8th International Workshop on Decentralized Wastewater Treatment in Asia

分散型汚水管理分野における水系感染症対策
Wastewater Epidemiology for Novel Coronavirus in Japan

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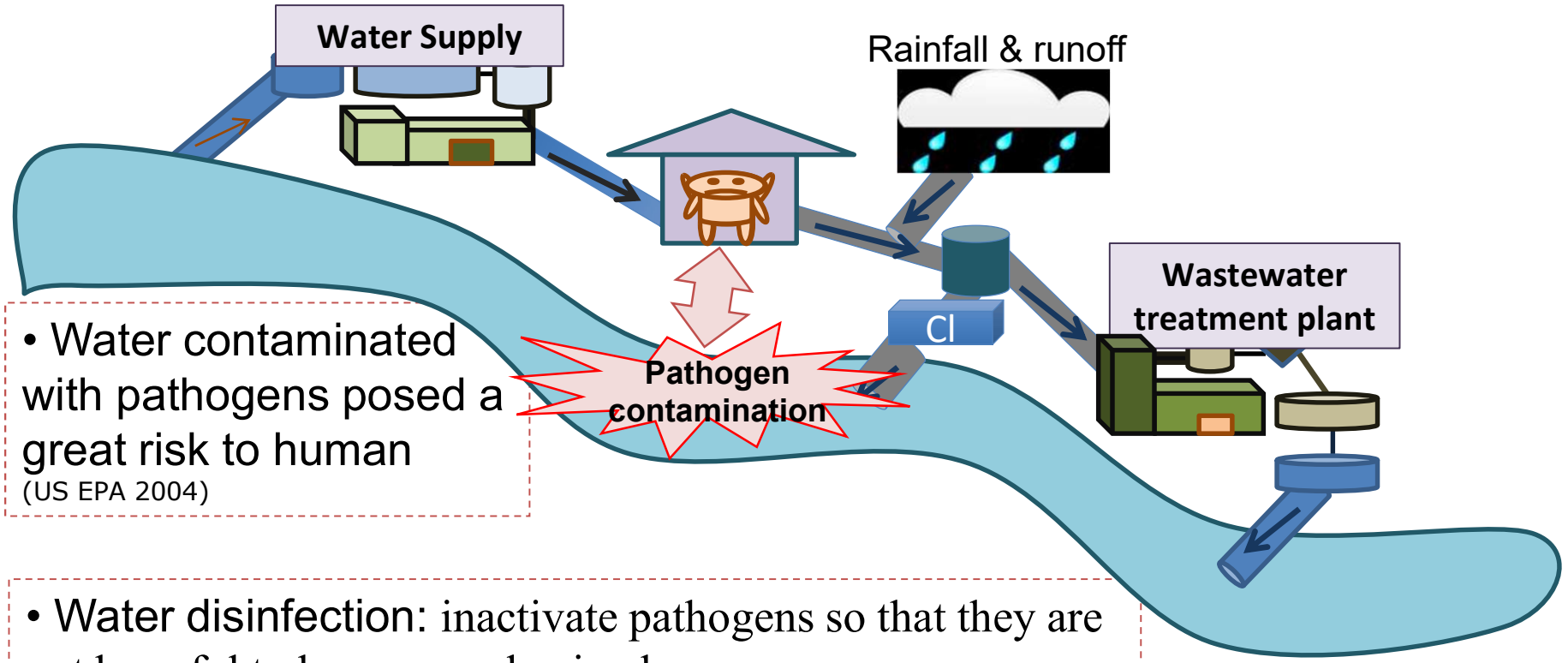
Importance of disinfection



- Water contaminated with pathogens posed a great risk to human
(US EPA 2004)

- Water disinfection: inactivate pathogens so that they are not harmful to humans and animals

Chlorine Reactions with Ammonia

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O$$

$$NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$$

$$NHCl_2 + HOCl \rightarrow NCl_3 + H_2O$$

- Free chlorine can react with organic nitrogen in the water to form chloramines.
- The chloramines are not very effective against viruses and are a weaker disinfectant.

"combined available chlorine",
"combined chlorine residual"

CT value for 2-log reduction

Table CT values (mg/min l^{-1}) for 99% inactivation at 5°C

Microorganism	Free chlorine (pH 6–7)	Preformed chloramines (pH 8– 9)	Chlorine dioxide (pH 6–7)
E. coli	0.034–0.05	95–180	0.4–0.75
Poliovirus 1	1.1–2.5	770–3740	0.2–6.7
Rotavirus	0.01–0.05	3810–6480	0.2–2.1
Phage f2	0.08–0.18	–	–
G. lamblia cysts	47–>150	–	–
G. muris cysts	30–630	1400	7.2–18.5

Adapted from Hoff (1986)

Chloramine is not effective to viruses

Ozone

- Moderately effective to inactivate Cryptosporidium

- Highly reactive

  Residual ozone then enough virus inactivation

  → Need ozone destruction of off-gas

  → Combination with activated carbon

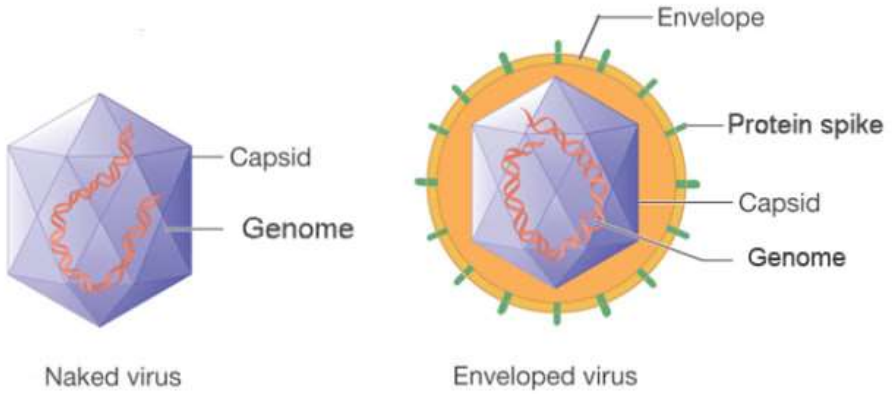
- Disinfection by-product: Bromate anion (BrO_3^-) : drinking water quality guideline <0.01mg/L

- Depends on temperature

Coronavirus

enveloped viruses are usually unstable in water than non-enveloped virus

a positive-sense single-stranded RNA genome



- The genome size of coronaviruses ranges from approximately 27 to 34 kilobases, the largest among known RNA viruses
- The name coronavirus is derived from the Latin corona, meaning "crown"

Coronavirus

- cause respiratory tract infections
- typically mild, such as the common cold,
- SARS, MERS, and COVID-19 can be lethal
- COVID-19 is named SARS-CoV-2

Outbreak	Virus type	Deaths
2003 severe acute respiratory syndrome outbreak	SARS-CoV	774[31]
2012 Middle East respiratory syndrome coronavirus outbreak	MERS-CoV	Over 400[32]
2015 Middle East respiratory syndrome outbreak in South Korea	MERS-CoV	36[33]
2018 Middle East respiratory syndrome outbreak	MERS-CoV	41[34]
2019–20 coronavirus outbreak	SARS-CoV-2	Over 3000[35]

SARS-CoV is sensitive to Cl_2

Table 4
Disinfection of SARS-CoV in wastewater by chlorine and chlorine dioxide[a]

Disinfectants	Dose (mg/L)	Inactivation rate (%)			Free residue chlorine (mg/L)
		SARS-CoV	f_2 phage	*E. coli* (8099)	
Chlorine					
	5	68.38	30.91	0	0.11
	10	100	27.27	0	0.40
	20	100	79.09	100	0.50
	40	100	100	100	0.82
Chlorine dioxide					
	5	0	0	0	0.00
	10	94.38	32.73	0	0.00
	20	82.22	42.73	0	0.00
	40	100	60.00	99.46	2.19

[a] SARS-CoV, $10^{1.75}TCID_{50}$/ml; f_2, 1.1×10^5 pfu/L; *E. coli*, 1.3×10^6 cfu/L; temperature, 20 °C; disinfection for 30 min. Results from three experiments.

Journal of Virological Methods 126 (2005) 171–177

SARS-CoV is weaker than *E. coli*

Table 5
Effect of contacting time on inactivation of SARS-CoV in wastewater with low-concentration disinfectants[a]

Disinfectants	Contacting time (min)	Inactivation rate (%)			Free residue chlorine (mg/L)
		SARS-CoV	f_2 phage	*E. coli* (8099)	
Chlorine					
	1	43.77	15.79	0	0.39
	5	68.38	15.79	0	0.33
	10	100	18.32	14.29	0.40
	20	100	21.05	26.09	0.40
	30	100	31.58	20.21	0.35
Chlorine dioxide					
	1	43.77	42.11	0	–
	5	68.38	26.32	17.39	–
	10	68.38	17.79	0	–
	20	68.38	26.32	14.29	–
	30	55.33	47.37	21.74	–

[a] Concentration of chlorine and chlorine dioxide was 10 mg/L. SARS-CoV, $10^{1.6}TCID_{50}$/ml; f_2, 1.9×10^5 pfu/L; *E. coli*, 4.6×10^5 cfu/L; temperature, 20 °C.
(–) Not detected. Results from three experiments.

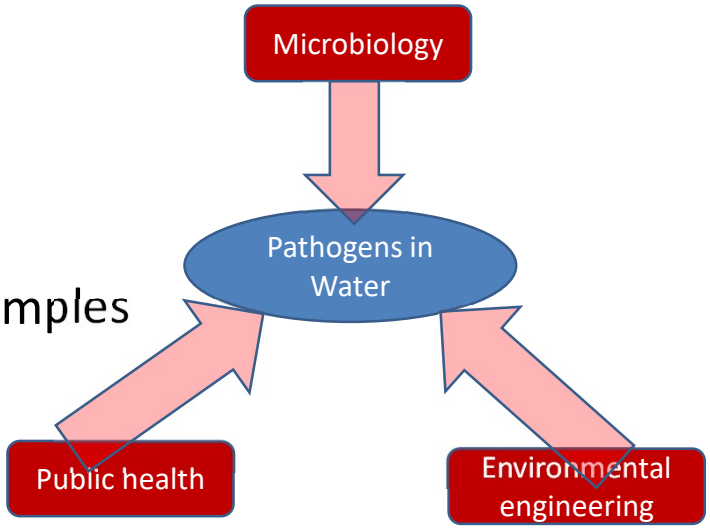
SARS-CoV2 in water

- Cause less risk than other enteric virus
- If properly managed, then water sectors are not posing risk
- Water tap may be a possible spreading source

- Another issue: monitoring of COVID in the community.

PCR : Game changer

- Before PCR
  - Medical microbiology, Virology
  - Culture methods
  - Emphasis on identifying viruses
- After PCR
  - From 1990s, Applied to environmental samples
  - Active enrollment of Researchers in Environmental Engineering
  - Importance of Quantitative results



Water Research 135 (2018) 168–186

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Review

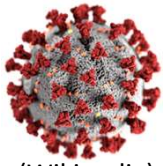
A review on recent progress in the detection methods and prevalence of human enteric viruses in water

Eiji Haramoto [a, *], Masaaki Kitajima [b], Akihiko Hata [c], Jason R. Torrey [d], Yoshifumi Masago [e], Daisuke Sano [f], Hiroyuki Katayama [g, h]

Viral quantification as a vital process to implement WBE

■ Concept of wastewater-based epidemiology (WBE)

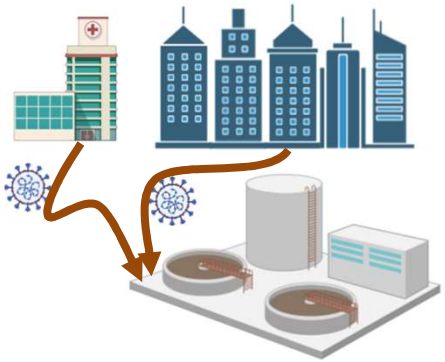
SARS-CoV-2

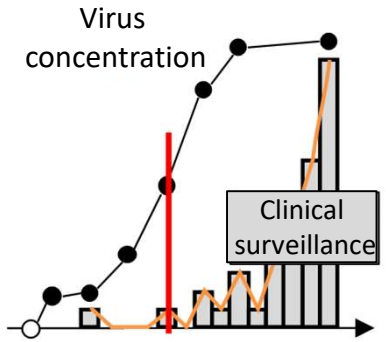


(Wikipedia)

Viral RNA are excreted in feces

(Wölfel et al., 2020)



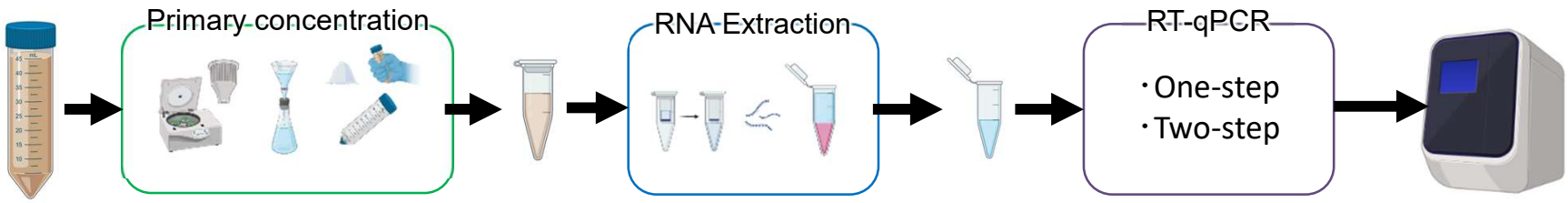


Capturing infections trend

Detection of SARS-CoV-2 RNA → Presence of infected people in the catchment.

■ Detection process

Previous studies: Optimized for non-enveloped viruses in most cases



Research gaps

Are the virus concentration methods applicable to the recovery of enveloped viruses?

WBE on Norovirus in Japan

Environmental Surveillance of Norovirus Genogroups I and II
for Sensitive Detection of Epidemic Variants

(Kazama et al. *Appl Environ Microbiol*. 2017)

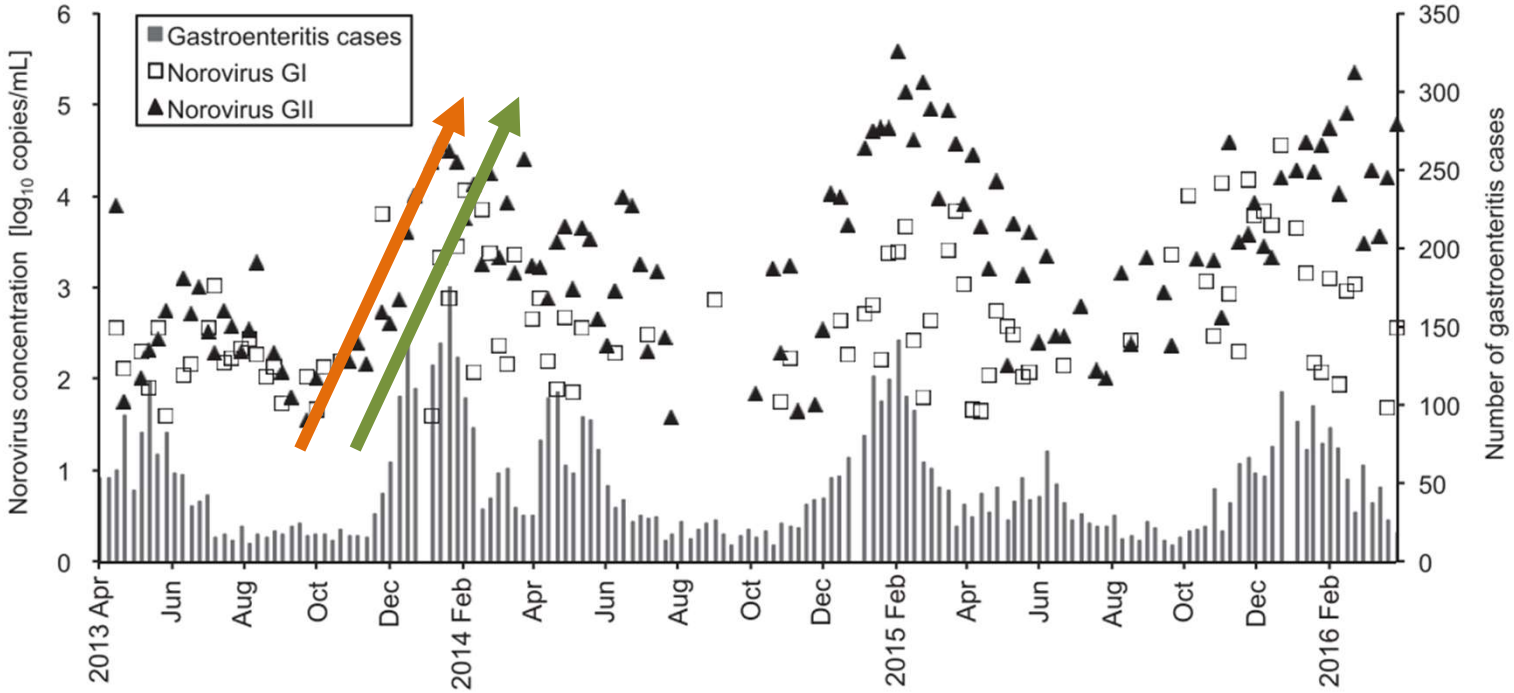
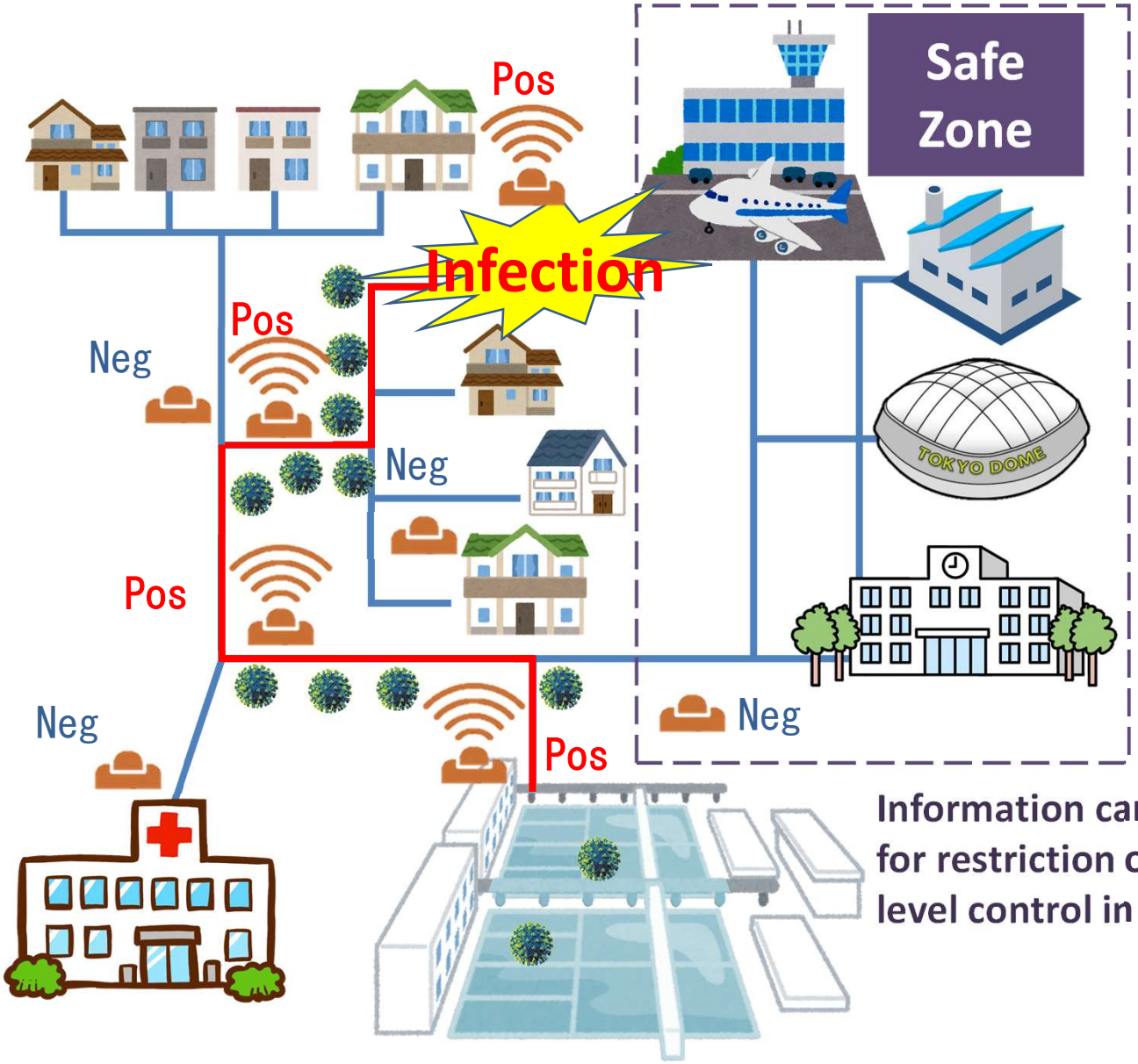


FIG 1 Concentrations of norovirus GI and GII in sewage and numbers of gastroenteritis cases reported in the study area. The norovirus GI and GII detection limits are 1.5 and 1.6 $\log_{10}$ copies/ml, respectively.

Virus concentration go up before increase of patients -> Possibility of early warning?

Same approach for SARS-CoV-2 ?

Initial Idea on WBE



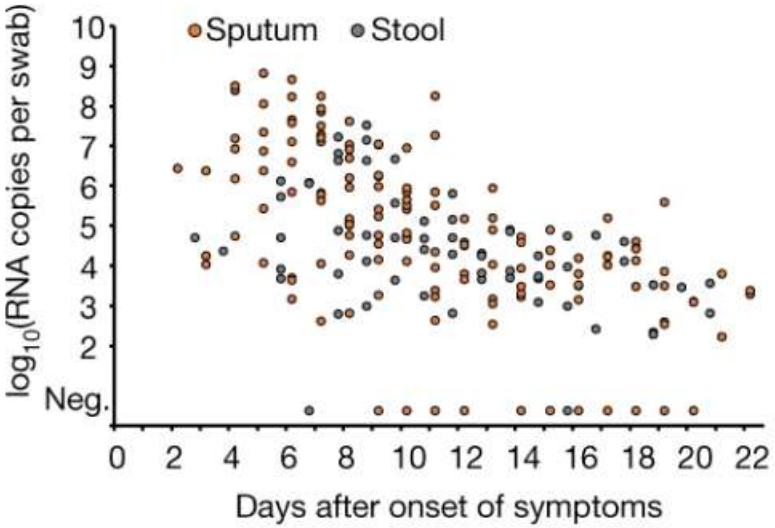
Information can be used for restriction or activity level control in the area

Limitation of wastewater-based epidemiology

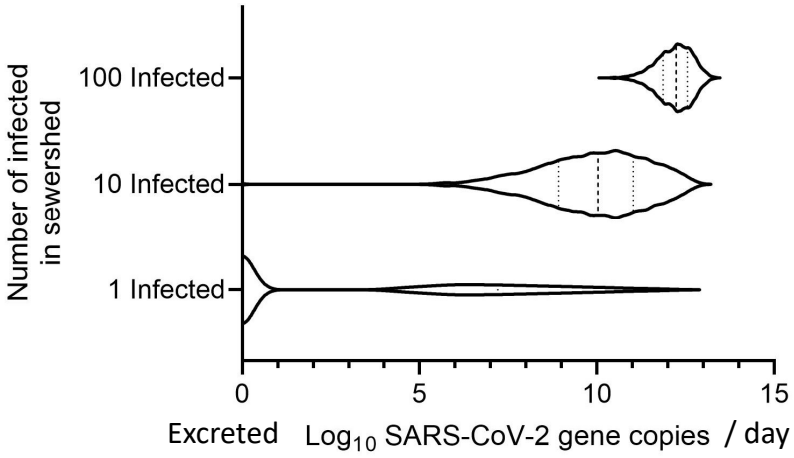
Sampling

Interpritation

➢ Highly fluctuated virus emission in feces



(Wölfel et al. 2020. *Nature*)
https://doi.org/10.1038/s41586-020-2196-x



https://twitter.com/psychedonwata/status/1282757230945763330/photo/1

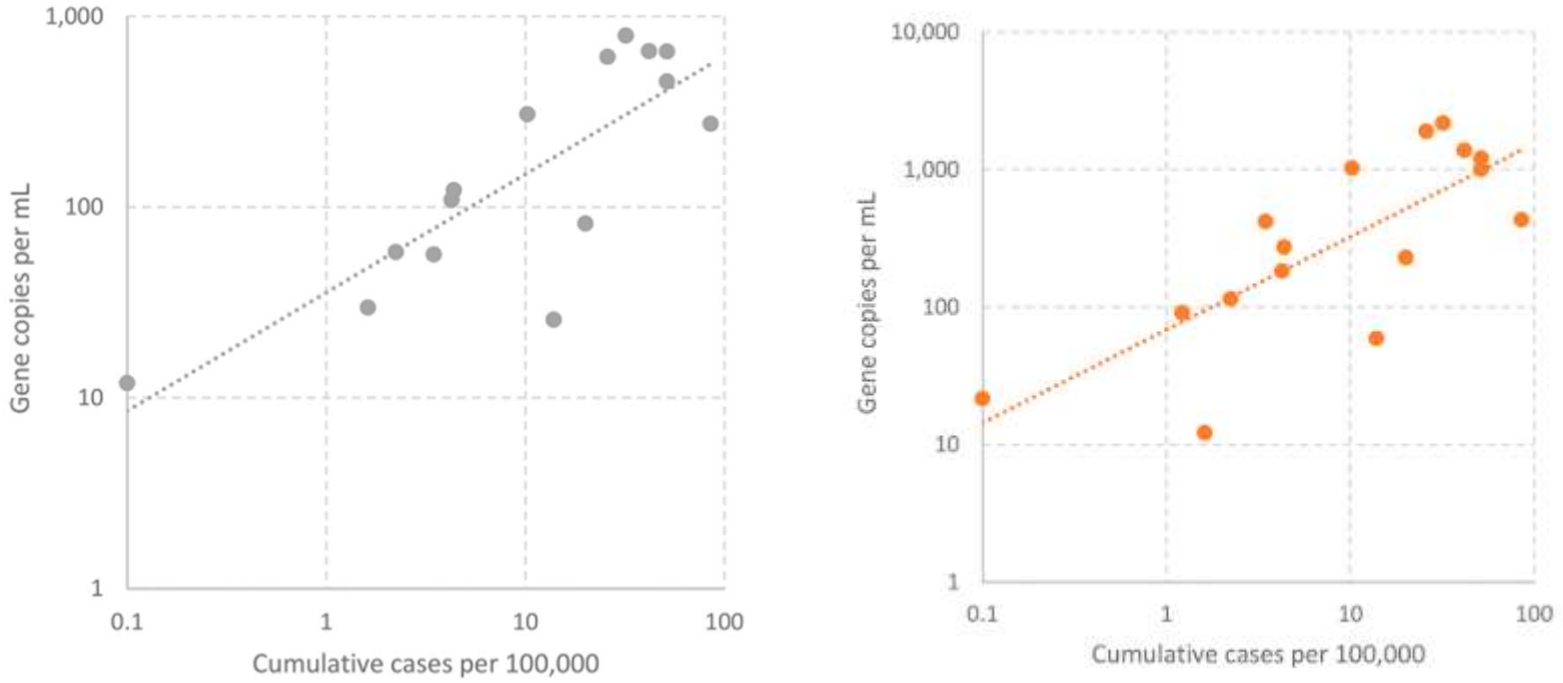
・Possible false negative in case of small number infection
・Limitation of WBE should be discussed

Report from oversea

Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands

(Medema *et al*. *ES&T* letters. 2020)

A.



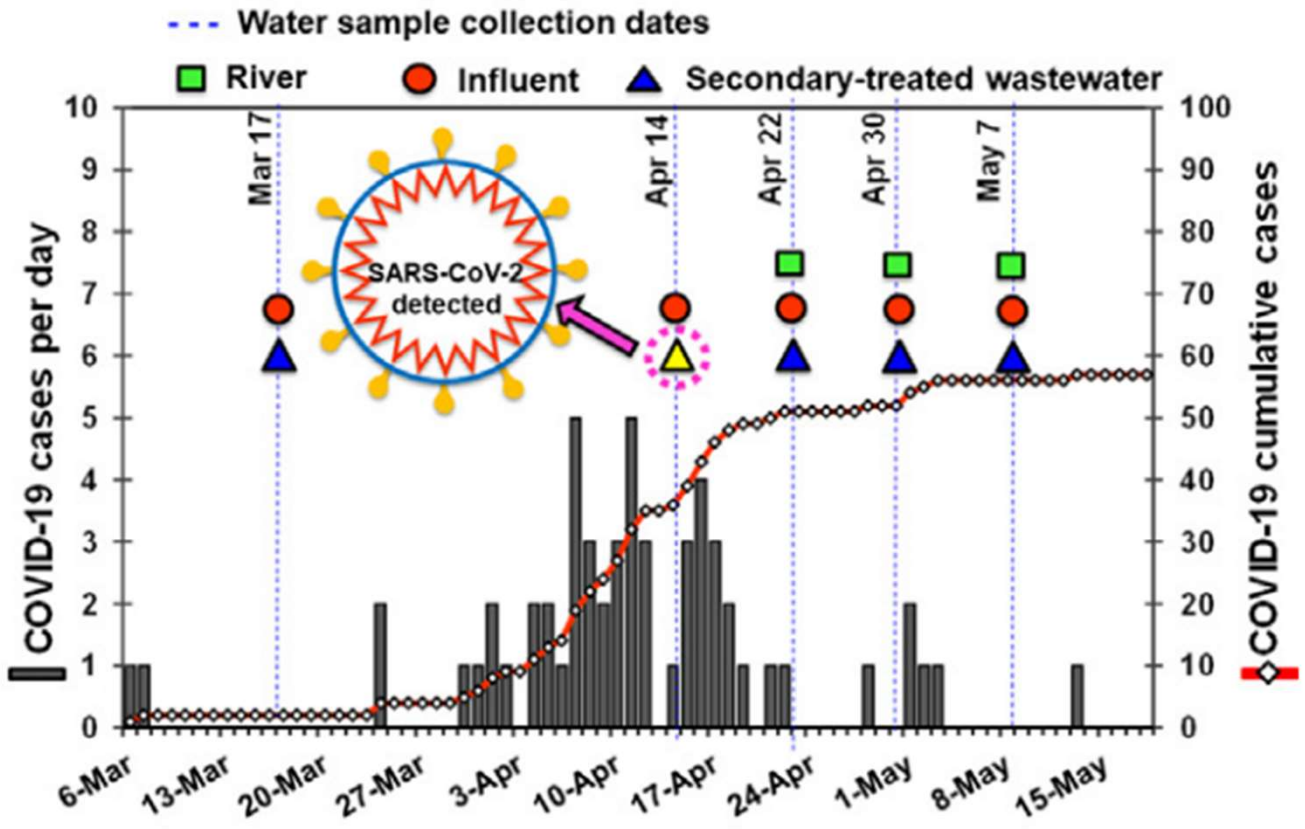
・Correlation between number of patients and SARS-CoV-2 level

・Variation ← Stability of concentration method, difference of PCR method

Other report in Japan 1

First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan

(Haramoto *et al*. Sci. Total Environ. 2020)

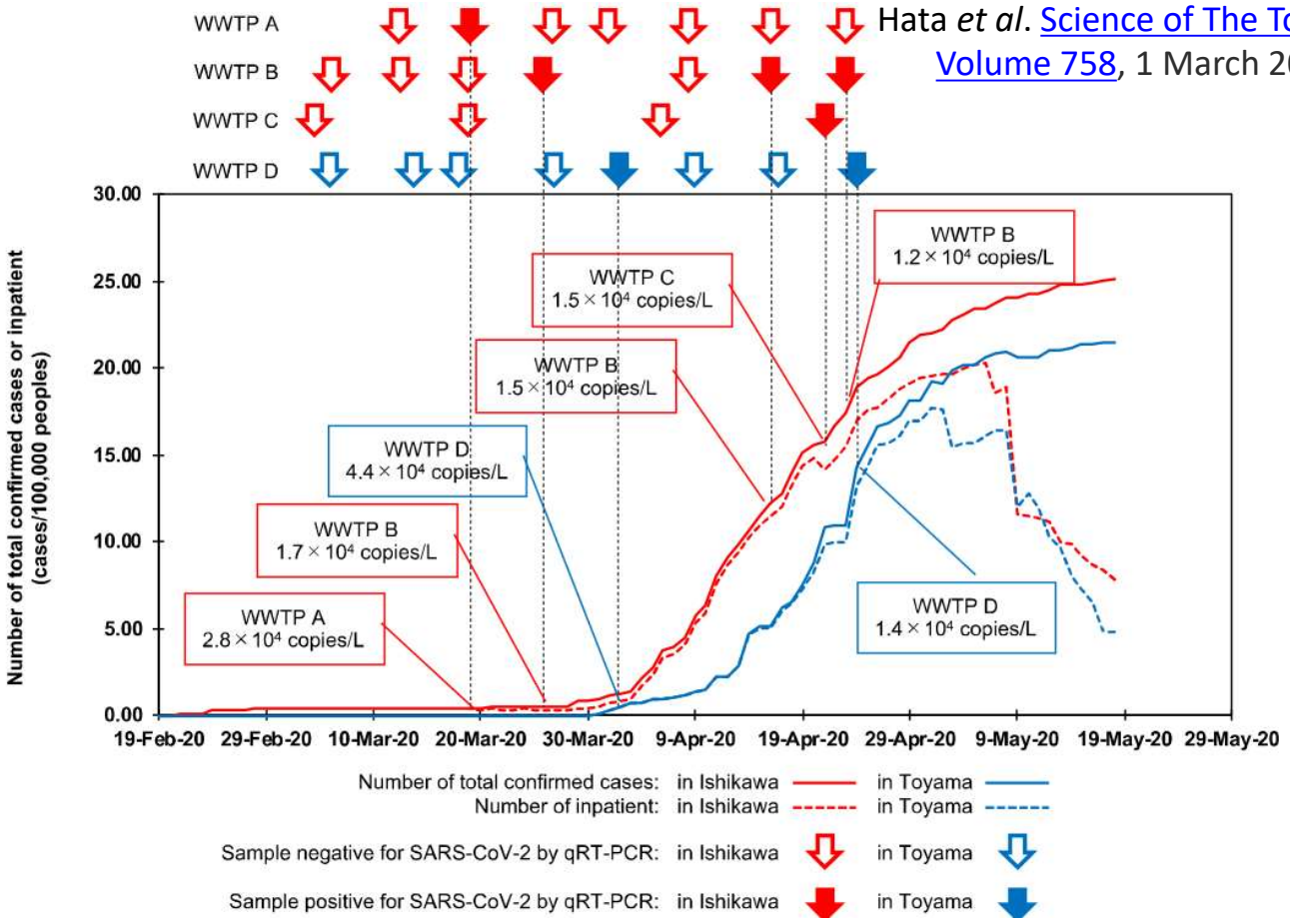


Detected from treated wastewater in Yamanashi Prefecture

Other report in Japan 2

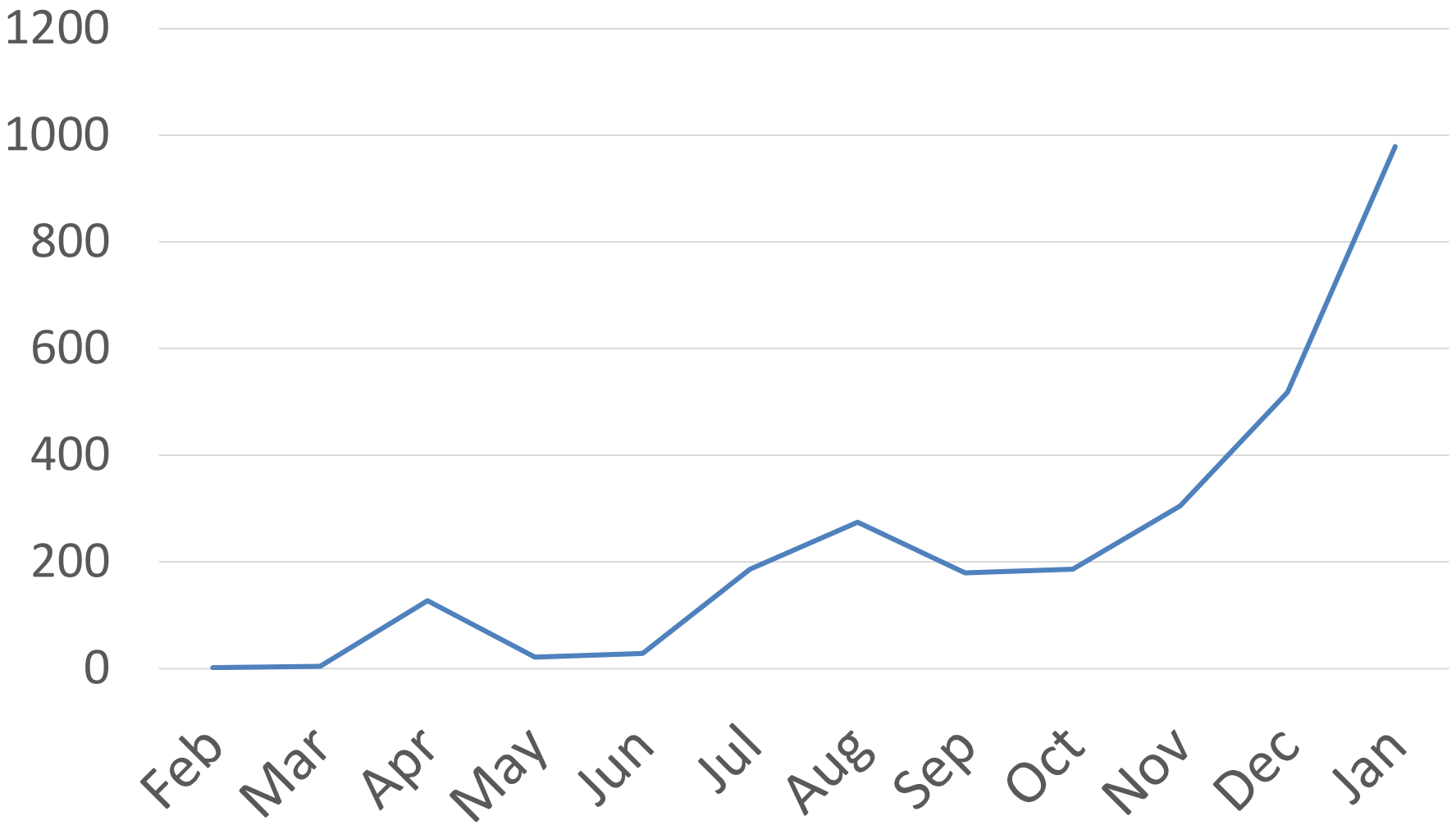
Identification of SARS-CoV-2 in wastewater in Japan
by multiple molecular assays -implication for wastewater-based epidemiology (WBE)

Hata *et al*. Science of The Total Environment Volume 758, 1 March 2021, 143578.



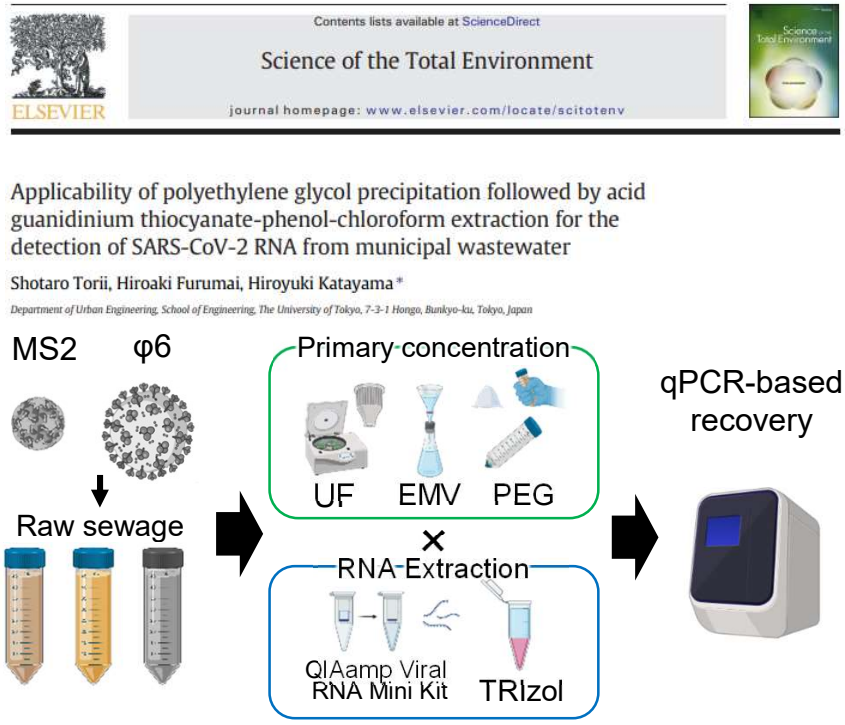
In Toyama and Kanazawa, PCR positive before outbreak

Daily newly confirmed positive in Tokyo



Out of 13 million population in Tokyo district.
7-day average in the middle of the month, except for Jan 5th

Detection of SARS-CoV-2 RNA by polyethylene glycol precipitation followed by acid guanidinium thiocyanate-phenol-chloroform extraction from municipal wastewater in Tokyo, Japan



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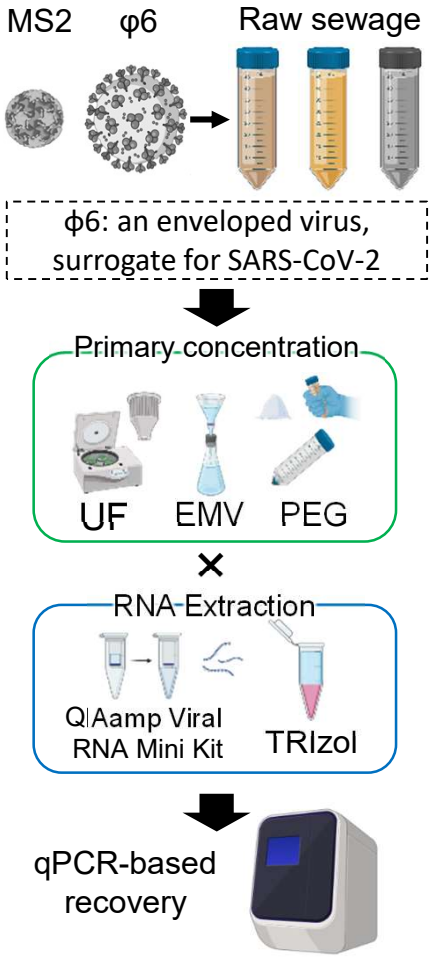
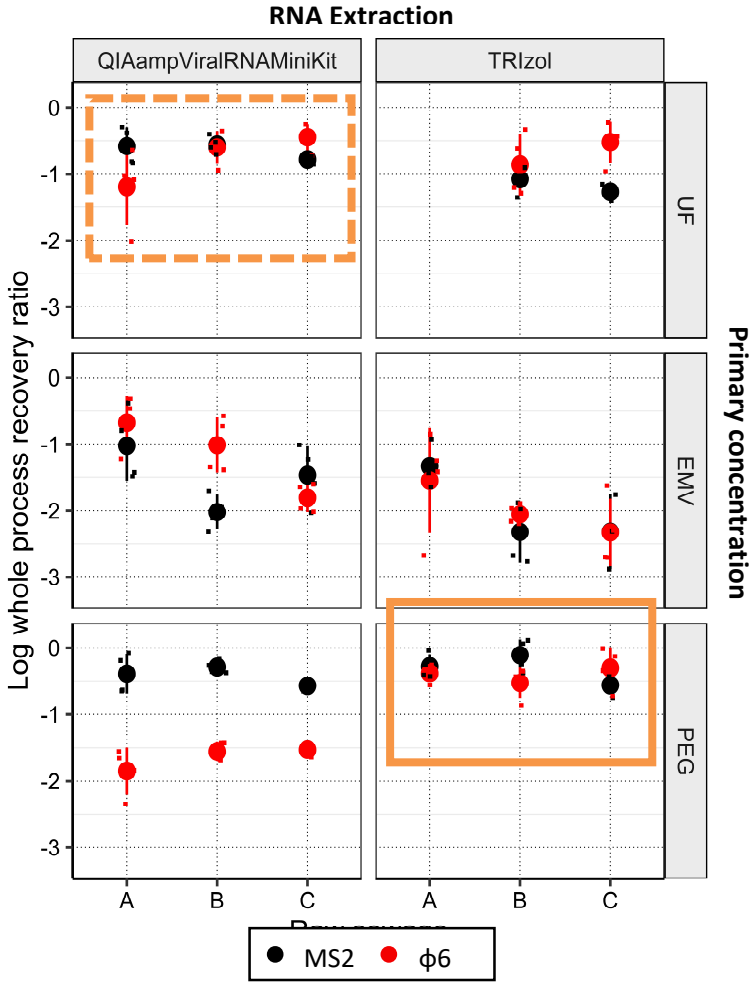
Comparison of whole process recovery

Materials and methods



Results and discussion



Whole process recovery

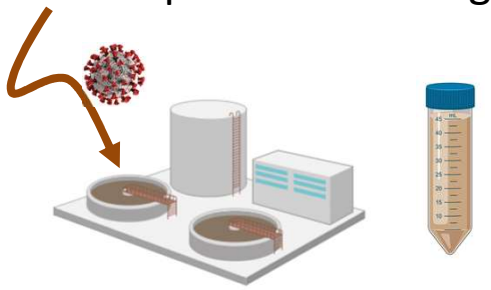
$$\frac{\varphi 6\ recovered}{\varphi 6\ seeded}$$

PEG+TRIzol provided the highest recovery for ϕ6, followed by UF+QIAamp

Detection of SARS-CoV-2 RNA from raw sewage

Weekly sampling

Grab sample of raw sewage



In June and July 2020

Processing

Frozen 40 mL | PEG | Modified TRIzol | RT-qPCR

Occurrence in raw sewage

Date	$\log_{10}$ W[a]	SLOQ[b] (copies/mL)	SARS-CoV-2
June 30	-0.41	16.1	Neg
	-0.76	38.5	Neg
July 7	-0.79	40.6	**Pos**
	-0.65	27.5	Neg
July 16	0.04	6.0	**Pos**
	-0.29	13.2	Neg
July 22	-1.14	86.6	Neg
	-0.34	13.5	Neg
July 29	-0.36	14.3	**Pos**
	-0.26	11.3	**Pos**
August 5	-0.2	10.4	Neg
	-0.68	31.3	Neg

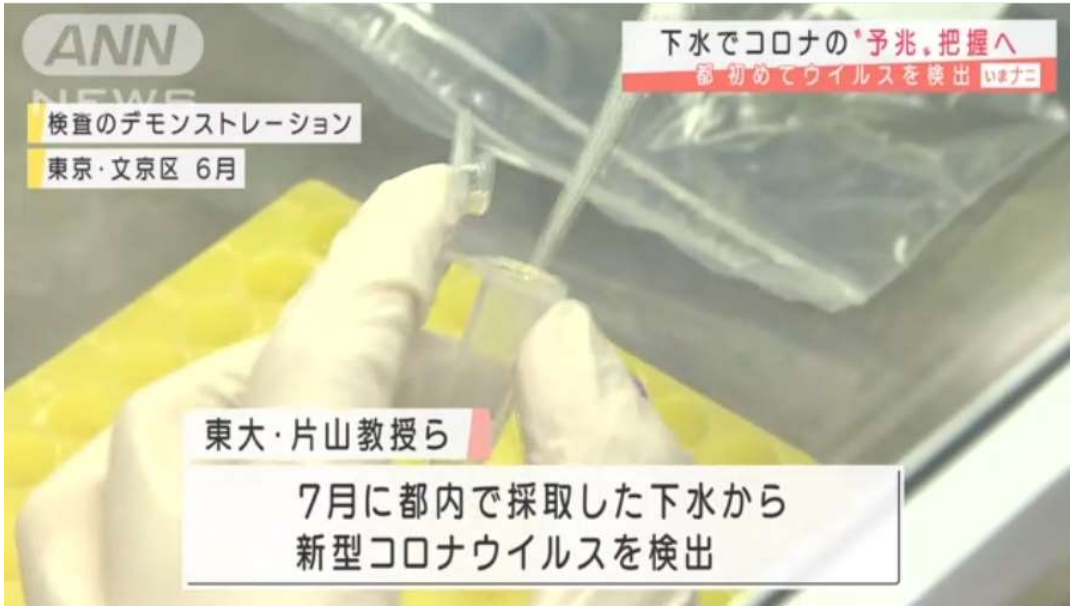
Pos indicates positive for the target while Neg indicates negative

[a]: Log whole process recovery [b]: Sample limit of quantification (SLOQ)

Of the 12 grab samples, 4 were positive for SARS-CoV-2 by RT-qPCR

Summary

- Wastewater-based epidemiology is useful tool to monitor COVID-19 in a community
- SARS-CoV-2 RNA was detected from wastewater in Japan



Shift of Idea on WBE

At first

- It is possible for WBE to detect earlier than human cases.
- Rapid detection system is needed.
  - Right after arrival of sample, how long does it take?
- We want positive data.
  - For news value
  - To convince the method is OK

After first wave

- WBE is meaningful for society because of low number of infection.
- It is impossible to detect earlier than human PCR.
- We need efficient workflow for WBE.
  - Many samples from many places, mostly negative, but constantly monitor.
  - When positive then we need action. How?

COVID-19 Taskforce in JSWE

- Wastewater sectors are not positive to provide samples at first.
- Japan Society for Water Environment launched COVID-19 Taskforce in May 2020.
- Many wastewater works started to cooperate with researchers.
- Most of the members belongs to International Water Association (IWA) specialist group on Health-related Water Microbiology
- Ministry of Land, Infrastructure, Transport and Tourism is now supporting its activity.
- We published a laboratory manual draft for detection of novel coronavirus from wastewater in Dec 2020