Tracking community infection dynamics of COVID-19 by monitoring SARS-CoV-2 RNA from wastewater in Japan

Masaru Ihara

Research Center for Environmental Quality Management, Graduate School of Engineering, Kyoto University,
Faculty of Agriculture and Marine Science, Kochi University

Wastewater surveillance for SARS-CoV-2 RNA

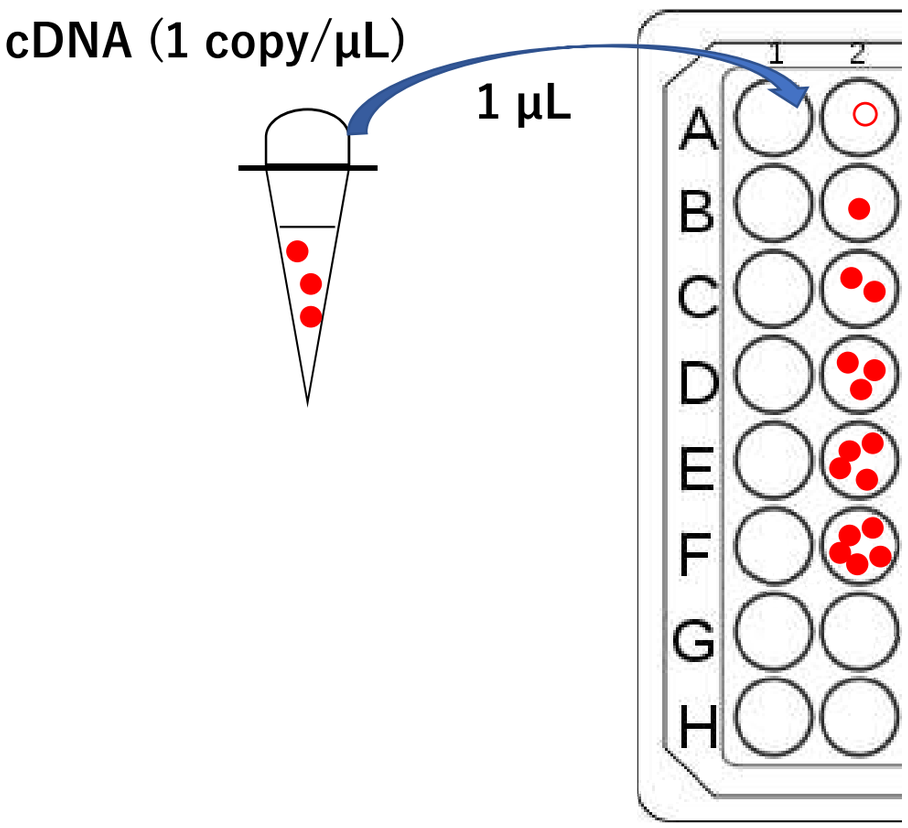
- ✓ Sampling site : **WWTP A** in the city B (population: 300,000) in Kansai region, Japan
- ✓ Sample type: Primary effluent, 120 mL
- ✓ Period : **October 2020～July 2021**
  (correspond to third and fourth waves of the infection in Japan)
- ✓ Sampling time : 10 am, spot sampling
- ✓ Frequency : weekdays, from Monday to Friday

- ✓ Virus concentration method : PEG precipitation

- ✓ RT-qPCR for SARS-CoV-2 :
  - ■ used CDC N1 and N2 primer sets.
  - ■ lots of samples were positive, but all of them were unquantifiable.
    How to get meaningful information in WBE in low prevalence area is still key challenge.
    So we applied the **positive count method** based on the Poisson distribution.

Poisson distribution

It has been reported that, in PCR, when initial target molecule number is less than 10, the probability an aliquot contains a particular number of target molecule is given by the Poisson distribution.



Initial copy number estimated by the Poisson distribution

Positive/negative ratio in repeated qPCR reaction

0 copy, $P = 36.7\%$ → Negative in qPCR. 36.7%

1 copy, $P = 36.7\%$

2 copies, $P = 18.4\%$

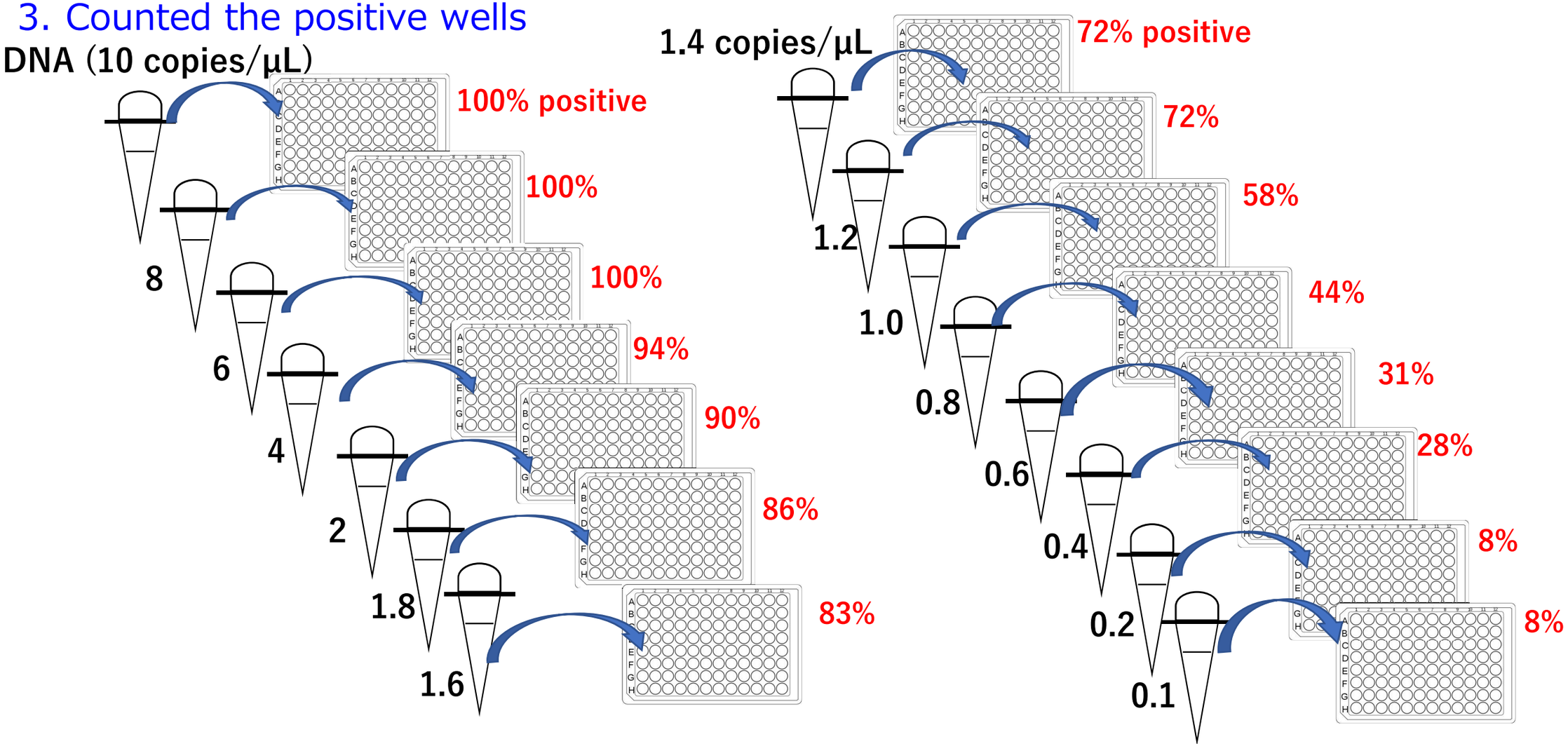
3 copies, $P = 6.1\%$ → Positive in qPCR, 63.3%

4 copies, $P = 1.5\%$

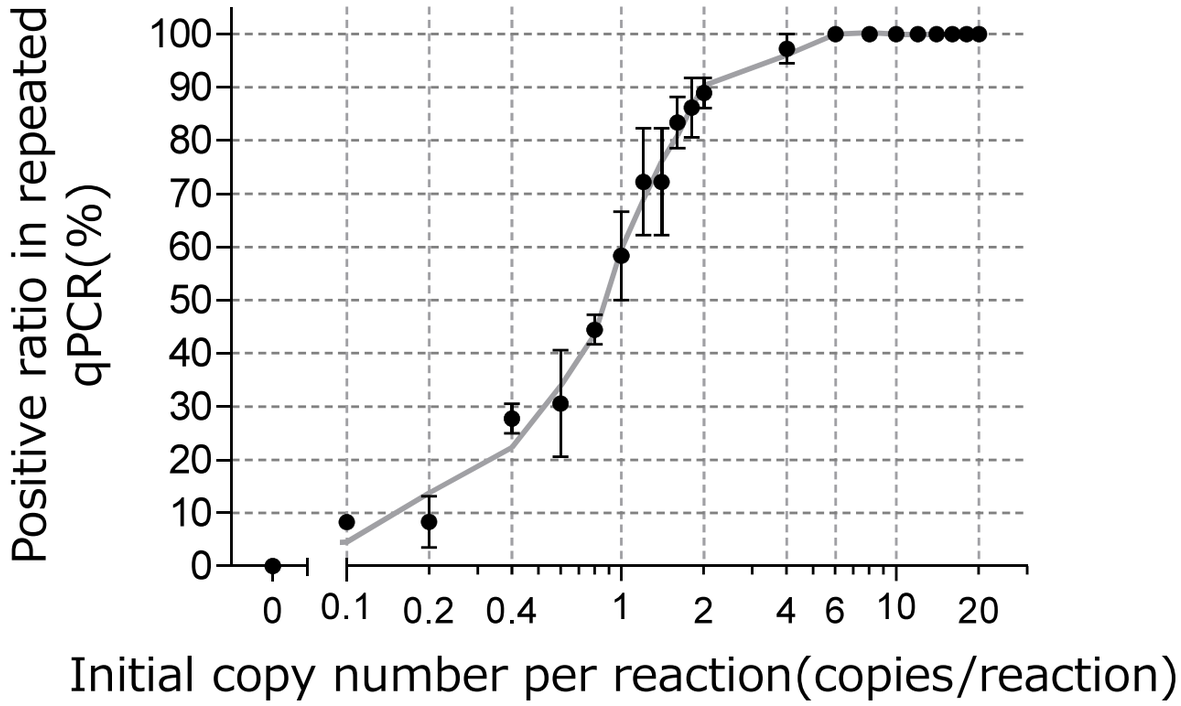
5 copies, $P = 0.3\%$

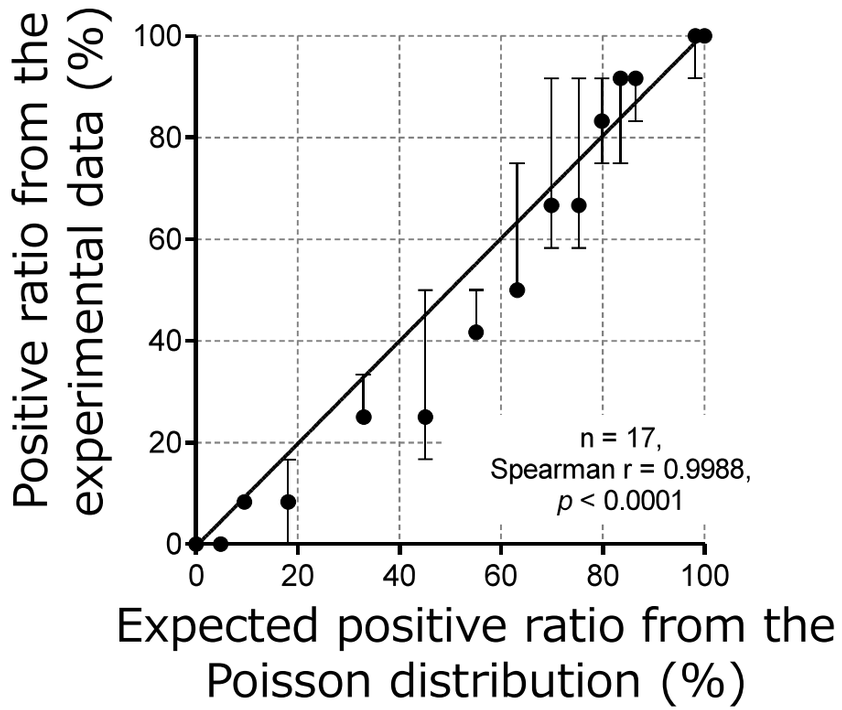
Verification of the positive ratio in qPCR for low-target copy number samples

1. We prepared the dilution series of oligo DNA for SARS-CoV-2,
2. Conducted qPCR in 12 wells, and repeated three times (n=36) for each dilution,
3. Counted the positive wells



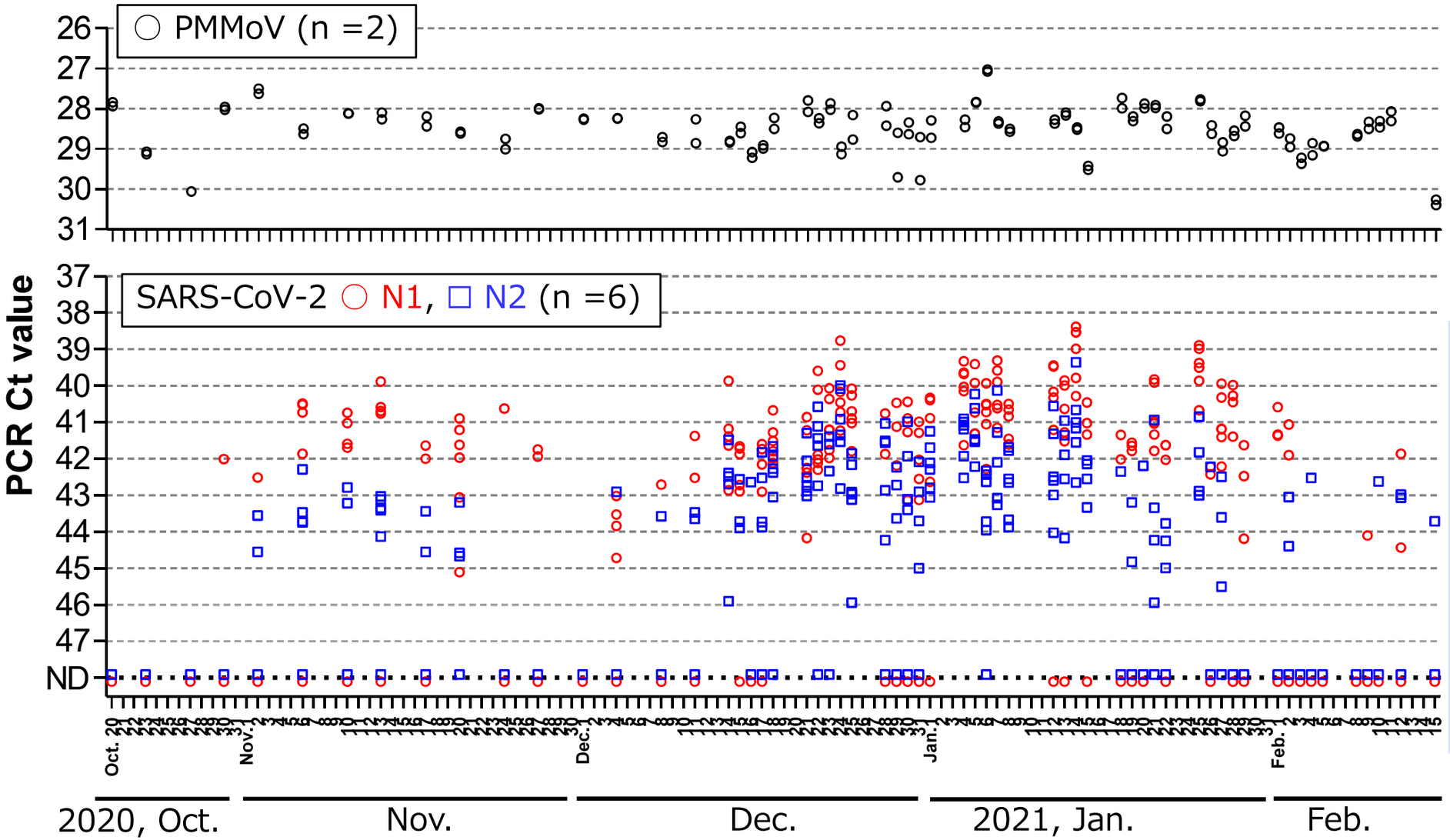
Pattern of positive and negative results is in accordance with the Poisson distribution





✓ By counting positive reactions in repeated qPCR, we could monitor the changing of the SARS-CoV-2 RNA level in wastewater even they are unquantifiable level

qPCR threshold cycle (Ct) value of PMMoV and SARS-CoV-2 in wastewater samples during the 3rd wave of the infection in Japan



PMMoV : all positive、Ct values were stable

⇒Sample preparation was stable through the campaign

N1 :
Ct values, ND～38

N2 :
Ct values, ND～40

Ct values are unquantifiable, but positive numbers increased at the peak of the third wave.

Positive number of CDC-N1 and N2 assays for SARS-CoV-2 RNA in wastewater in the 3rd wave of the infection

✓unquantifiable, but positive numbers increased at the peak of the third wave

Comparison between the SARS-CoV-2 RNA signal in wastewater and the epidemiological data, in the 3rd wave of the infection

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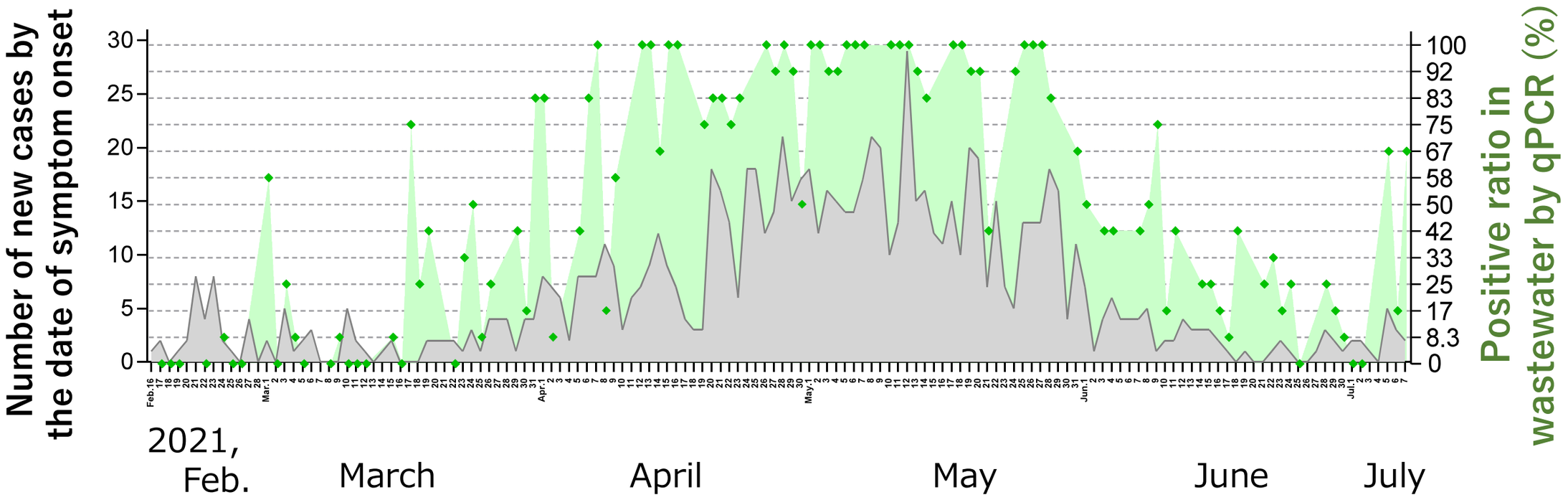
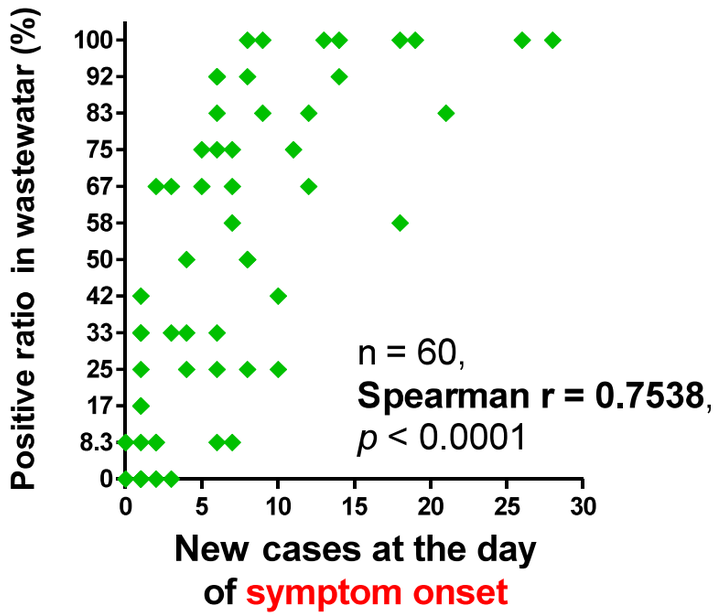
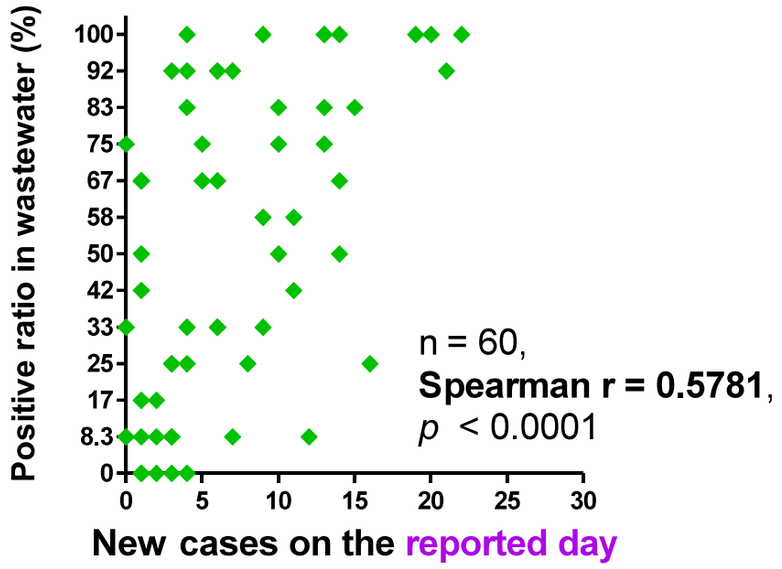
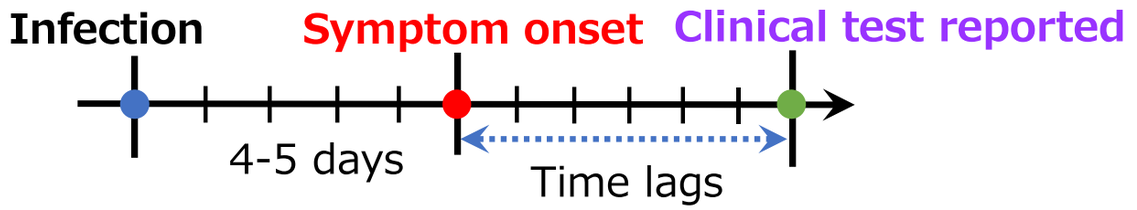
Comparison between the SARS-CoV-2 RNA signal in wastewater and the epidemiological data, in the 4th wave of the infection



Correlation analysis (Spearman's r) shows a better correlation in the new cases by the date of symptom onset than by the date reported







WBE, if analyzed and reported on the same day as sampling, is the earliest estimate of the viral spread in the community


2021, April
May
June
July
August
Sep.

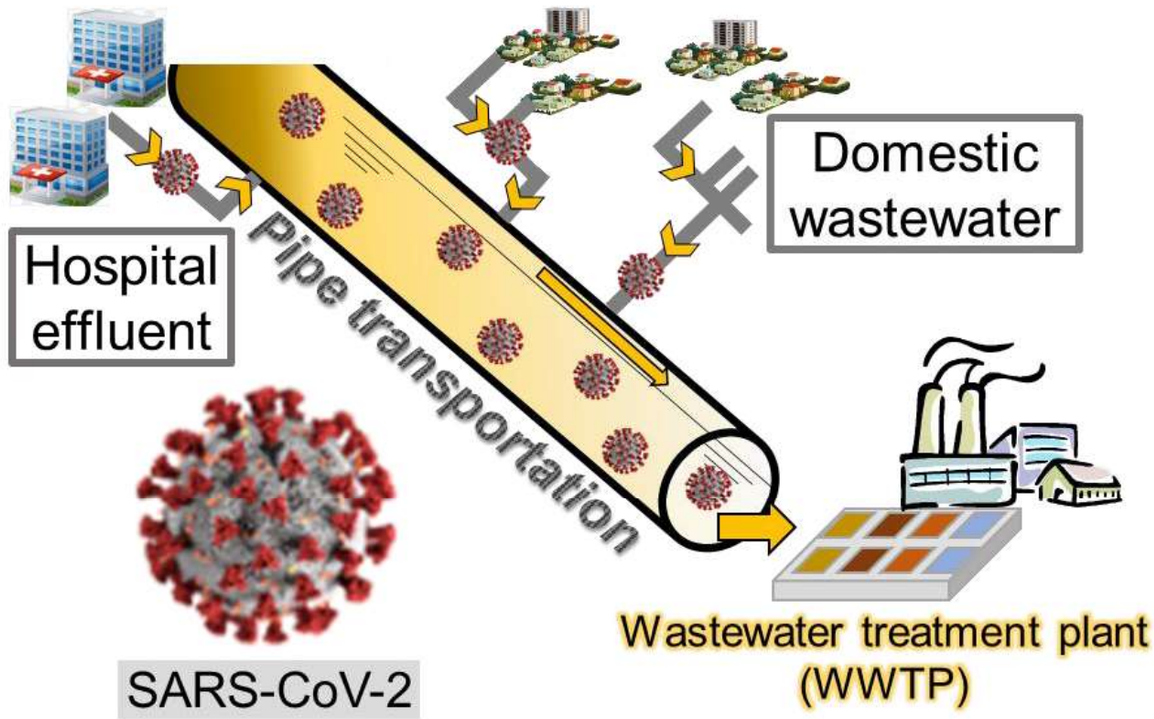
Summary

- ✓ **We demonstrated that positive ratio in repetitive qPCR for viral RNA in wastewater showed significant correlation with the number of new cases by the date of symptom onset.**
- ✓ **Notably, our results indicate that although the SARS-CoV-2 RNA concentrations in wastewater were lower than the LOQ, the positive count method could trace the prevalence of COVID-19 in the community.**
- ✓ **This method cannot quantify the genome copy numbers of SARS-CoV-2 in wastewater. However, for the purpose of tracking the virus infection dynamics in the community, exact values of virus concentration are not always necessary. It is enough to see the changing of the viral RNA levels in wastewater, and the positive count method is thus useful.**

Surveillance of SARS-CoV-2 RNA in wastewater from manholes in the city

Objective 1:
To investigate the spacial variation of the viral RNA in different manholes in the city

Objective 2:
To investigate the monthly variation of the viral RNA in different manholes



- Sampling site: 25 manholes in the city F in Japan

Area A:
10 manholes in residential area, connected to WWTP F

Area B:
3 manholes in residential area, connected to WWTP G

Area C:
6 manholes in residential area, connected to WWTP F

Area D:
6 manholes in industrialized area, connected to WWTP H

- Sampling method: spot sampling, 120 mL at each sampling site
- Sampling date: Nov., 2020 (Current infected people in the prefecture≒ 200)
  Dec. (≒ 700)
  Jan., 2021 (≒ **1,700**)
  Feb. (≒ 800)
  May (≒ **1,300**)
  June (≒ 200)
  Aug., (≒ **6,000**)
  Oct. (≒ **100**)

Positive ratio of CDC-N1 and N2 qPCR assays at manholes (%) in the city F

- ✓ At 2021 Jan. and Aug., positive ratio were higher at many manholes than other days, which seems related with the number of cases.
- ✓ At the manholes in the area A, positive ratio were higher than other area.

Summary

- ✓ **Different manholes showed different positive ratio of CDC-N1 and N2 qPCR assays detection, indicating spacial variation of SARS-CoV-2 RNA inside the city.**
- ✓ **January and August, 2021 with higher current infected people, showed higher positive ratio than other sampling days.**
- ✓ **SARS-CoV-2 RNA surveillance in wastewater from manholes might be effective to monitor the progress of COVID-19 in the community from positive detection of CDC-N1 and N2 qPCR assays.**

Thank you for your listening!