infectious Enterovirus.
Some examples: Detection of pathogenic viruses in tapwater without the presence of fecal indicators

In South Korea

Lee and Kim (2002): between 40 et 50% + (0.002 – 0.03 NPPUC/L) for infectious *Adenovirus* and *Enterovirus*
FAO et WHO (2008): an increase of food or water outbreaks

Some outbreaks in Europe

- Oysters
  - Hepatitis A virus
  - N = 111
  - France
  - (Guillois-Becel et al., 2009)

- Raspberries
  - Norovirus
  - N = 200
  - Finland (Maunula et al., 2009)

- Christmas diner (salad ?)
  - Norovirus
  - N = 22
  - Portugal
  - (Mesquita et al., 2009)

- Dry tomatoes
  - Hepatitis A virus
  - N = 11
  - NL (Petrignani et al., 2010)

- Tap water
  - Astrovirusr / Enterovirus
  - Rotavirus / Norovirus
  - N = 299
  - Italy (Scarcella et al., 2009)

- Oysters
  - Norovirus
  - N = 334
  - F / UK / S / N / D
  - (Westrell et al., 2010)

- Figatelli
  - Hepatitis E virus
  - N = 20
  - France (ANSES, 2009)

Eurosurveillance (http://eurosurveillance.org)
Why do we observe such outbreaks?

Viruses are most resistant than bacteria which are currently used as indicators (*E. coli*, *enterococci*)

Microbiological criteria:
- $> \text{limits} = \text{high viral risk}$
- $< \text{limits} = \text{some outbreaks may still be described due to viruses}$

« Some criteria may be defined for enteric viruses in mollusc and water as soon as the analytical tools will be developed. (Règlement 2073/2005/CE)
Two questions for prevention of viral outbreaks:

1. Are tools for detecting viruses enough developed to define criteria and regulations?

2. How can we select a model to describe virus survival (environment or during treatment)?
1. Diagnostic tools

Viral targets: Norovirus, HAV (HEV?)
Matrices: oysters, raspberries, salads, water, surfaces.

Cell culture not usable

Molecular tools (RT-PCR): only way

Define detection method

DOI 10.1007/s12560-010-9042-5

International Standardisation of a Method for Detection of Human Pathogenic Viruses in Molluscan Shellfish

David Lees · CEN WG6 TAG4

Lees (2010)
Now we have a standardized method

Extraction/concentration of viruses from the matrice
   Dissection, digestion with proteinase K (Jothikumar et al. 2005) : oysters
   Others : elution, filtration...

Extraction of nucleic acid
   Guanidine isothiocyanate et magnetic beads

Real time RT-PCR
   Primers in conserved regions (HAV, Norovirus GI et GII)
   Controls (control +/- for process, inhibition)
Presence of viral genome is not a proof of the presence of infectious virus

Example: Poliovirus 1, mineral water, 35°C (Gassilloud et al. 2003)

![Graph](image)

**FIG. 3.** Persistence of the PV1 genome (viral genome) and infectious PV1 (infectious virus) in mineral water at 35°C over time as described by equations 2 and 1, respectively.

Same results for a lot of situations: ClO₂; Ozone; UV... (Simonet et al. 2006; Sano et al. 2010... )
Relation between genome and infectivity depend on the inactivation mechanisms

Loss of the capacity to bind to the cell receptor (binding), to inject genome inside the cell (injection) and to replicate the genome (replication). Model: MS2 phage.

Wigginton et al. 2012
How can we interpret the presence of viral genome in term of viral risk?

- Detection of viral genome is an indicator of the presence of a viral pollution which may be recent or old. Their presence is not always correlated with the presence of infectious virus.

- Absence of viral genome (if the right control are made) correspond to the absence of the corresponding infectious virus.

- Absence of viral genome may not give any information about the global fecal pollution and the presence of other viruses.

- Molecular tools largely underestimate impact of treatments.
Presence or high level

Probability of the presence of infectious virus

Positive results

Detection by RT-PCR:
- Norovirus
- HAV
- Enterovirus
- Others

Negative results

Absence or low level

E. coli
Enterococci
Prevalence of Norovirus genome is sometimes very high!

- Oyster (production zone)

*76.2% (n= 844 ) (GB)  (Lowther et al. 2012)
60% > 100 cg/g ; 30% >1000cg/g some with 10 000cg/g !

*3.9% (4.4% HAV) (n= 390) (USA) (DePaola et al. 2010)

* de 9% à 23% NV (F)  (Beuret et al. 2003 ; Le Guyader et al. 2000)

- Red fruits

*7% et 34% en France et Belgique (Baert et al. 2011)

- Salads

*0.8% à 12.4% ( n=210)  (Adria Normandie – Prevavir 2011)
What should be done?

Use the standardized method to evaluate genome prevalence in different matrices (water, food)

Quantifying viral genome to evaluate viral genome pollution in different matrices

Try to make links between detection of viral genome and outbreaks

Develop studies to better understand viral inactivation mechanisms to discriminate infectious from non infectious viruses and define conditions which favor genome degradation

Don’t forget that other indicators can help! Fecal bacteriophages, hygienic indicators...
Are phages interesting in such context?

Bacteriophages: the most abundant biological entities in environment

97%: Caudovirales

3%: others

Ackermann H. Virologie (2001)
Phages are present:

- in human stools

<table>
<thead>
<tr>
<th>Bacteriophages</th>
<th>Frequency</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic coliphages</td>
<td>20%-90%</td>
<td>$10^4$-$10^6$ UFP/g</td>
</tr>
<tr>
<td>F-specific RNA phages</td>
<td>0%-57%</td>
<td>$10$-$10^3$ UFP/g</td>
</tr>
<tr>
<td><em>B. fragilis</em> phages</td>
<td>0%-15%</td>
<td>$10^2$-$10^8$ UFP/g</td>
</tr>
</tbody>
</table>

- in wastewater

$\log_{10}$ PFU or CFU/100mL

<table>
<thead>
<tr>
<th>Country</th>
<th>Sample</th>
<th>FC</th>
<th>FE</th>
<th>SRC</th>
<th>SOMCPH</th>
<th>FRNAPH</th>
<th>BFBRYCPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>$n = 36$</td>
<td>6.74</td>
<td>5.86</td>
<td>5.37</td>
<td>5.78</td>
<td>4.85</td>
<td>4.07</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5.66</td>
<td>4.44</td>
<td>4.60</td>
<td>4.95</td>
<td>3.54</td>
<td>2.00</td>
</tr>
<tr>
<td>Min.</td>
<td></td>
<td>5.32</td>
<td>4.85</td>
<td>4.00</td>
<td>3.15</td>
<td>4.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Max.</td>
<td></td>
<td>8.30</td>
<td>7.20</td>
<td>6.50</td>
<td>6.67</td>
<td>6.15</td>
<td>4.98</td>
</tr>
<tr>
<td>Colombia</td>
<td>$n = 38$</td>
<td>7.05</td>
<td>5.87</td>
<td>5.63</td>
<td>5.</td>
<td>5.24</td>
<td>3.75</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5.23</td>
<td>4.45</td>
<td>4.48</td>
<td>3.15</td>
<td>4.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Min.</td>
<td></td>
<td>5.36</td>
<td>6.90</td>
<td>6.60</td>
<td>7.00</td>
<td>6.34</td>
<td>4.98</td>
</tr>
<tr>
<td>Max.</td>
<td></td>
<td>8.36</td>
<td>6.90</td>
<td>7.00</td>
<td>7.00</td>
<td>6.34</td>
<td>4.98</td>
</tr>
<tr>
<td>France</td>
<td>$n = 38$</td>
<td>6.65</td>
<td>5.95</td>
<td>4.76</td>
<td>6.14</td>
<td>5.69</td>
<td>4.65</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5.32</td>
<td>4.85</td>
<td>2.00</td>
<td>4.41</td>
<td>4.04</td>
<td>2.54</td>
</tr>
<tr>
<td>Min.</td>
<td></td>
<td>5.19</td>
<td>5.61</td>
<td>3.48</td>
<td>6.34</td>
<td>4.36</td>
<td>3.95</td>
</tr>
<tr>
<td>Max.</td>
<td></td>
<td>7.93</td>
<td>7.00</td>
<td>6.71</td>
<td>7.95</td>
<td>6.89</td>
<td>5.89</td>
</tr>
</tbody>
</table>

FC : Fecal coliforms; FE : Fecal enterococci; SRC : Spores of sulphite-reducing clostridia; SOMCPH : Somatic coliphages, FRNAPH : F-specific RNA phages, BFBRYCPH : *B. fragilis* (RYC) phage

Lucena et al. (2004)
Somatic coliphages (E. coli WG5)

4 families: Myoviridae, Siphoviridae, Podoviridae and Microviridae

Murphy et al. 1995
2 families: *Leviviridae* and *Inoviridae*

F-specific RNA phages

F-specific DNA phages

F-specific phages

(*S. typhimurium* WG49 or *E. coli* C)
B. fragilis (HSP40 or RYC 2056)
1 family: *Siphoviridae*

Murphy et al. 1995
Is the replication of somatic coliphages in water environments significant?

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Keywords
coliphages, environment, replication, significance, water.

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Summary
Somatic coliphages are amid several groups of bacteriophages that have been suggested as indicators in water quality assessment. One of the limitations frequently endorsed to somatic coliphages as indicators is that they can replicate in the water environment. This review intends to evaluate the significance of this potential replication. In view of: the threshold densities of somatic coliphages and host bacteria needed for productive infection to occur, the densities of both host cells supporting somatic coliphages replication and these phages in water environments, and the poor contribution of lysogenic induction to the free somatic coliphage numbers in water, it can be concluded that replication of somatic coliphages in waters is very unlikely. Consequently, the contribution of replication in the environment of somatic coliphages is expected to have a non-noticeable influence on the numbers of somatic coliphages detected in water environments. Thus, the replication in the environment should not be argued as a limitation to the use of somatic coliphages as indicators.
Phages are fecal indicators which are not pathogenic, may not replicate in environment are easily detectable with low cost method and for which the infectious character can be easily verified.

Standardized method

Some phage have a similar structure compared to pathogenic viruses (Leviviridae)

They have a similar survival in a lot of situations
Some of them may discriminated the origin of fecal pollution

Blanch et al. (2006): 20 parameters + 18 associations
N= 103 samples of wastewater from known origin (81 + 22)
100% good discrimination

but also F-specific RNA phage genotyping
Absence or low level

E. coli Enterococci

Presence or high level

Bacteriophages (kind of phage important)

Positive or High level

Probability of the presence of infectious virus

Negative or Low level

+ + + +

+ + +

+ / -

-
2. Choose a good model to estimate global enteric virus inactivation

Use a cultivable model

For *Norovirus*

- **same family** : FeCV ou MNV (Canon et al. 2006)

- **same structure** : MS2 phage, Enterovirus,... (Casteel et al. 2009)

- the most resistant:
The most resistant:

2.1 UV = MS2 phage (Hijnen et al. 2006)
2.2 heat = ΦX174 phage ≈ *Lactobacillus helveticus* phages ≈ *Lactococcus lactis* phages (Bertrand et al. 2012)

Choose the right model is important

2.3 Ex: MS2 vs GA vs Qβ phages = same family and structure is not sufficient
Example for UV: simple conditions
MS2 Phage: (20-30 nm; RNA $\approx$3500 b)

**MS2 phage**: 14 publications for 64 Log reduction.

- **Dose (mJ.cm$^{-2}$)**
  - $y = -0.0354x - 0.2177$
  - $R^2 = 0.9337$

- **Abatement viral (Log$_0$ (Nt/No))**
  - $y = -0.0533x - 0.1578$
  - $R^2 = 0.9737$

z-value between 19 and 29 mJ.cm$^{-2}$
Conclusions for UV in simple media

(Hijnen et al. 2006 : review) + J. Simonet (thesis 2007) + COST 929

**UV sensitivity:**

ϕX174 phage > enteroviruses ≈ hepatitis A virus ≈ animal caliciviruses > rotaviruses > MS2 phage > adenoviruses (41)
Resistance to temperature

Inactivation/dégradation du genome= 652 TFL de 73 publications

heat = ΦX174 phage ≈ Lactobacillus helveticus phages ≈ Lactococcus lactis phages (Bertrand et al. 2012)
### Description of RNA F-specific bacteriophages

<table>
<thead>
<tr>
<th></th>
<th>MS2</th>
<th>Qβ</th>
<th>GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>20-30 nm</td>
<td>20-30 nm</td>
<td>20-30 nm</td>
</tr>
<tr>
<td>Genome (RNA)</td>
<td>3569 nts</td>
<td>4217 nts</td>
<td>3577 nts</td>
</tr>
<tr>
<td>IEP</td>
<td>3.9</td>
<td>1.9 to 5.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Amino acid seq.</td>
<td>• 20 % sim. between MS2 and Qβ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of capsid protein</td>
<td>• 62 % sim. between MS2 and GA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Similar fundamental structures**

Amino acid exposed at the capsid surface are different → Different interfacial properties?
Behavior of three bacteriophages during (physical) drinking water treatment

Schematic drinking water treatment at pilot scale

Q: Flow meter
Cond.: Conductivity

(Boudaud et al. 2012)
Behavior of three bacteriophages during drinking water treatment

Separate experiments for infectivity: log reduction

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MS2</th>
<th>Qβ</th>
<th>GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation/floculation + sand filtration</td>
<td>4.5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Membrane ultrafiltration</td>
<td>6</td>
<td>4</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The elimination efficiency in these treatments follows the phage hydrophobicity sequence

(Boudaud et al. 2012)
Choose the right tool to do the right thing....

1) Estimation of fecal/viral pollution in water
2) Estimation of virus removal by treatment
3) Tracking the origin of fecal pollution
Universal indicator does not exist:

Viral pollution

1) Define objectives and situations
2) Use the tool box

Fecal phages
Detection of viral pathogens by molecular tools
Bacterial fecal indicators
Recent fecal pollution
Old fecal pollution
Viral behavior
Treatment efficiency
Discrimination of fecal pollution?
Epidemiologic studies
Diversity of viral pollution
Absence of virus
Cost?
Thank you for your attention
Figure 2 : Logigramme d'interprétation d'un résultat positif par les techniques de biologie moléculaire