Determination of Murine Norovirus Aerosol Concentration During Toilet Flushing

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Background

Annually, norovirus causes 19-21 million cases of acute gastroenteritis, 56,000-71,000 hospitalizations, and 570-800 deaths, in the United States.[1]

The increasing incidence of norovirus outbreaks presents a risk of exposure to employee groups across several industries.[2]

Limited evidence suggests that norovirus can become aerosolized with toilet flushing hypothesized as an aerosolization source.[3,4]

Among toilet types, flushometer type toilets produce the highest concentrations of particles when flushed. These toilets are used commonly in governmental, educational, and healthcare facilities.[4]

Therefore, direct investigation of flushometer toilets is needed to determine if norovirus is aerosolized during flushing.

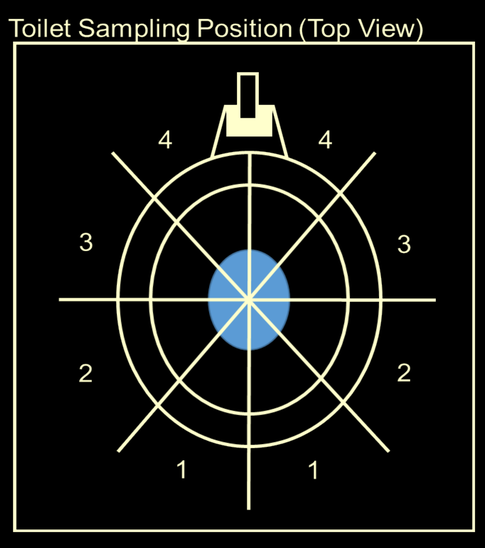
Objectives

1. Characterize the particle plume produced from the flushing of a flushometer (FOM) type toilet to inform bioaerosol sampler placement
2. Quantify the concentration of aerosolized murine norovirus (MNV, a human norovirus surrogate) from flushing a FOM type toilet using two bioaerosol samplers
3. Quantify the concentration of viable aerosolized MNV from flushing a FOM type toilet using two bioaerosol samplers

Methods

Particle size and count distribution of toilet plume were measured:

- FOM toilet located at the University of Iowa in the Information Technology Facility
- Heights: 0, 0.15, and 0.25 m above the toilet bowl rim
- Positions: 4 positions around the toilet (figure below)
- Side: Left and right side were sampled simultaneously for each height/position combination
- Sampling occurred 5 minutes prior to flush and for 30 minutes post flush
- TSI AeroTrak Optical Particle Counter (Shoreview, MN) was used



Aerosolized MNV toilet seeding, collection, extraction, and quantification:

- 50 mL of MNV at concentrations of either 10^5 or 10^6 plaque forming units (PFU)/mL
- Two bioaerosol samplers were used to collect aerosolized MNV (Figure 1)
- QIAamp Viral RNA Mini Kit spin method extraction kit (Qiagen, Hilden, Germany)
- Viral membrane integrity of MNV was determined using propidium monoazide (PMA) dye (Biotium, Fremont, CA)
- PMA treated and untreated samples were quantified using reverse transcription – droplet digital polymerase chain reaction
- There were three trials for each seeding concentration (n=30)

Mean particle concentration for each height & position combination at minute six were analyzed across trial and side. Particle concentrations were compared between the left and right side of the toilet using a Wilcoxon Signed Rank test with a Type 1 error rate of 0.05.

Experimental Setup

Figure 1. Sampling setup showing a SKC BioSampler (12.5 L/min) and Coriolis µ sampler (150 L/min) containing Hank's Balanced Salt Solution placed 0.15 m away from the toilet to collect aerosolized MNV.

Results

Height 0.15 m at Position 4 had the highest mean particle concentrations

Particle concentrations on the left side of the toilet were significantly higher than the right side for two particle sizes:

- 0.3 µm (p-value = 0.002)
- 0.5 µm (p-value = 0.002)

Table 1. Concentrations of MNV from all trials for control, toilet, and aerosol samples.

Trial n=30	Seeding Concentration (PFU/mL)	Control		Samples		
		Toilet	Air	Toilet (total copies)	SKC	Coriolis (copies/m³)
1	10^5	ND	ND	6.40×10^5	ND	ND
2	10^5	ND	ND	2.18×10^5	ND	ND
3	10^5	ND	ND	3.43×10^5	ND	ND
4	10^6	ND	ND	9.35×10^6	ND	684
5	10^6	ND	ND	4.76×10^6	ND	505
6	10^6	ND	ND	9.65×10^6	ND	383

The mean relative percentage of MNV from intact capsids for post-seeding toilet water was 67%. All other control and bioaerosol samples were ND's.

Conclusion

Flushing a FOM toilet was identified as an indoor aerosolization source for MNV, a human norovirus surrogate

- Control measures should be considered (*e.g.,* lid, flushing chamber, waterless toilet, and reducing water pressure)

A toilet plume was created in every direction around the toilet, with higher particle concentrations toward the rear of the toilet

- Surface contamination at the rear of the toilet from flushing should be evaluated

Viability of MNV was maintained in toilet water, with chlorine having minimal effect on viability

Future Work

Perform norovirus sampling in healthcare settings (*e.g.,* hospitals and long-term care facilities

Continue characterization of toilet plume from different heights, positions and distances

Continue evaluation of the effect of flushing on the viability of MNV

Acknowledgements & References

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