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Evaluation of the microbiological quality of coastal waters by quantifying human and animal viruses

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Epidemiological studies conducted at bathing beaches have shown a significant increase in incidence of illness among those who engage in water-based recreational activities. Several viruses including adenoviruses, enterovirus, hepatitis A virus and noroviruses have been shown to cause recreational water-borne disease outbreaks.

The adequacy of using bacteria as indicators of the microbial water quality has been questioned since viruses and protozoan cysts have shown to be more resistant to treatment and disinfection processes commonly applied in sewage treatment plants.

There is a public health requirement for additional parameters more reliably.

New molecular methodologies for the quantification of viral indicators to be used as markers of fecal contamination and as Microbial Source Tracking Tools (MST) as well as new methods for the concentration of the viruses present in large volume of water samples have been developed for our research group in the context of 3 European Commission funded projects: VIROBATHE (www.virobathe.org), EPIBATHE AND VIROCLIME (www.viroclime.org).

PROJECT

VIROBATHE (2006-2007): Methods for the detection of Adenoviruses and Noroviruses in European Bathing Waters with reference to the revision of the Bathing Water Directive 76/160/EEC.

EPIBATHE (2006-2008): Assessement of human health effects caused by bathing waters. The study investigate the level of the risk associated with bathing water exposure.

VIROCLIME (2010-2012): Impact of Climate Change on the Transport, Fate and Risk Management of Viral Pathogens in Water.

OBJECTIVES

- To evaluate methods for detecting in water noroviruses and adenoviruses.
- To develop tests to detect these agents rapidly in marine and fresh recreational waters (Phase I).
- To demonstrate and further refine the tests in a 20 weeks surveillance program (Phase II) including selected recreational waters across 9 countries in the EU.

- Seawater samples from two different locations and four sampling days, were processed for HAdV and NoV analysis using a concentration method based on a direct flocculation protocol.

- Tools and methods developed in our laboratory will be used to conduct case studies on 5 selected sites (Sweden, Spain, Hungary, Greece and Brazil) vulnerable to climate change (principally rainfall events). Figure 4: Spanish case study site.
- The empirical baseline data accrued will be used in mathematical models constructed to estimate changes in exposure under defined conditions. Exposure levels will then be used to estimate risk of disease associated with such changes.

METHODS

- Comparison of methods for the rapid concentration of viruses from recreational waters (Figure 1).
- Development of nested and quantitative PCR assays for the detection and quantification of human adenoviruses and noroviruses in recreational waters.

- The procedure applied represents a low cost and efficient methodology for the routine quantification of HAdV and NoV in seawater and for further risk assessment studies.
- Viruses detected in seawater samples, often containing very low levels of bacterial indicators, may reflect the presence of diffuse sources of contamination and, potentially, contamination contributed by the bathers in the experimental area.
- Results are summarized in Table 1

- Quantitative PCR of human adenoviruses and JC polyomaviruses as human fecal indicators (Bofill-mas et al., 2006)
- Quantification of porcine adenoviruses (Hundesa et al., 2009) and bovine polyomaviruses (Hundesa et al., 2010) as animal fecal indicators

RESULTS

- Improved rapid concentration and detection methods for waterborne noroviruses and adenoviruses (Calgua et al., 2008; Bofill-Mas et al., 2010).
- Surveillance data on the target viruses through a range of EU recreational water (Bofill-Mas et al., 2010): Figure 1and 2.

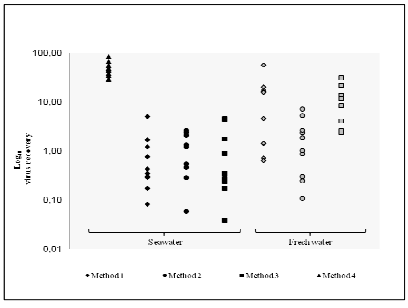

Figure 1. Intra-laboratory variability of viral concentration methods in artificial seawater and freshwater. recovery values obtained after spiking sets of ten 10-l samples with 2, concentrating by: Method 1: electronegative filters of nitrocellulose and glycine 0.05 M pH 9.5 – skimmed milk buffer. Method 2: electronegative filters of nitrocellulose and glycine 0.25 M pH 9.5 – beef extract buffer. Method 3: a column of glass and glycine 0.25 M pH 9.5-beef extract buffer. Method 4: Direct organic with skimmed milk and quantifying the recovery by qPCR according to Bofill-Mas et al. (2006) and Girones et al. (2010)

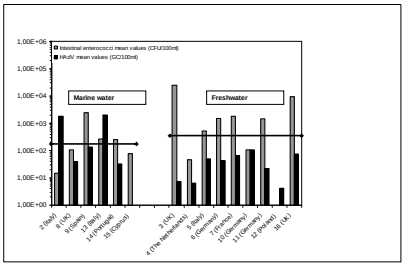

Figure 2. Comparison between mean value of IE and HAdV GC per 100 ml of water in the studied sites. Lines in bold indicate the maximum level of IE per each type of water (coastal and transitional or inland) required for good quality waters (based upon a 95-percentile evaluation) as established in the European Bathing Water Directive (2006/7/EC).

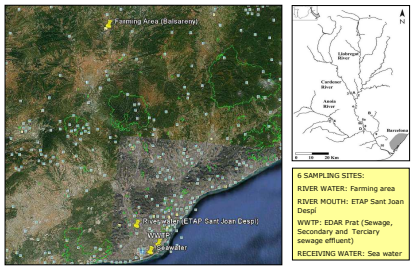

Figure 3. Spanish case study site that will be studied during VIROCLIME

	Sampling day	Analyzed samples	Viral analysis GC/L: Mean values detect every sampling day HAdV	NoV	Positive samples (%) and mean values detect every bathing season HAdV	NoV	Viral strains detected HAdV	NoV	Bacteriological analysis * CFU/100 mL *E. coli*	Enterococci
Bathing season 2006	A	24	$2{,}27x10^2$ ($7{,}80x10^0$ -$5{,}60x10^2$)	NR	12.5% $1.42x10^2$	2.1% $1{,}76x10^2$	Species F (five samples)	GG II	33 P95	25 P95
	B	24	$5{,}8x10^1$ ($1{,}4x10^1$ -$1{,}4x10^2$)	$1{,}76x10^2$						
Bathing season 2007	A	24	$1{,}20x10^3$ ($4{,}04x10^0$ - $9{,}81x10^3$)	NR	97.8% $9.98x10^2$	NR	Species F (two samples) Type 2 (seven samples)	-	1501 P90	563 P90
	B	23	$7{,}36x10^2$ ($5{,}13x10^1$- $4{,}11x10^3$	NR						

Table 1. Detection and quantification of HAdV, NoV and bacterial indicators present in seawater samples during bathing seasons 2006 and 2007. * Data from Figueras MJ. and collaborators, partners of the Epibathe project.

CONCLUSIONS AND BIBLIOGRAPHY

Viral concentration and quantitative PCR assays for the concentration and quantification of human adenoviruses, JC polyomaviruses, porcine adenoviruses and bovine polyomaviruses in different water matrices have been developed in the context of three different European projects. Data on the occurrence of these agents in recreational waters have been obtained and data on the presence of these viruses and others will be obtained during VIROCLIME.

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