

Overview

Viruses pose one of the biggest threats to human wellbeing being responsible for numerous infections and millions of deaths worldwide each year. Worryingly, it is clear that the burden of waterborne disease is likely to increase in Europe in the future in response to increased pressure on wastewater infrastructure (due to population rise), sewer misconnections and a greater incidence of storms and flood events causing the release of untreated sewage (stormwater discharge) into river networks and the coastal zone. As our knowledge of both pathogenic and non-pathogenic viruses in the environment was poor, the VIRAQUA project set out to:

- (i) **develop new approaches to monitor viral pollution in waterbodies,**
- (ii) **determine how infective these viruses are,**
- (iii) **explore the diversity of viruses in the environment, and**
- (iv) **develop new and improved risk assessment tools for protecting human health.**

Over a 4-year period, the universities of Bangor, Liverpool and Cambridge alongside CEFAS and the Centre for Ecology and Hydrology came together to address these goals. The work was funded under the NERC Environment Microbiology and Human Health programme with additional support provided by the Food Standards Agency.

Approaches for the accurate detection of enteric viruses in environmental matrices

Our research team at Bangor University successfully validated novel and simple methods for the concentration of riverine, sea-, wastewater and sediment for the detection of enteric viruses. For water samples, a two-step concentration method was developed using tangential flow ultrafiltration (TFUF) and polyethylene glycol (PEG) precipitation¹. For sediment samples, viruses are first eluted from the surface of sediment particles and then precipitated using PEG². We developed multiplex reverse transcription quantitative PCR assays for noroviruses, sapovirus, hepatitis A and E viruses³. **Using multiplex assays up to three targets can be quantified simultaneously, significantly reducing the time and costs of viral detection. The concentration methods have very limited effect on viral integrity and hence the infectivity of the viruses can be assessed in the concentrates.** For that propose, a viral capsid integrity assay using porcine gastric mucin-coated magnetic beads (PGM-MBs) was also validated⁴. This commercially-available protein has high affinity to the norovirus capsid proteins. Hence, norovirus particles in the environmental concentrates bind to the coated beads and can be easily separated from degraded particles and free viral nucleic acids using magnetic force. **All these methods enable sample process and viral detection within 24 hours without the use of expensive equipment and can be standardised for regulatory proposes.** Benchtop protocols are available on our website (<http://www.viraqua.uk/publications>).



The use of indicator viruses to assess wastewater contamination



As the direct detection of pathogenic viruses in the environment can be challenging, indicators are often used to track wastewater contamination and for risk assessment. Our research team at CEFAS have been investigating the usefulness of infectious F-RNA bacteriophages as a proxy for viral contamination in shellfish. This is a low cost, non-molecular based method. Monitoring the levels of both genogroup II F-RNA bacteriophages and norovirus has shown that F-RNA bacteriophages provide a good indicator of sewage contamination in coastal waters and shellfish beds. This viral-based assay complements existing methods for the routine monitoring of faecal bacteria in bathing waters and shellfish. This is an important development as faecal bacteria are poor predictors of viral contamination risk. Our method therefore now allows regulatory authorities to gain a more holistic assessment of microbiological pollution in the coastal zone and the risk this poses to human health.

At Bangor University, we have investigated the usefulness of alternative indicators, namely human adenoviruses, polyomaviruses and the recently discovered crAssphage (cross-assembly bacteriophage). The results of our environmental monitoring campaign suggested that adenovirus and crAssphage are persistent viruses and present in wastewater-polluted aquatic environment and mussel at high concentrations⁴⁻⁶. **Hence, these viruses are well suited to tracking human wastewater contamination and pollution risk assessment in aquatic environments.**

Viral survival and infectivity in the aquatic environment

The joint research of the team members at Cambridge and Bangor Universities revealed that the PGM-MG assay assessing norovirus integrity shows good correlation with actual viral infectivity in animal cell lines. Further research revealed extensive viral decay and hence loss of infectivity in environmental samples that was not noted when only molecular detection (qPCR) was used⁴. Our research suggests that viral integrity assays, such as **the PGM-MB assay, give a good estimate of infective norovirus titres in environmental samples significantly improving current viral risk assessment**. Our ongoing research will reveal the actual decay rates of human noroviruses in the aquatic environment, which as yet has not been described (due to the lack of *in vitro* techniques for human norovirus culturing).



Viromics and public health

Our research team at the University of Liverpool used state-of-the-art metagenomics approaches to study the wide diversity of pathogenic and non-pathogenic viruses in wastewater treatment plants, the river network and the coastal zone. We developed an optimised pipeline for the sequencing of RNA and DNA viruses and the bioinformatics reconstruction of their genomes. As a result, we were able to infer the presence of several enteric viruses, e.g. a new strain of norovirus GI, two sapovirus strains along with several possibly zoonotic rotavirus species in wastewater⁷. Our results suggest that **the viromics analysis of municipal wastewater provides a valuable insight into the health status of inhabitants, which can be extremely useful for public health management, and can aid in the identification of novel pathogens to include in routine environmental monitoring**.



Transport of viruses in the aquatic environment



Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Our researchers at the Centre for Ecology and Hydrology and at Bangor University have modelled the fate of adenovirus down the river Conwy, through the estuary and out to sea. This used measured river and sewage works flow and adenovirus concentration data. This simulation included actual tidal cycles in the estuary and sea, a range of river flows and flow combinations and a range of relationships between these flows and the tidal cycle. Our results show that the greatest concentrations of virus are associated with high flow events. Higher river flows led to higher concentrations of virus entering the estuary and ultimately the coastal zone. Transport of viruses from the estuary to the sea was also found to be influenced by the tidal cycle, with strong ebbing tides carrying viruses out of the estuary. A strong ebbing tide combined with high river flows poses a higher risk of high adenovirus counts in coastal waters, shellfisheries and beaches. **Our modelling approach enables the identification of safe zones for bathing and shellfish harvesting in UK waters**. All the animations produced by the project showing simulations and predicted transit of viruses can be viewed on our website (<http://www.viraqua.uk/models>).

References

All Viraqua publications are open access. For the full list of publications with direct links to full text please visit <http://www.viraqua.uk/publications>.

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