

NORWEGIAN SCHOOL OF VETERINARY SCIENCE

Virological Survey of Surface waters Ricardo C Rosado(Ricardo.Rosado@nvh.no), Mette Myrmel (Mette.Myrmel@nvh.no)

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The project VISK is part of the INTERREG IV A programme, with the ultimate goal of reducing susceptibility to waterborne viral diseases in Scandinavia, in spite of climate change.

Drinking water in Scandinavia is subject to different risks, seen on the illustration to the right. Climate change studies show an increase by 65% of extreme events in Europe between 1998 and 2007, events which affect both the quantity and quality of water resources. At this time, there is little information on outbreaks in Scandinavian raw water:

Finland – 18, 1998-2003 (Maunula et al., 2005)

Sweden – 4 in 2010, 900 affected (SMI) Norway - 13, 1988-2002, 6480 affected (Nygård et al., 2003)

Objectives

- Establish filtration methods for virus detection in raw water commonly used in the project area.
- Survey surface water at the intake of the Nedre Romerike water plant for the presence of viruses implicated in human waterborne disease outbreaks.
- Survey both inlet and outlet waste water from sewage treatment plants in the project area for the same viruses.
- Produce data on virus quantities in sewage and drinking water in relation to season and precipitation.

cDNA synthesis

cDNA total

volume

(20 ul)

SuperScript® III

(11 ul RNA)

Realtime PCR (2ul)

Risks to drinking water Wastewater contaminati **Public confidence Poor warning Inssuficient treatment**

Water (5-10L) mengovirus (8e+05 PCR units / L) **Primary concentration** Elution (40 ml) Negative Process control NanoCeram® (0.05 M KH2PO4, control (40 (1 ml water + 2 142 mm disc 1.0 M NaCl, 0.1% ml elution ml lysis buffer) (v/v) Triton X-100, buffer) pH 9.2) **Secondary concentration** PEG precipitation Centrifugation Ressuspension $(10,000 \times g, 45)$ (80 g/L PEG 8000, (3 ml lysis 17,5 g/L NaCl, pH min, 4°C), removal buffer) 7,3) of supernatant DNA/RNA isolation Centrifugation Several wash Elution 50 μl magnetic /ressuspension $(25 \mu l)$ (400 ul) (400-500 ul) Norovirus / **Adenovirus** Mengovirus

Viruses surveyed

Norovirus

- leading cause of viral diarrhea worldwide in every age group, with
- 20 million cases in the USA every year - over half of all foodborne outbreaks
- first detected as an agent of waterborne disease in 1997

Adenovirus (hAdV)

- third most common cause of infantile gastroenteritis after
- rotavirus and norovirus, - acute gastroenteritis, primarily in children.
- almost half are subclinical
- hAdV are often detected in the environment and have been proposed as a molecular index of viral contamination of human origin. Testing for hAdV in the Glomma is of interest for two different reasons:
- to assess the presence of this human pathogen
- as an indicator of faecal contamination

Surface water: NRV plant Wastewater: •Rånåsfoss (Sørum kommune) •Fjellfoten (Nes)

Project area - Norway

	Results Virus detection in the Glomma river						
	Norovirus GI Norovirus GII Adenovirus Rain Temperature	05					
3500		25					
2800		20					
Genome equivalents		01+ (mm/ c)					
700		10 0					
0		0					
	January March May July September November January March May February April June August October December February April June						
	Months .						

	Positives	Average	Max
Adenovirus Glomma	52,47%	1,08E+03	1,22E+04
Adenovirus wastewater inlet	100,00%	2,16E+04	8,39E+04
Norovirus GI Glomma	43,83%	3,41E+02	1,86E+03
Norovirus GI wastewater inlet	52,38%	7,79E+03	3,54E+04
Norovirus GII Glomma	38,27%	1,32E+03	6,56E+03
Norovirus GII wastewater inlet	80,95%	2,73E+04	2,43E+05
			-

Summary

- 162 samples over 55 weeks
- 139 positive for at least one virus
- 29 positive for all viruses
- 23 negative for all viruses,
- only 2 months out of 18 had no positives

Inhibitors

~20% of samples showed signs of inhibition

Virus recovery

- 7-34% using spiked surface water
- best recovery norovirus GI

Conclusions

- The method has consistent results, but variable recovery. No cause for this has been identified.
- Adenovirus was detected most often, but norovirus GII had the highest average concentration, questioning how useful it is as an indicator in the Glomma region.
- Large difference between winters of 2011/2012 complicates study of virus concentration in relation to observed weather events.







