Assessing Virus Reduction/Inactivation by UV during Municipal Wastewater Treatment

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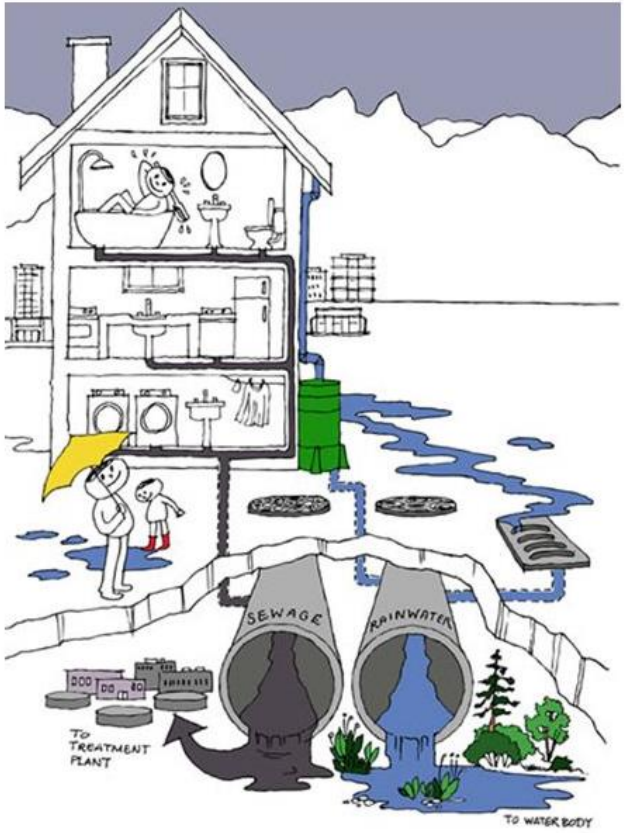
Municipal wastewater

- Source of wastewater: household waste; Rain/storm water; Industrial waste; Livestock waste
- Wastewater contains: Macro-solids; Small particles; Pathogens
- Pathogens: Bacteria, Viruses, Protozoa



Wastewater treatment plant (WWTP)

- Wastewater treatment: to remove the contaminants from the wastewater and make it safe enough to return to the aquatic environment or being reuse

Wastewater treatment process

1 - Preliminary treatment: remove the large solid matter and grit
2 - Primary treatment: remove the heavy and light solid
3 - Secondary treatment: biological nutrient removal
4 - Tertiary treatment: UV disinfection of microorganisms

UV disinfection

- UV radiation damages the nucleic acids, proteins and enzymes of microorganisms to prevent their reproduction and infection
- Factors impact the effectiveness of UV disinfection: UV intensity, exposure time, turbidity and transmittance
- Sensitivity of microorganisms to UV light varies due to their different characteristics, such as cell structures, genome contents and DNA repair enzymes.

Current regulation for wastewater treatment discharge

- Current municipal wastewater treatment uses the bacteriological indicators, such as *E.coli* and total coliform
- No regulatory standards for virus removal during wastewater treatment
- Bacterial indicators did not correlate well with the presence of viruses

Viruses in wastewater

- Viruses are a major cause of human waterborne and water-related diseases, such as gastroenteritis, hepatitis
- Human enteric viruses are the most common causes of gastroenteritis, and they have been found in wastewater with high amount accoss the world
- Limited data on the UV disinfection of human enteric viruses during municipal wastewater treatment

Aims

- To monitor the occurrence of human viruses at two municipal wastewater treatment plants in Calgary

- To assess the virus reduction/inactivation by UV disinfection during wastewater treatment

Wastewater treatment plants in this study

	WWTP1	WWTP2
Population served	250,000	1,000,000
Average Capacity	100 ML/d	500 ML/d
Preliminary treatment	Screens and grit removal	Screens and grit removal
Primary treatment	Primary clarification	Primary clarification
Secondary treatment	Activated sludge; Secondary clarifier, Cloth-media disk filter	Activated sludge; Secondary clarifier
Tertiary treatment	UV disinfection (UV dose: 24 mJ/cm^2)	UV disinfection (UV dose: 30 mJ/cm^2)

Wastewater sample collection

- Wastewater samples were collected monthly before UV treatment (pre-UV) and after UV treatment (post-UV)
- 10 liter samples were collected directly from the flowing streams of wastewater within the UV channel
- Samples were placed in cooler with ice packs and transported to the laboratory within 24 hours for processing
- A total of 29 pre- and 28 post-UV samples were collected from WWTP1 between October 2014-June 2016, 22 pre- and 22 post-UV samples from WWTP2 between November 2014-December 2015

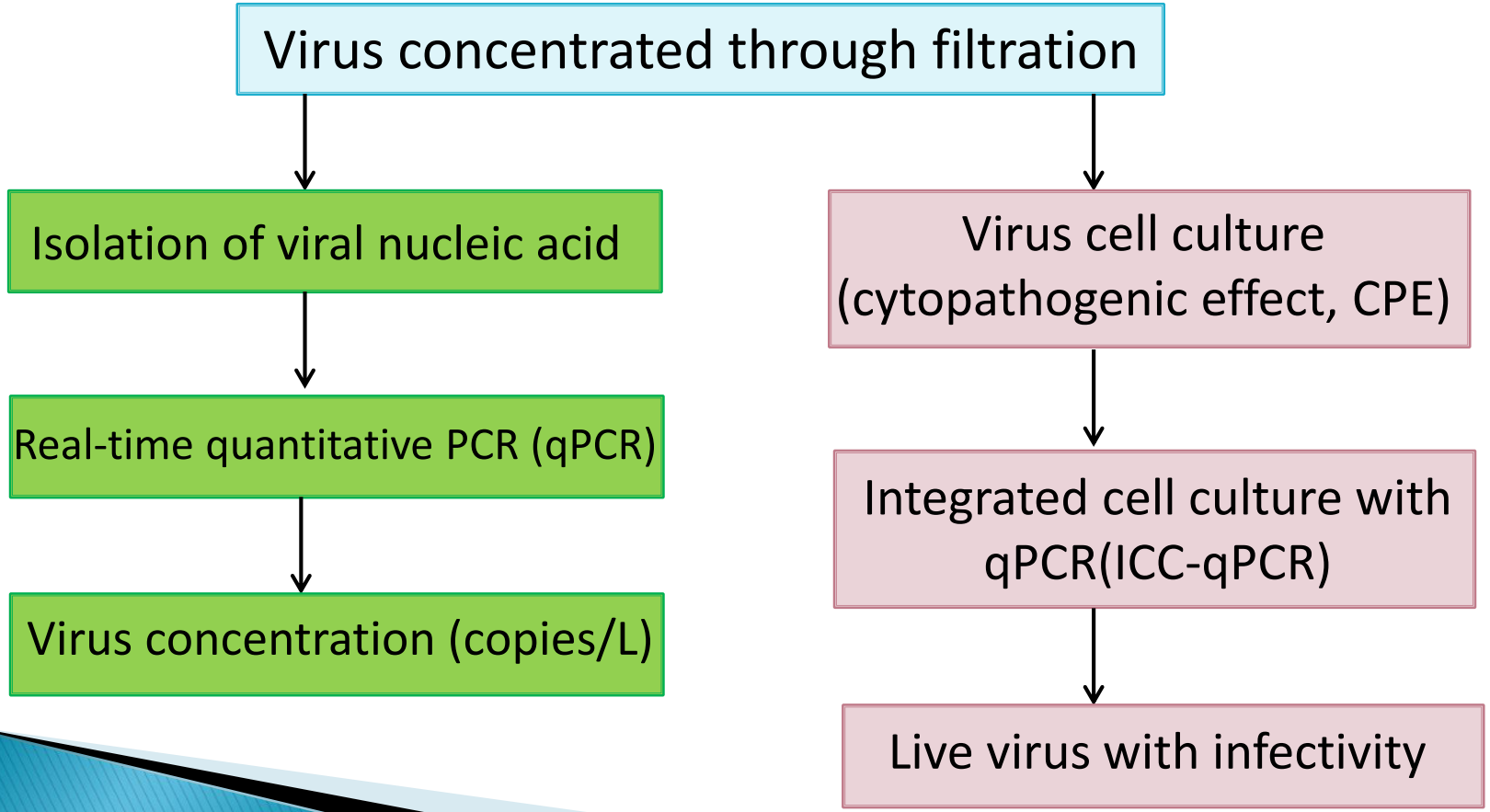
Virus targets

- **Enteric viruses**
  - ✓ Norovirus GI and GII (NoV)
  - ✓ Sapovirus (SaV)
  - ✓ Rotavirus (RV)
  - ✓ Reovirus (ReoV)
  - ✓ Astrovirus (AsV)
  - ✓ Adenovirus (AdV)

- **Enteroviruses (EV)**
  - ✓ Coxsackievirus
  - ✓ Echovirus

- **Human polyomavirus**
  - ✓ JC virus (JCV)

Procedures of virus detection in wastewater



Virus Concentration

Filtration Adsorption using electropositive charged membrane filter

1-2 hrs

Elution Elution with the pH adjustment, pH 9.75

0.5-1hr

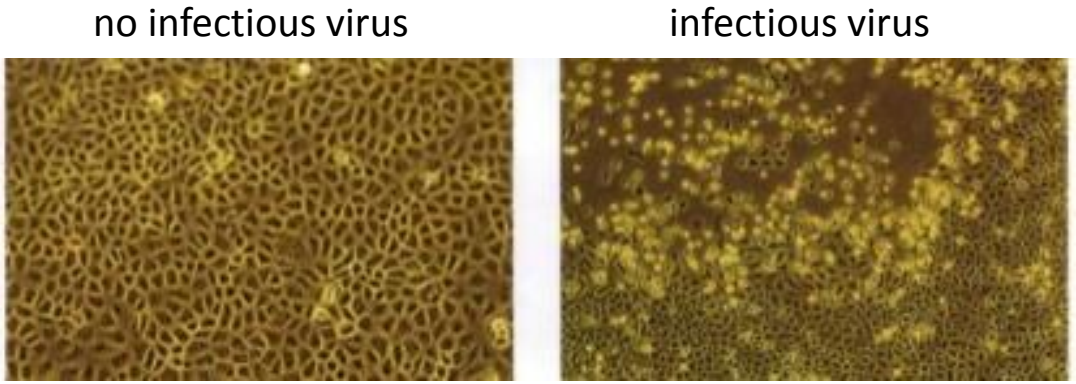
Flocculation pH 3.5

1-2 hrs

15 ml concentrated viral specimen

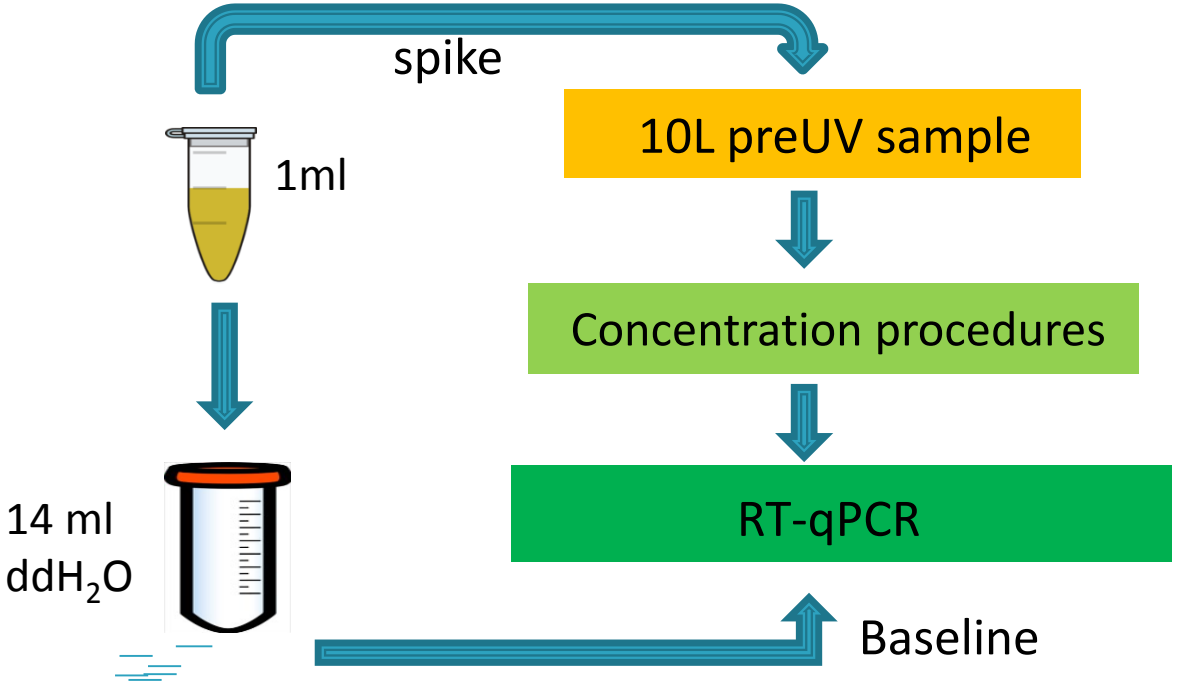
Infectious virus detection

- Detection of infectious virus using cell culture as observed by the change of cell morphology (CPE)
- ICC-qPCR to identify the virus type: Rotavirus, Adenovirus, Enterovirus and Reovirus
- Infectious virus concentration was determined using a Most Probable Number (MPN) method



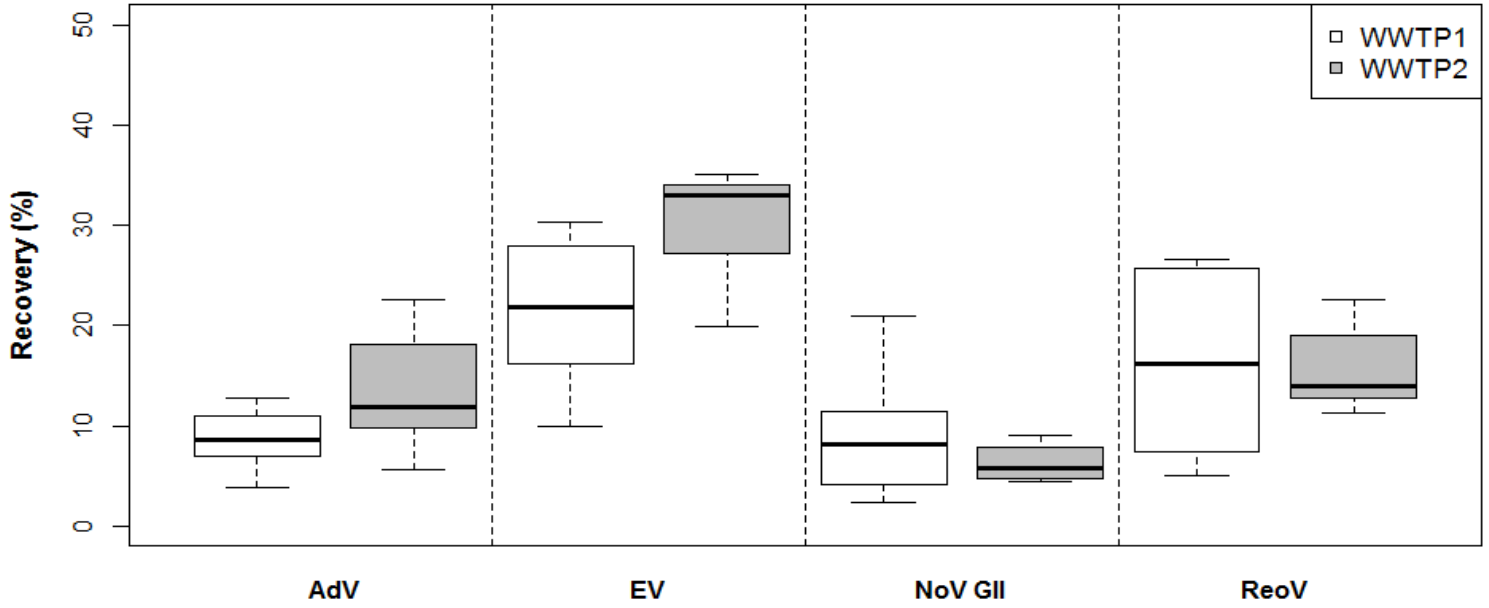
Virus recovery efficiency

Mixed viruses:
- NoroGII
- Adenovirus
- Enterovirus
- Reovirus



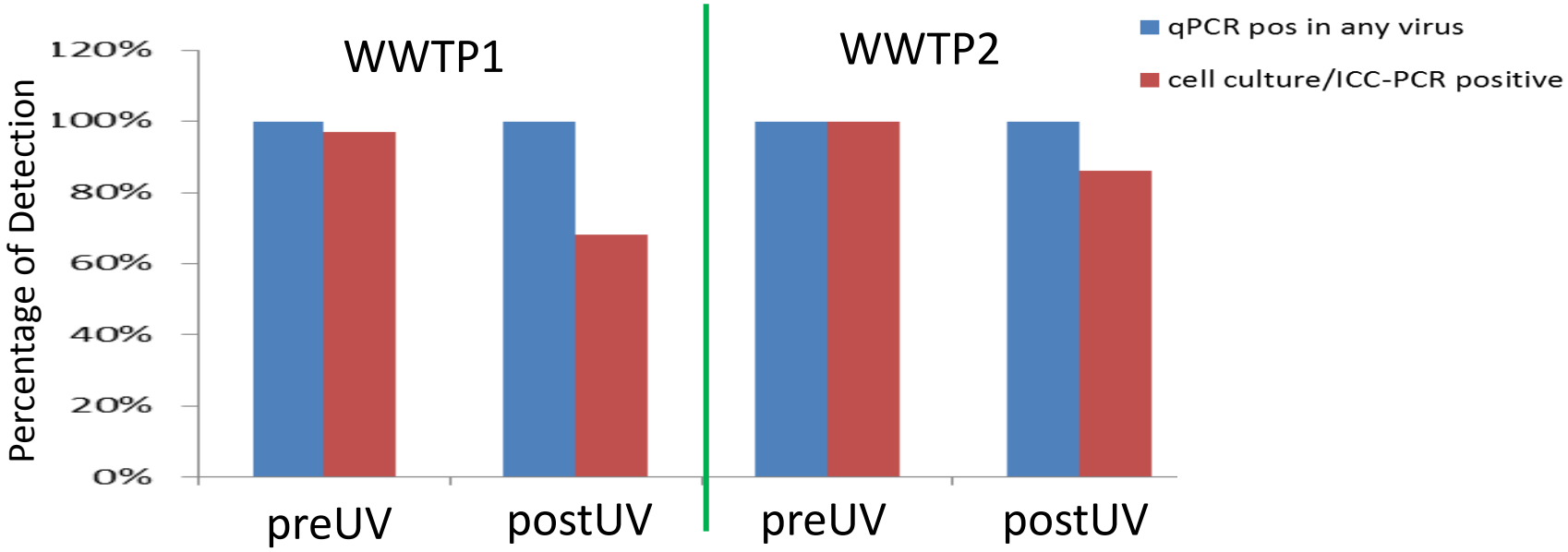
Recovery efficiency (%) = (virus concentration from spike sample- virus concentration in the original non-spiked sample)/virus concentration in the baseline x 100

Recovery efficiency



- EV has the best recovery among all the viruses tested
- The recovery of four viruses is comparable to other studies

Detection of virus in wastewater

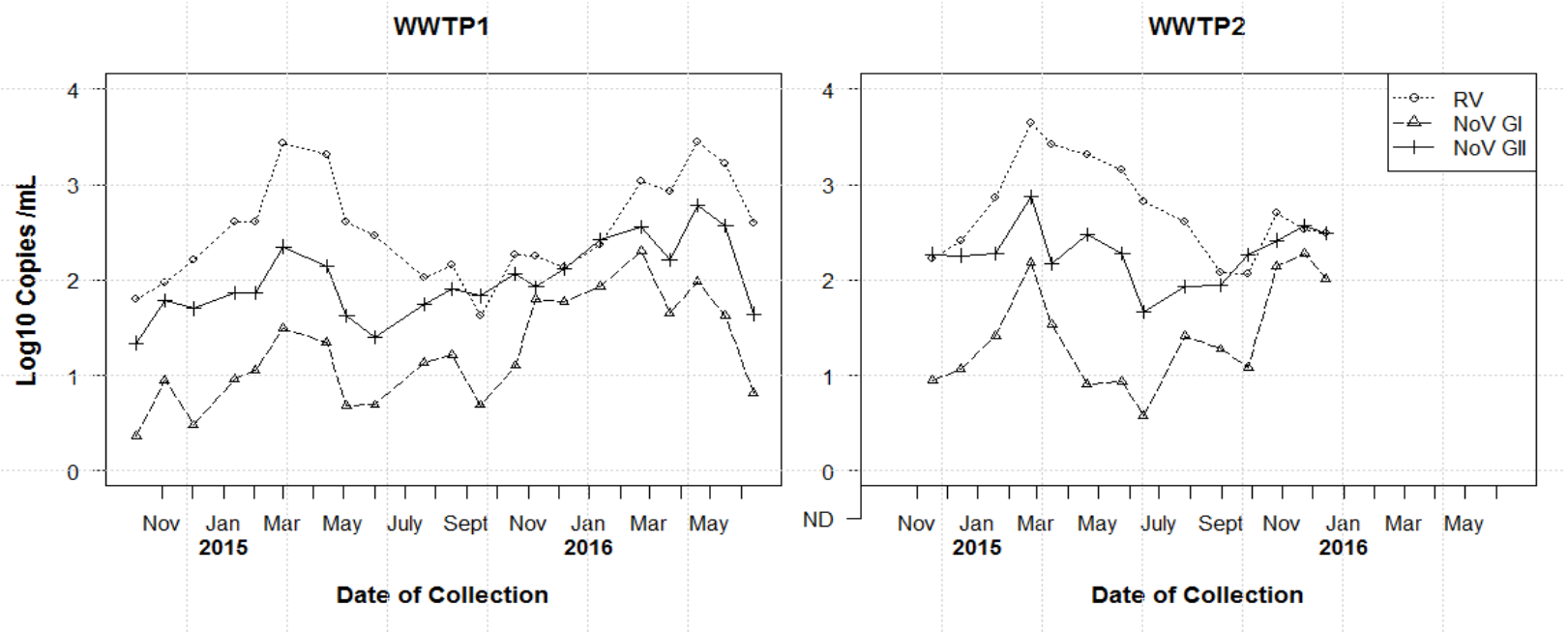


- Viruses were detected in all samples by qPCR
- Infectious viruses were detected in less postUV samples

Virus level in wastewater by qPCR

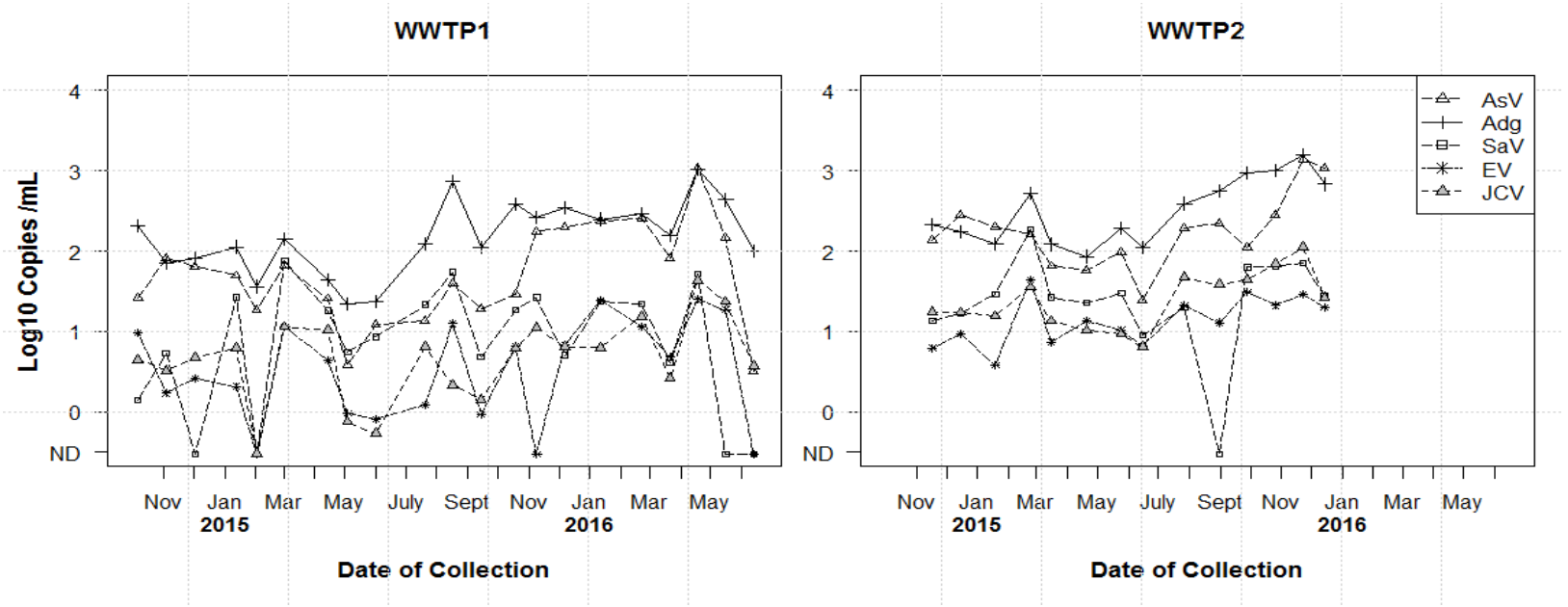
Virus	WWTP 1		WWTP 2	
	Virus concentration (log_{10} copies/L)			
	Pre-UV mean ± SD	post-UV mean ± SD	Pre-UV mean ± SD	post-UV mean ± SD
NoV GI	4.19 ± 0.49	4.02 ± 0.52	4.35 ± 0.53	4.12 ± 0.64
NoV GII	4.96 ± 0.36	4.86 ± 0.36	5.23 ± 0.29	5.12 ± 0.31
RV	5.51 ± 0.55	5.41 ± 0.55	5.75 ± 0.49	5.68 ± 0.45
SaV	4.01 ± 0.6	3.85 ± 0.57	4.37 ± 0.48	4.16 ± 0.49
AsV	4.58 ± 0.59	4.59 ± 0.53	5.16 ± 0.43	5.1 ± 0.44
EV	3.62 ± 0.46	3.46 ± 0.39	4.13 ± 0.31	3.82 ± 0.32
AdV	5.15 ± 0.46	4.97 ± 0.49	5.49 ± 0.41	5.35 ± 0.46
JCV	3.69 ± 0.46	3.61 ± 0.42	4.37 ± 0.37	4.17 ± 0.47

Virus level in preUV samples



- Rotavirus concentration peaked in the spring
- Norovirus also had some of the highest concentration observed in spring

Virus level in preUV samples



- No distinct temporal patterns of virus occurrence observed for any other viruses

Virus reduction by UV disinfection---qPCR

*Average of virus reduction	NoV-GI	NoV-GII	RV	SaV	AsV	EV	AdV	JCV
WWTP1	0.17	0.11	0.09	0.31	0.05	0.23	0.19	0.15
WWTP2	0.45	0.11	0.07	0.34	0.07	0.31	0.13	0.3

*log reduction =$\log_{10}$ (preUV concentration/postUV concentration)

- qPCR alone under-estimates UV inactivation of viruses

Infectious virus detection

Virus detected by ICC-qPCR and/or CPE	Positive sample number (%)			
	WWTP1		WWTP2	
	pre-UV	post-UV	pre-UV	post-UV
CPE positive, n (%)	27/29 (93%)	11/28 (39%)	22/22 (100%)	15/22 (68%)
Virus identified by ICC-qPCR				
RV	0	1 (4%)	0	0
EV	1 (3%)	2 (7%)	0	0
AdV	0	3 (11%)	0	1 (5%)
ReoV	12 (41%)	4 (14%)	5 (23%)	9 (41%)
RV+ReoV	1 (3%)	2 (7%)	0	0
EV+ReoV	6 (21%)	1 (4%)	7 (32%)	3 (14%)
AdV+ReoV	5 (17%)	2 (7%)	8 (36%)	3 (14%)
RV+EV+AdV	0	1 (4%)	0	0
EV+AdV+Reo	0	0	1 (5%)	0
RV+EV+AdV+ReoV	1 (3%)	0	1(5%)	0
Overall infectious virus detected	28/29 (97%)	19/28 (68%)	22/22 (100%)	19/22 (86%)

Infectious virus detection

Virus or CPE	Positive sample number (%)					
	WWTP1			WWTP2		
	Pre-UV	post-UV	Reduction of detection	Pre-UV	post-UV	Reduction of detection
Overall detection	28/29 (97%)	19/28 (68%)	29%	22/22 (100%)	19/22 (86%)	14%
RV	2 (7%)	4 (14%)	N/A	1 (5%)	0	5%
EV	8 (28%)	4 (14%)	14%	9 (41%)	3 (14%)	27%
AdV	6 (21%)	6 (21%)	N/A	10 (46%)	4 (18%)	28%
ReoV	25 (86%)	9 (32%)	54%	22 (100%)	15 (68%)	32%

Log reduction of infectious virus

Virus or CPE	WWTP 1			WWTP 2		
	Mean concentration, Log_{10} (MPN/L) (Min, Max)		Log_{10} reduction	Mean concentration, Log_{10} (MPN/L) (Min, Max)		Log_{10} reduction
	Pre-UV	Post-UV		Pre-UV	Post-UV	
RV	0.27 (0.26, 0.29)	0.09 (-0.04, 0.29)	0.18	-	-	n/a
AdV	0.26 (0.26, 0.26)	0.29 (1 sample)*	n/a	0.38 (0.26, 1.26)	NQ	n/a
EV	0.95 (-0.04, 2.26)	NQ	n/a	1.07 (-0.05, -2.26)	2.02 (1 sample)*	n/a
ReoV	1.05 (-0.04, 3.02)	-0.04 (-0.04, -0.04)	**1.05**	1.69 (0.26, 3.02)	0.16 (-0.04, 0.26)	**1.53**
CPE	1.54 (0.54, 2.56)	0.59 (-0.05, 1.02)	**0.95**	2.07 (1.54, 3.02)	0.69 (0.54, 1.56)	**1.38**

NQ: not quantifiable

Summary

- NoV, RV, SaV, AsV, EV, AdV and JCV were frequently detected in preUV and postUV samples
- Infectious viruses were more prevalent in preUV samples (98%) than postUV samples (76%), indicating that UV has some effect on inactivation of viruses
- Overall, there is no significant difference for the log reduction of infectious viruses in two plants
- The presence of infectious viruses in UV-treated wastewater effluent released to the river suggests potential risk to human and environmental health, as well as the need for monitoring viruses in treated wastewater

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Questions