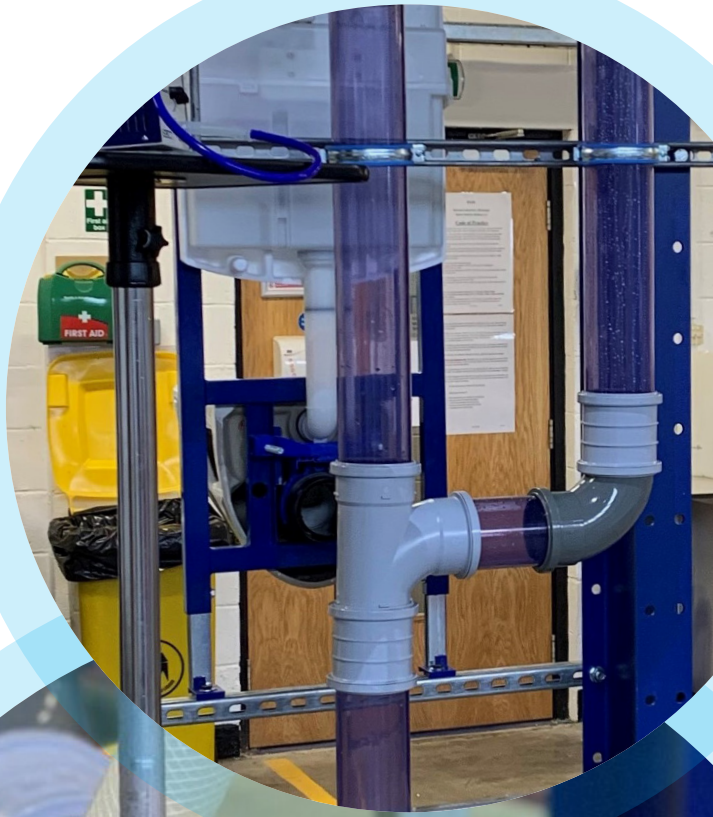
CREW CENTRE OF EXPERTISE FOR WATERS

Aerosol/droplet sampling of wastewater for SARS-CoV-2



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Executive summary

Objectives

This project focussed on a proof of concept for the detection of viral RNA from aerosolised particles which are naturally generated inside the sanitary plumbing system (pipework, including the main vertical pipe or 'stack') in a building. The hypothesis is that when a toilet (which contains faeces, urine, or vomit of a person infected with SARS-CoV-2) is flushed, the virus will be present in aerosols generated within the system and so, will then be carried in the system airstreams. Sampling the airstreams will capture the aerosols, which can be tested for the presence of viral RNA. This information can then be used as part of the suite of surveillance tools used to inform public health policy.

The objectives of this project were:

1. Complete a rapid literature review to scope the current state of the art, covering aerosol generation and appropriate sampling methodologies;
2. Set up a physical test-rig of a two-storey building sanitary plumbing system;
3. Establish sampling method(s) using existing or modified bio-samplers;
4. Run a series of experiments to establish the extent of aerosolisation of water dosed with a surrogate virus (PRRSv) and analyse air samples for the presence/absence of viral RNA;
5. Establish the most effective location for viral sampling.

Background

Prevalence of the novel coronavirus SARS-CoV-2 in the community has been successfully demonstrated in previous research based on wastewater samples taken from various locations in the sewerage transport and treatment process. This technique relies on reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) testing to identify the presence of SARS-CoV-2 RNA in the wastewater samples. This previous work established protocols for sample testing and identified a suitable surrogate virus, Porcine Respiratory and Reproductive Syndrome virus (PRRSv) for validation of the sampling technique. The same RT-qPCR technique was used in this research and heat de-activated PRRSv was used to dose the water in the system to avoid the use of SARS-CoV-2.

This methodology could be applied to buildings such as schools, care homes, prisons or university residences, which have been linked with disruptive outbreaks in the past; all of which have much wider societal contexts.

This methodology could be used to expand existing surveillance protocols and could be linked directly to the work of Public Health Scotland (PHS), Scottish Environmental Protection Agency (SEPA) and Scottish Water (SW), so if viral RNA was detected in or very near buildings then SEPA/SW should be notified to increase sampling of wastewater treatment plants (WWTP) in the vicinity (catchment).

Research undertaken

A series of experiments were carried out on a two-storey test rig in which water dosed with PRRSv was flushed into the system using a number of pipework system configurations representative of those found in practice. Aerosols were sampled in the following ways:

1. Using an aerodynamic particle size sampler – which enumerated the number, size and concentration of aerosols produced by the flushing process;
2. Aerosol sampling for the detection of viral RNA at a number of locations in the test rig. Samples were taken using the following instrumentation:
    i. An SKC[1] BioSampler (impinger type sampler);
    ii. A passive in-line stainless-steel sampler.
    iii. An IOM[2] filter-based sampler with gelatin filter;
    iv. A wet cyclone air sampler.
3. All samples were sent for RT-qPCR analysis at the Roslin Institute (University of Edinburgh).

Key findings

- Viral RNA can be detected using bioaerosol sampling methods within a building's sanitary plumbing system, at distance from the source.
- Detection was demonstrated:
    - Both below and above the source (the source being defined as the inlet to the vertical (stack) pipe connected directly to a toilet).
    - Below the source this was from the horizontal collection drain leading to the main sewer.
    - Above the source, detection was achieved 1 m above the inlet. No viral RNA was detected beyond this point under the conditions described in this report.
- The inoculum composition, concentration and sampling methods were potentially factors in detection distances.
- Viral RNA was detected from the passive samplers

1 SKC is a manufacturer of bio air samplers.
2 Institution of occupational medicine (IOM)

and the wet cyclone sampler. No positive samples were detected from the SKC BioSampler or the IOM filter-based sampler.

- Sampling aerosols from sanitary plumbing systems is heavily influenced by environmental and water chemistry conditions, e.g. relative humidity and dissolved salts.
- Sampling aerosols is preferential over direct wastewater sampling because samples are aggregated over time rather than taken from discrete points in time.
- The most effective location to detect viral RNA from sanitary plumbing systems is from the horizontal collection drain at the bottom of the vertical stack, just before it connects with the main sewer.

Policy Implications

1. Detection of viral RNA from building drainage systems is possible and could form part of the on-going surveillance efforts for SARS-CoV-2 and COVID-19, particularly where source questions arise, for example in care homes, prisons and university halls of residence.
2. The aerosol generating processes investigated show a significant public health implication for the spread of many other diseases, particularly those more amenable to faecal/oral route of infection. The safe management of wastewater and sanitary systems should be updated to include aerosol and droplet management.

Recommendations

1. Further work is recommended in a real building setting based on the learning obtained from this project. The findings could be exploited in a number of settings which may still require increased surveillance in the future, particularly care homes, prisons and university halls of residence.
2. Further research is needed to adapt the passive filter so that it can be simply and safely installed in real-world settings for widespread sampling.
3. The work could be expanded to the investigation of the presence of viral RNA in mains sewer pipes. This work could be calibrated against the wastewater epidemiological work already being carried out using wet samples.
4. Whilst not a direct objective of this project, the results have implications for the transmission of disease through sanitary plumbing systems within buildings, and the inclusion of this mode of transmission as a public health imperative through increased regulation and monitoring is strongly advised.

Background

Prevalence of the novel coronavirus SARS-CoV-2 in the community has been successfully demonstrated in previous research sponsored by CREW (Corbishley et al., 2020) and has been based on wastewater samples taken from various locations in the sewerage transport and treatment process. The technique relies on reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) testing to identify the presence of SARS-CoV-2 RNA in the wastewater samples. Previous work established protocols for sample testing and identified a suitable surrogate virus, Porcine Respiratory and Reproductive Syndrome virus (PRRSv) for validation of the sampling technique. The same RT-qPCR technique, and heat de-activated PRRSv, were used to dose the water in the system in this study to avoid the use of SARS-CoV-2.

The rationale for this project focuses on the necessity from a public health point of view to establish if testing for COVID-19 in a much smaller population (i.e. the occupants of a building) can be conducted alongside the wider-scale monitoring offered by wastewater sampling at a treatment plant or catchment level. An indication of the presence of infected people within a building could help public health officials to monitor outbreaks or clusters of cases with more accuracy and shorter timescales, especially when prevalence in the wider community is relatively low. This could be applied to buildings such as schools, care homes, prisons or university residences, which have been linked with disruptive outbreaks in the past, all of which have much wider societal contexts.

This methodology could be used to expand existing surveillance protocols and could be linked directly to the work of Public Health Scotland (PHS), Scottish Environmental Protection Agency (SEPA) and Scottish Water (SW); if viral RNA were detected in or very near buildings, then SEPA/SW should be notified to increase sampling of WWTP in the vicinity (catchment).

The hypothesis being tested in this research is that when a toilet (which contains faeces, urine, or vomit of a person infected with SARS-CoV-2) is flushed, the virus will be present in the aerosols and droplets generated within the system, and then be carried in the airstreams. Sampling the airstreams will capture the aerosols and droplets which can then the tested for the presence of viral RNA. This information can then be used as part of the suite of surveillance tools used to inform public health policy.

The objectives of this project were:

1. Complete a rapid literature review to scope the current state of the art, covering aerosol generation and appropriate sampling methodologies;
2. Set up a physical model of a two-storey building sanitary plumbing system to simulate a real in-building setting to include realistic updraughts from the base to the top of the vertical stack;
3. Establish sampling method(s) using existing or modified bio-samplers;
4. Run a series of experiments to establish the extent of aerosolisation of water dosed with a surrogate virus (PRRSv). Analyse samples for presence/absence of viral RNA;
5. Establish the most effective location for viral sampling.

Literature review

A rapid review of available sampling techniques was performed to ascertain appropriate viral bioaerosol experimental methods for the study. The full published review (Dight and Gormley, 2021) is shown in Appendix C. This section reviews the current state of the art in methods to identify airborne virus as this was central to this study. Since virus viability was not an objective for this study, sampling methods which optimised collection of viral RNA were prioritised.

The aerosolisation of a virus often occurs at a different rate to the aerosolisation of bulk fluid, for example sprays and jets can cause aerosols which do not contain any virus. The reasons behind this are complex and not solely determined by the virus structure, which make the interpretation of results from the laboratory, or disparate fields of research, difficult. Only some factors require attention in the wastewater context.

Whilst virus survival was not investigated in this study it is useful to consider some important parameters affecting survivability as this is associated with the integrity of the virus particle. It has widely been shown that virus survival will vary with temperature and relative humidity (Verreault et al., 2008; Xagoraraki et al., 2014). These factors have been shown to interact with the influence of organic matter in the suspension fluid. Smither et al. (2020) showed that the influence of artificial saliva on the survival of aerosolised SARS-CoV-2 was reversed depending on the relative humidity. Ijaz et al. (1985) found that human rotavirus aerosolised from a human faecal suspension lost viability at a lesser rate than the same virus in tryptic soy broth.

Particles and aerosols once formed, deviate from the path of the bulk fluid under various influences: - settling or buoyancy and inertial effects play a more important role in the motion of larger particles, the mass of which is greater compared to their air resistance; random walk type effects such as Brownian motion and turbophoresis, cause more mobility in smaller particles. Electrostatic effects

also exert a larger influence on small particles, relative to their mass. Finally, filtration depends to some degree on the physical obstruction of larger particles (Verreault et al., 2008; Pan et al., 2019). The differing roles of various forces in removing particles from the bulk air mean that different sampling methods capture particles of different sizes with roughly equal efficiently. The interaction of a particular aerosol with a particular sampler cannot be characterised purely in terms of size, as factors such as density, morphology, and electrical characteristics also influence their propensity to be sampled. Nevertheless, empirical results often show that there is a trough at around 300 nm at which neither inertia nor diffusion facilitate the efficient capture of aerosols; efficiency at 300 nm is therefore used to quote filter efficiency, and to set standards (EN12341:2014 [European Committee for Standardisation, 2014]). The physics associated with aerosols and aerosolised virus particles is extremely complex and evaluation of sampling techniques cannot ignore this complexity as can be seen below.

Verreault et al. (2008) conducted a systematic review of viral bioaerosol sampling up to 2007. Their study classed samplers as either; cyclone, liquid impinger, slit sampler, electrostatic precipitator, filter, and other; this last included sample collection from existing air handling filters and plant, and the exposure of animal hosts to aerosols. Pan, et al. (2019) classified samplers as impactors and cyclones, liquid impingers, filters, electrostatic precipitators, and water-based condensation devices. Given the merits and weaknesses of different samplers, some authors report on chimeric devices; sequentially targeting different size fractions of the aerosol population. The principles of the major sampling technologies will briefly be set out below.

Impactors, cyclones, and impingers are built to rely on particle inertia. Slit samplers are perhaps the simplest, with air forced through fine holes in a 'sieve' at high velocity; and subsequently deflected by a plate; larger particles follow the deflection of the airstream less closely, and so impact on the backing plate. It is convenient to use an agar plate where virus viability is to be tested by bacteriophage. Filter media or stainless steel plates can also be used, and washed with a sampling fluid, or dissolved, as appropriate. Impactors capture smaller particles more efficiently at higher air speeds, so multi-stage impactors employ progressively finer sieves (implying faster air flow) to capture progressively smaller particles for analysis. Slit samplers discharge only onto part of a sampling plate (agar in practice); by moving the backing plate at a known rate, a population density of bioaerosols can be captured as a function of time.

Impingers take the form of a vial containing a small volume of collection fluid to be tested for virus. Air passes through a nozzle or nozzles at sonic velocity, which provides a constant flow rate which is a function which is a characteristic of the device rather than the back pressure, over its operating window. Air is directed towards the bottom of the vial, where it impacts on the fluid, or against a vial surface which is washed by the fluid in motion; deposition is dominated by particle inertia as with impactors. The first widely deployed impingers were of the All-Glass Impinger (AGI) design, which come in smaller (c.5ml) and larger (c.30 ml) variants, and feature a single, vertical nozzle. The BioSampler, featuring three nozzles which impart a rotational component to the air's flow, was proposed as a development of the AGI; both are now seen as reference machines for the performance of impingers. Some authors report having found the BioSampler to be as effective or more effective for the capture of viable virus at subsonic velocities (Hogan et al., 2005; Lednicky et al., 2016).

Cyclones are conical devices; air enters at the flared end of the cone, at an angle (often tangential – Kenny et al., 2017) such that it has a certain rotational momentum. Air is exhausted at the tip end, meaning that the rotational momentum causes the air to spin at an increasing rate; particles are removed from the air stream by their inertia. The exhaust is often taken out through a pipe which passes through the cone's flared end; this means that with the tip pointing down, cyclones can hold water or some other sample collection fluid; they may alternatively be run dry. Cyclones can vary hugely in size, with Lindsley et al. (2006) reporting the development of a cyclone stage for a personal air quality monitor which was most efficient at 3.5 l/s (although effective down to 2 l/s), while devices measuring hundreds of litres per second are used for air quality monitoring and research, and functionally identical machines are used to clean industrial exhaust gases. Cyclones are generally used to capture larger particles of 10 µm or more, although models do exist which somewhat extent this range.

Filters interrupt aerosols in sampled air by impaction due to inertia, random walk, and electrostatic effects arising within the filter. They can be deployed near to the source to sample naturally occurring air flows, and in this instance may also capture droplets and larger particles which would not be carried to a remotely located sampler. Filters can take various geometries, depending on the desired function and the material required for a given application. Glass fibres, quartz fibres, PTFE fibres, and PTFE-coated glass fibres are stipulated in EN12341 (European Committee for Standardisation, 2014), cited as the standard by Setti et al. (2020), whereas membrane filters are often made from materials such as gelatin, polycarbonate, and more recently stainless steel. Filter efficiency is sensitive to temperature and humidity, and gelatin filters are prone to disintegrate in hot or humid conditions. The sampling of 'non-lab' filters found on air handling equipment has been used to test for the presence of airborne pathogens or contaminants in the field. Although filters often achieve high particle removal

efficiencies, the rate of collection of viable virus is often low, possibly due to virus inactivation by desiccation.

Electrostatic precipitators use a corona discharge device to impart a charge on aerosols, which are then captured on electrodes. This can be a quiet, compact, and energy-efficient way of sampling aerosols, although it is more effective for larger particles. This process also generates ozone, which impairs virus viability.

Condensation-based devices increase the relative humidity of the air, causing existing aerosols to grow, or simply harvesting condensate from cooling fans. Lednicky et al. (2016) have demonstrated the use of their VIVAS device to sample aerosolised influenza virus; this sampler cools the air before passing it down a warm, wet-walled tube, causing larger aerosols to nucleate around existing nuclei. These are then collected using an impactor-type stage, and in this instance the VIVAS was shown to collect viable virus more efficiently than the BioSampler. This principle was used by Gormley et al. (2017) in detecting aerosolised bacteria in a sanitary plumbing system. The stainless steel plate or 'passive sampler' contained perforations which allowed airflow with minimal disruption to the pressure regime and enough surface area for aerosols to condense and bacteria to stick to the plate. This method is more appropriate for larger droplets. The passive sampler benefits from location in an area where relative humidity is high.

Hogan et al. (2005) compared the performance of an AGI-30, a BioSampler, and an immersed frit-glass bubbler. All devices were less than 10 % efficient at collecting particles in the 20-100 nm range, however in a longer, 50 min sample run, the frit glass bubbler seemed to show a sharp uptick in efficiency in the collection of 25 nm. Bacteriophage culture showed similar low recovery of viable virus from the frit bubbler as from the other devices. It was suggested that the frit glass acted like a filter, initially preferentially accreting bioaerosol solids on the solid surfaces of the sampler until an equilibrium was approached between the adhesion and resuspension of organic matter.

Agranovski et al. (2002) demonstrated the collection of viable influenza and vaccinia viruses from a sampler with a submerged frit / filter medium, attributed to the formation of tiny bubbles within the medium, encouraging diffusive and gravitational aerosol collection.

In summary, a number of sampling technologies exist, however all face the intrinsic problem that capturing particles in the 300 nm range is physically difficult; a good bioaerosol sampler must achieve high efficiency in particle capture, particle recovery, and in many cases maintain virus viability, although this was not necessary for this project. The interaction of these factors across sampling technologies and sample media might suggest the use of a given technology in a particular application, however, there remains a place for experiment and empiricism, especially in novel applications. Therefore, a number of different technologies were used in this project.

Methods

Physical model

A full-scale physical model was constructed to represent the sanitary wastewater system of a standard two-storey building, as illustrated in Figure 1. The system consisted of a main vertical (stack) pipe with a branch connection at each of the two floors. At Floor 1, the branch connection provided the wastewater system inlet via a flushing toilet or manual flush-valve (both providing around six litres of flushed wastewater). At Floor 2, the branch connected to a small chamber (representing a bathroom with extract fan) with an open-end (i.e. no U-bend or water trap seal connected). The base of the stack connected to a horizontal drain which discharged to a collection tank. The system was constructed to conform to BS EN 12056 (the British and European Standard for the design and construction of sanitary wastewater systems in buildings) and was constructed with 100 mm diameter clear PVC-u rigid pipework, and standard fittings. A full description of the sampling approach is given below and in Appendix A.

Test locations

A number of test-points were incorporated around the system in order to measure viral content throughout the different stages of testing. These include the wastewater inlets: (i) the virus solution [A], and (ii) the toilet bowl [G]; and the wastewater outlets: (iii) the horizontal drain discharge [B, C, D], and (iv) the collection tank. They also included various points within the system to sample the air: (v) the horizontal drain [H, K, L, P, S, W], (vi) the main vertical stack [I, Q, T], (vii) the branch connection to the chamber [J, M, N, R], (viii) at the branch open-end inside the chamber [F], and (ix) the top of the main vertical stack [U, V].

Airflow

The airflow rate within the test-rig was controlled using the extract fan in the small chamber and corresponded to that generally found in real systems. For some tests, the extract fan was switched off to assess the effect of the natural buoyancy of the air within the system.

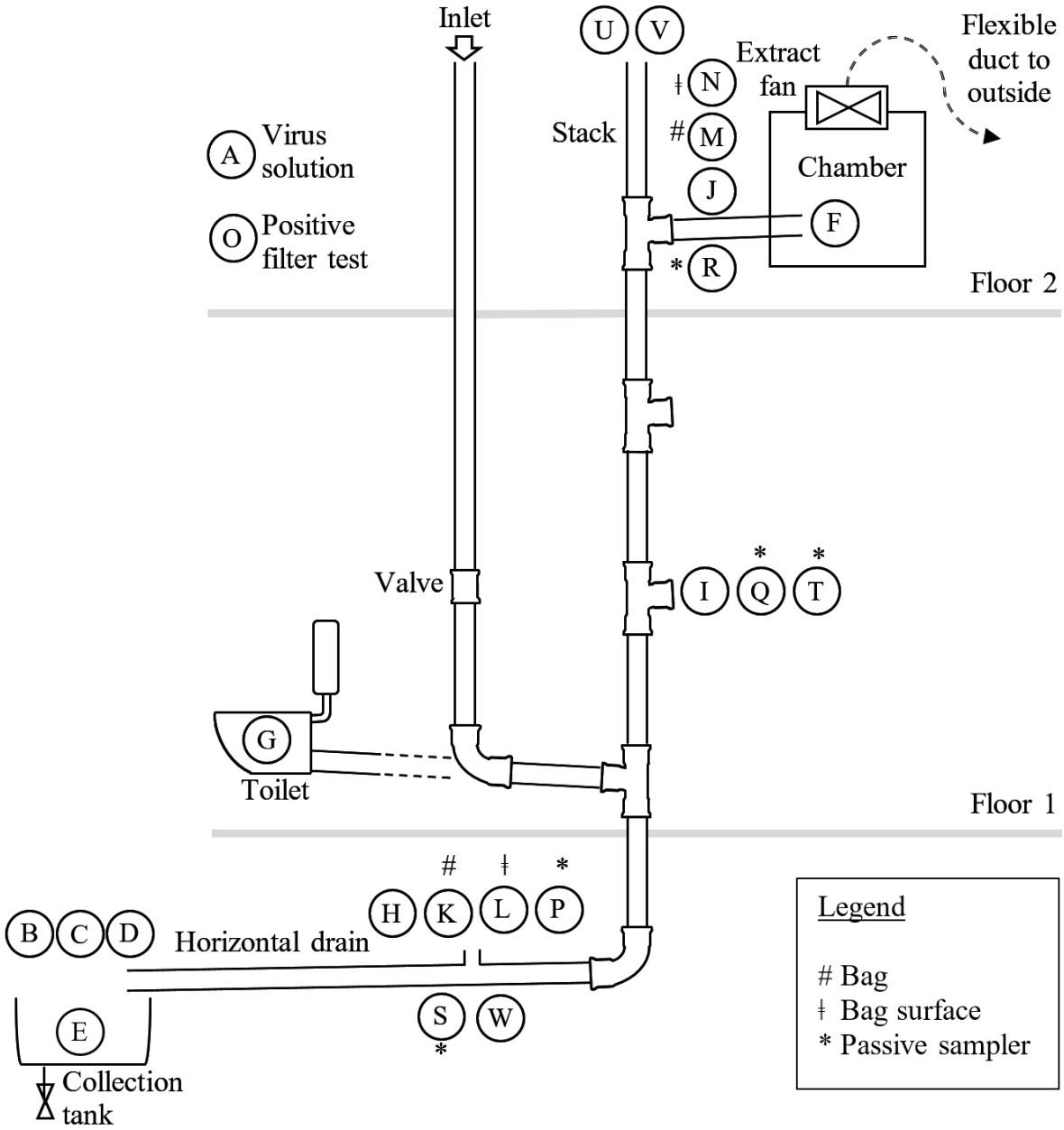


Figure 1. Bioaerosol test-rig showing the configuration of the system and the test-points.

Indirect sampling

A series of indirect sampling experiments were carried out using a containment bag within the small chamber in order to better collect and test all of the air within the system following the wastewater flush. A standard refuse bag (with nominal volume of 300 l) was attached and sealed to the open-ended branch inside the chamber. A second smaller bag (with nominal volume of 15 l) was attached at the horizontal drain, housed within a customised cylinder. Extract fans connected to the small chamber and customised cylinder allowed these spaces to be depressurised, thereby drawing the air from the system into the bags.

Aerosol/droplet sampling

A TSI APS3321 aerodynamic particle sizer (APS) was used to define the extent of aerosol/droplet generation and propagation throughout the system. APS was used to identify locations where the presence of aerosols was optimal before bio-sampling. APS sampling was carried out in a number of locations – (i) at top of stack; (ii) in the chamber; (ii) test points along the stack and (iv) on the horizontal drain. Sampling occurred for the following flush permutations: water only; water and virus; water, virus and salt (sodium chloride). These permutations were designed to provide a range of aerosol/droplet results for the same flush conditions since the rate of aerosolisation is affected by the water chemistry of the flush.

Dosing

The system was dosed with an inoculum consisting of mains tap water, heat treated PRRS virus stock and sodium chloride (NaCl) solution (0.9%w/v or 'normal' saline) in various permutations shown in Appendix B. The PRRS virus stock was obtained from the Roslin Institute and was the same stock used as the control spike for wastewater sampling for SARS-CoV-2.

The inoculum was stored at -20 °C at the end of each set of experiments. A 20 ml sample was taken of the thawed inoculum at the start of a series of experiments and sent for RT-qPCR testing. The re-use of the inoculum did not lead to a significant drop in concentration, however over time concentration reduced due to losses in the system. The magnitude of the concentration reduction due to losses was small and does not impact the overall conclusions.

It was hoped to use a high virus concentration in the inoculum (between 10^7 and 10^9) to simulate a flush from a heavily infected individual however this was not possible due to availability of virus stock. In addition to this limitation, a significant 'foaming' of the inoculum was observed. It was not possible to establish the importance of this phenomenon, but since it changed the chemistry of the mix it may have affected the way virus was aerosolised.

Bio-sampling

Four different bioaerosol sampling approaches were used in the experiments: 1) SKC BioSampler (impinger sampler); 2) passive sampler; 3) IOM head and gelatin filter sampler and; 4) cyclone sampler (see Appendix A for details).

RNA detection RT-qPCR tests

RNA extraction and PCR testing – see protocols (Corbishley et al., 2020) developed at the Roslin Institute for extraction and RT-qPCR testing of samples. Due to the explorative nature of this research, samples were returned with a CT value and a genome copies/litre figure (Appendix B) from a standard Cq/viral load curve, however, of greater interest was the presence or absence of virus in the samples so a positive/negative result was used to determine further investigation.

Results and discussion

Flushing characteristics

Initial experiments used a 6 litre toilet flush discharged into the drainage system. Seven millilitres of virus stock were added to the water in the toilet bowl, mixed and sampled. Following the flush, mixing was observed in the pipework such that a reduction in concentration was measured from three consecutive samples as shown in Figure 2(a). The flow rate vs time profile (characteristic) of the flush is shown in Figure 2(b).

Due to the reduction in concentration, it was decided to premix the inoculum to a consistent concentration so that experiments could be made more repeatable.

Table 2 shows a summary of the results obtained from a positive/negative perspective (full results are given in Appendix B).

A positive result was recorded when a sample met the following conditions:

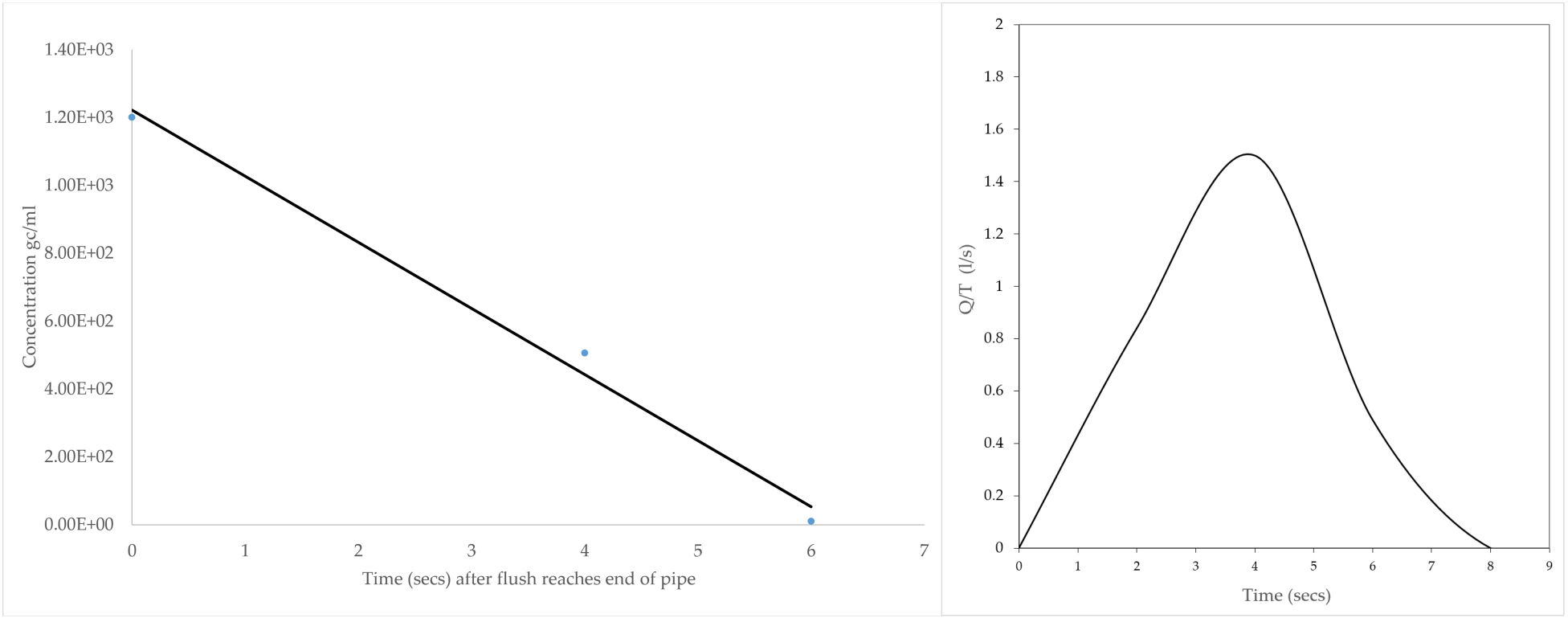
- The sample returned an amplified signal in less than 40 PCR cycles.
- The genomic count was above the limit of detection.
- The result was confirmed by duplicate PCR tests.

Some samples were returned as positives by the external laboratory that were below the limit of detection or only showed amplification on one of the two PCR tests. While it cannot be ruled out that these were true positives, confidence in the result is low. The low dosages used in the inoculum and inefficiencies with bioaerosol sampling meant that most samples were near the lower end of detection.

Positives were recorded for the horizontal drain using both the passive sampler and the wet cyclone (Table 2). A positive result was also recorded with the passive sampler 1 m above the source.

No virus was detected either at the top of the stack or in the chamber (Table 2). The APS data indicated significant number counts and concentrations of aerosol particles <20 µm aerodynamic diameter particularly when salt (sodium chloride (NaCl) was present in the water (Figure 3).

Positive results in the horizontal drain and in the stack at 1 m above the location of the flushing toilet junction were from samples collected using the passive sampler and the wet cyclone. It is likely that these methods have detected large droplets (> 20 µm) due to their proximity to the turbulent source and the predisposition of the methods towards the detection of virus in larger droplets. As the natural system updraughts cause aerosolised particles to drift up through the system, virus concentrations are reduced due to prevailing drainage system conditions including evaporation, dilution and settling of large droplets.



(a) (b)

Figure 2. (a) Attenuation of concentration through the flush volume of initial tests and (b) flow rate against time for a 6 litre toilet flush.

Table 2. Positive / negative results summary from RT-qPCR.			
Location	**Sampler**	**Approach**	**Result**
Horizontal drain	Wet cyclone (4)	Direct	Positive
	SKC BioSampler	Direct	Negative
	SKC BioSampler	Indirect	Negative
	IOM	Indirect	Negative
	Passive (2)	Direct	Positive
1 m above source	Passive (2)	Direct	Positive
	SKC BioSampler	Direct	Negative
Chamber	SKC BioSampler	Direct	Negative
	SKC BioSampler	Indirect	Negative
	IOM (3)	Indirect	Negative
	IOM (3)	Direct	Negative
	Passive	Direct	Negative
	Wet cyclone (4)	Direct	Negative
Top of Stack	SKC BioSampler (1)	Direct	Negative
	Wet cyclone	Direct	Negative

Sampling methodologies and considerations

Following on from the rapid literature review and the implementation of the sampling strategy, some specific observations were made in relation to sampling in a sanitary plumbing system, particularly on the importance of aerosol particle size on sampling efficiency.

- Larger particles have a higher inertia relative to their surface or projected area – that is, they respond more slowly to changes in the speed and direction of the air flow being sampled. Samplers can exploit this, using an abrupt change in the direction of fast-flowing air to 'throw' aerosols out into a collection liquid, or onto a solid collection surface.
- Smaller particles have lower mass, and their motion is therefore influenced by the buffeting of individual molecules in the air, causing a 'Brownian Motion', or 'random walk'. By presenting a large surface area to the airflow – using filters, or bubbling it through a collection fluid, aerosols can be captured as they drift to the collector surface.
- Samplers such as impactors, impingers, and cyclones, rely heavily on sampling by particle inertia, and so are more effective for larger particles, while diffusive effects and inertial effects both play a significant role in filter particle capture. It is not uncommon to build samplers for particular applications which pass air through a number of different instruments, capturing different sizes of particles and aerosols.
- Different samplers also sample air at different rates; high air flow rates facilitate the detection of contaminants on very diffuse aerosols, but the extraction of large volumes of air may interfere with the dynamics of the system being sampled; the efficient sampling of high volumes of air is also technically challenging.
- Other common particle sampling methods use electrical effects to sort particles by size or manipulate the temperature and humidity in the air to increase the mass of smaller particles so that they can more easily be captured. These methods were considered beyond the scope of this pilot project.
- The transient nature of aerosol plumes generated inside the sanitary plumbing system means that sampling flow rate is of considerable importance. Most samplers rely on pumps to draw the air sample into the device so that a process of extraction (or transfer) of the micro-organism from the air to a liquid solution or through a filter which can later be processed to extract the micro-organism for analysis. Samplers are very sensitive to the size of particle to be sampled. This particle can be a micro-organism or a micro-organism containing aerosol or droplet. Matching these sizes to samplers can be challenging. Passive sampling presents minimal influence on system airflow, is unobtrusive and non-destructive.
- All extraction techniques are subject to low efficiencies which are cumulative through the process. The ability to get a positive result is therefore heavily reliant on the initial viral load. It is now understood that the viral load in faeces, diarrhoea and urine will vary during the time that a person is infected and shedding (Lewis et al., 2021).

Aerosol Sampling

It was necessary to evaluate the extent of aerosolisation within various locations in the system in order to target bio-sampling. This is distinct from sampling for virus-laden aerosols and droplets described above. This section gives a brief overview of the findings and shows typical examples of aerosol data appropriate to this research.

The shape of the aerosol plume formed by the process was found to follows that of the generating flush. The data was gathered from the TSI APS3321 and it should be noted that the maximum size measurable was 20 µm, precluding the analysis of larger droplets within the system.

Figure 3 shows the effect of adding salt (as sodium chloride) to the water in terms of aerosolisation. This indicates how sensitive the process is to water composition and needs to be taken into account when analysing a particular installation.

Humidity

Sanitary plumbing systems contain a lot of moisture in the air, often up to 100% relative humidity (Gormley et al., 2014). The effects of humidity on the generation and propagation of aerosols and droplets can be seen in Figure 4, where humidification was added. It can clearly be seen that as relative humidity increases the number and concentration of aerosols, in number of particles per cm^3 also increase. Note; there is no change in the distribution of particle size throughout.

The analysis of aerosols confirmed the location where sampling could be optimised, however, it is not possible to evaluate the presence of viral particles in the aerosol plumes, but it is likely that viral concentration diminishes with distance from the source.

The change in aerosol count and concentration with water composition also highlights the need for further work on the inclusion of detergents and organic waste as these are also likely to affect aerosol concentrations.

Key findings:

- Viral RNA can be detected using bioaerosol sampling methods within a buildings' sanitary plumbing system, at distance from the source.
- Detection was demonstrated both below and above the source (the source being defined as the inlet to the vertical pipe connected directly to a toilet).
- Below the source, this was from the horizontal collection drain leading to the main sewer.
- Above the source, detection was achieved 1 m above the inlet. No viral RNA was detected beyond this point.
- The inoculum composition, concentration and sampling methods were potentially factors in the detection distances.
- Viral RNA was detected from the passive samplers and the wet cyclone sampler. No positive samples were detected from the SKC BioSampler or the IOM filter-based sampler.
- Sampling aerosols from sanitary plumbing systems is heavily influenced by environmental and water chemistry conditions, e.g. relative humidity and dissolved salts.
- Sampling aerosols is preferential over direct wastewater sampling because samples are aggregated over time rather than taken from discrete points in time.
- From a practical perspective, the most effective location to detect viral RNA from sanitary plumbing systems is from the horizontal collection drain at the bottom of the vertical stack, just before it connects with the main sewer.

Policy implications

1. Detection of viral RNA from building drainage systems is possible and could form part of the on-going surveillance efforts for SARS-CoV-2 and COVID-19, particularly where source questions arise, for example in care homes, prisons and university halls of residence.
2. The aerosol generating processes investigated in this project show a significant public health implication for the spread of many other diseases, particularly those more amenable to faecal/oral route of infection. The safe management of wastewater and sanitary systems should be updated to include aerosol and droplet management.
3. There is limited evidence of the transmission of COVID-19 via wastewater systems, however, the evidence that does exists points to aerosol and droplet formation as the most likely mode of transmission involved in conjunction with the unavoidable airstream transport found in all drainage systems.

Recommendations

1. Further work is recommended in a real building setting based on the learning obtained from this project. The work has established relationships between concentrations of aerosols and the detection of viral RNA which could be exploited in a number of settings which may still require increased surveillance in the future; particularly care homes, prisons and university halls of residence.

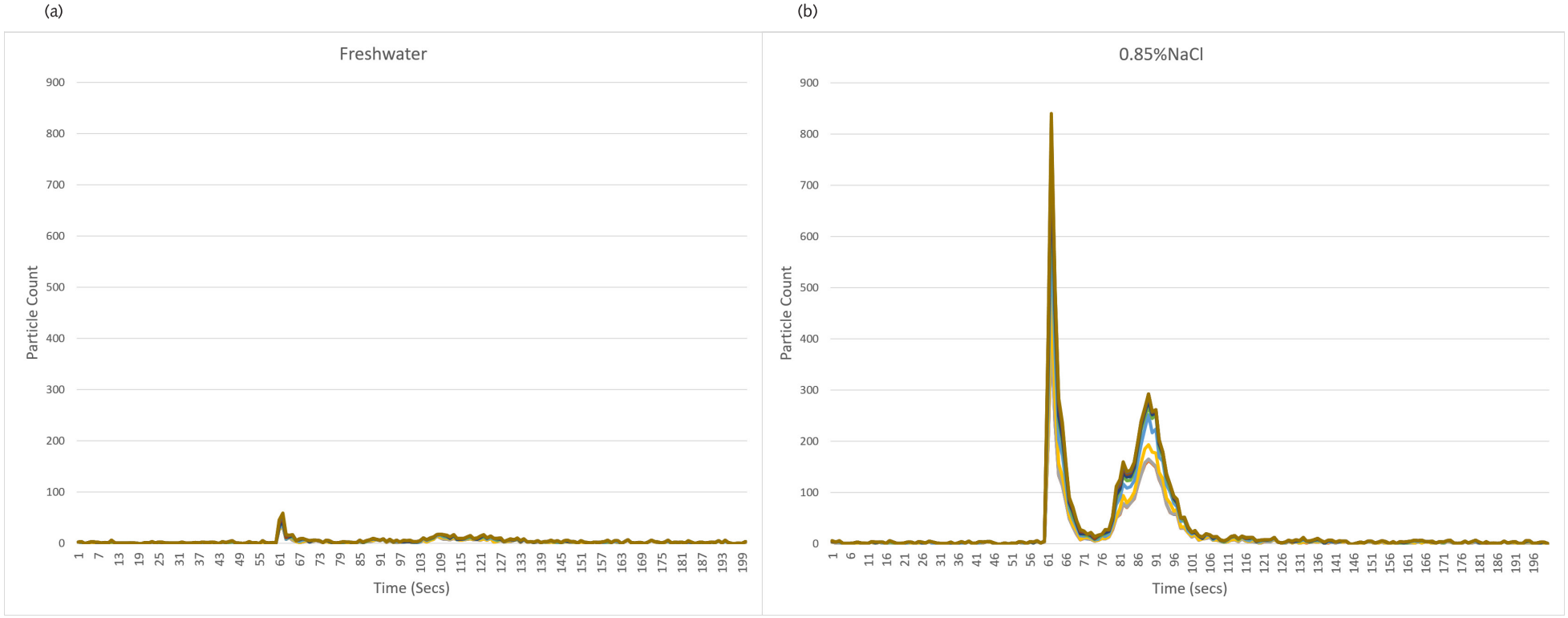


Figure 3. Effect of sodium chloride in water. Comparison of 6 litre toilet flush using tap water only (a) vs a saline solution (0.85% NaCl) (b).

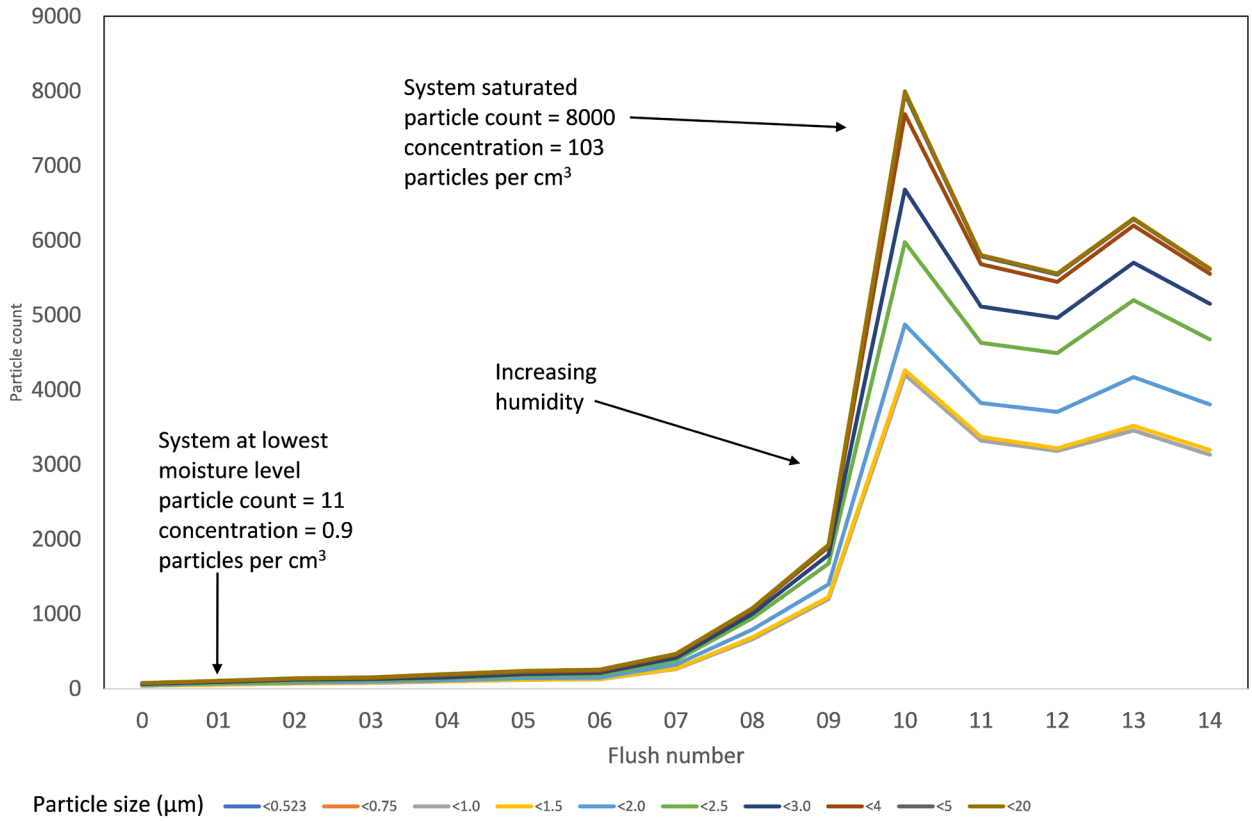


Figure 4. Effect of relative humidity on the generation of aerosols. (Particle sizes less than 20 µm).

2. The work could be expanded to the investigation of the presence of viral RNA in mains sewer pipes. This work could be calibrated against the wastewater epidemiological work already being carried out using wet samples.
3. Whilst not a direct objective of this project, the results have implications for the transmission of disease through sanitary plumbing systems within buildings, and the inclusion of this mode of transmission as a public health imperative through increased regulation and monitoring is strongly advised.

Future research

While this research successfully detected viral RNA in two locations in the system – the horizontal drain pipe and in the vertical stack 1 m above the discharge point where the branch enters the vertical stack – it is considered that these are related to the detection of large droplets (> 100 µm rather than the more prevalent and further travelling aerosols typically < 10 µm). This work has led to posing of several questions which should form the basis for future research;

1. Influence of viral stock properties on the generation and aerosolisation of virions. It is contended that the 'foaming' observed in laboratory investigations had heavily influenced the surface tension of the solution and directly affected the aerosolisation process. Surface tension and the energy required for virions to release into the air are central to the understanding of the process.
2. The chemical composition of the inoculum mix would benefit from further research to make it a better analogue of real effluent. This would include (but not necessarily limited to) the following factors: (i) dissolved salts unique to geographical regions in the UK (soft <20 ppm $CaCO_3$) to hard (>180 ppm $CaCO_3$); (ii) detergents ranging from toilet cleaners, handwash gels and soaps etc.; and (iii) emulsified or solid waste. These factors will change the surface tension, conductivity and pH of the flush, which have measurable effects on the hydrodynamics of the flush, the resulting spray and airflows generated in the system. This is readily visible as changes in foaming. A standard mix is required.
3. Detection techniques, particularly the indirect methods used in this research require further investigation. Automatic sensing of air pressure surges which pre-indicate the arrival of an aerosol plume would greatly enhance extraction efficiencies and merit further investigation.

4. Many of the samplers investigated showed very poor efficiencies (not uncommon in the detection of aerosolised virus in the air) however, these could be more finely tuned to the detection of viral RNA in a sanitary plumbing system.
5. The passive sampler gave good results and would benefit from further development particularly since they could be fitted and left for a period of time then recovered for testing.
6. Further research on the attenuation of viral load in the system would help quantify the limit of detection for the sampling technologies used. This would require a much higher initial viral load.
7. The application to a real-world setting is worth exploring, even based on the limited positive results observed in these experiments. Application in locations where high concentrations may be present – prisons, care homes and university halls of residence are worth exploring.
8. Further research is needed to adapt the passive filter so that it can be simply and safely installed in real-world settings for widespread sampling. The current passive filter could be adapted for connection to existing building drainage systems, possibly at an access rodding eye, allowing it to be installed and replaced easily by non-technical staff.

Publication

Investigators from this project have authored a systematic review of the evidence on transmission of SARS-CoV-2 in sanitary plumbing systems. The full text is given in Appendix C.

Dight, T. & Gormley, M. (2021). What's in the pipeline? Evidence on the transmission of SARS-CoV-2 via building wastewater plumbing systems, Frontiers in Built Environment. DOI: 10.3389/fbuil.2021.641745.

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Appendices

Appendix A Laboratory setup and experimental procedures

Test procedure

Step 1: At the beginning of every test, the wastewater virus solution was prepared and placed either within the toilet bowl, or within the pipe above the manual flush-valve. A sample of the wastewater was taken to record the viral load of the solution [A].

Step 2: Regardless of model design and test point, the air sampler was switched on and allowed to run for 60 seconds before the wastewater was flushed into the system. Following the flush, the air sampler was run for a further 10 minutes for general tests.

Step 3: When the airflow within the system was being controlled using the extractor fan in the small chamber, the airflow was allowed to stabilise before the air sampler was switched on.

Step 4: For tests using the containment bags within the small chamber and at the horizontal drain, once the wastewater was flushed, a valve on the system-side of the bag was opened whilst the fan depressurised the chamber/cylinder to fill the bag with air from the system (over 1-2 minutes). Once the bag was full, the valve was closed to contain the air, and the air sampler was switched on to draw air through the system for sampling, deflating the bag (over 20-22 minutes). The inside surface of the bag was also washed with 50 ml of water which was then collected for testing.

Dosing and virus concentration

Concentration of the stock was between 1.5×10^7 and 4.25×10^7 genomic equivalent (gc)/ml. The stock was received in batches of 7, 100 and 40ml throughout the series of experiments. The stock was diluted by making up to 6 litres of water for flushing, producing an initial concentration of approximately 10^6 gc equivalent per litre and a total qPCR input of between 10^2 and 10^3 genomic copies (gc). Concentration of the inoculum was sampled at the beginning and end of every experiment and were sent for qPCR testing alongside test samples.

BioSamplers used

(1) The SKC BioSampler was sterilised by autoclaving and filled with 20 ml of sterile deionised water prior to experimentation. The BioSampler was connected to an SKC biolite+ pump with flow rate previously adjusted to achieve sonic flow with no pressure drop (total flow rate 12.5 l min^{-1}). The flow rate was verified with a SKC Chekmate digital flow meter (range 5-30 l min^{-1}). The SKC BioSampler was run for a maximum of 30 mins. Following experimentation, the BioSampler trap water volume was determined to calculate loss and trap sample transferred to a sterile 50 ml centrifuge tube.

(2) The passive sampler (Gormley et al., 2017). Stainless steel 'upper discs' from domestic cafetieres (or coffee presses) were adapted as novel in-line passive air samplers (i.e. not described in BS EN ISO 14698–1:2003) and inserted into test points indicated in Figure 1A. Each test point was carefully sealed whether in use or not. Each sampler was sterilised in sealed bags by autoclaving prior to use. After use, each sampler was carefully removed from its test point and washed in a solution of distilled water. This water was then collected and sent for PCR testing.

(3) An IOM sampler head with pre-sterilised gelatin filters was connected to a vacuum air sampling pump (Solidsense Ltd, Glasgow, UK). The flow rate through the IOM head with gelatin filter was previously calibrated to 2 l min^{-1} using a Dwyer rotameter (0-5 l min^{-1} range) and calibration adaptor. Sampling using the IOM sampler head was limited to 30 mins duration to avoid deterioration of the filter. Following experimentation, the filters were transferred to a sterile 50 ml centrifuge tube for RT-qPCR testing.

(4) A wet cyclone sampler consisting of glass body and external pump was supplied by the University of the West of Scotland. The glass body was rinsed with sterile deionised water prior to use and 10 ml of deionised water was then added to the body for experimentation. Following sampling, the 10 ml was extracted from the body using a syringe and transferred to a sterile tube (as above) for RT-qPCR testing.

The active samplers (1, 3 and 4) were used in experiments independently or in combination with the passive sampler (2).

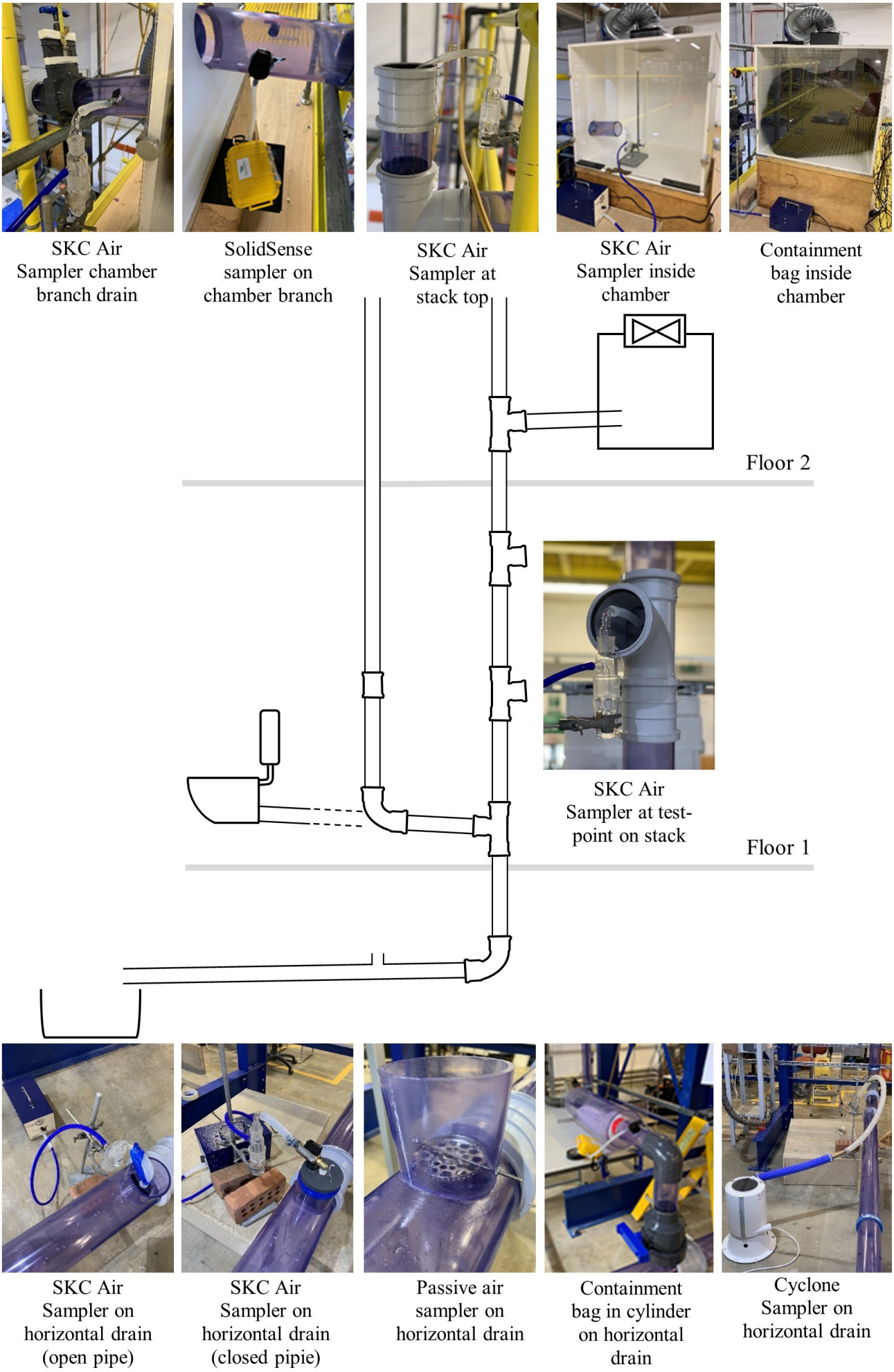

Figure 1A. Physical model with images of sampling methods used.

Appendix B Full results of all experiments

Table 1B: Results of all experiments carried out.

Test ID	Description	Flush Type	Extract Fan	Sampler	Gen. eq/L	Cq
1A	Control water [sample]	-	-	Direct		
1F	Branch end in chamber	Toilet	ON	SKC	6.96E+03	40
2A	Virus solution [sample]	-	-	Direct	9.02E+05	33
2B	Flush discharge 1	Toilet	ON	Direct	1.18E+06	33
2C	Flush discharge 2	Toilet	ON	Direct	5.10E+05	34
2D	Flush discharge 3	Toilet	ON	Direct	1.20E+04	39
2E	Collection tank	Toilet	ON	Direct	8.16E+04	37
2F	Branch end in chamber	Toilet	ON	SKC	7.18E+03	40
2G	Toilet bowl post flush	Toilet	ON	Direct		
3A	Control water [sample]	-	-	Direct		
3B	Flush discharge 1	Toilet	ON	Direct	6.83E+03	40
3F	Branch end in chamber	Toilet	ON	SKC		
4A1	Virus solution [sample] (Double flush)	-	-	Direct	3.82E+05	34
4A2	Virus solution [sample] (Double flush)	-	-	Direct	3.85E+05	34
4F	Branch end in chamber	Toilet	ON	SKC		
5A	Virus solution [sample]	-	-	Direct	3.54E+06	30
5H	Horizontal drain [cap open]	Valve	ON	SKC	8.46E+03	39
6A	Virus solution [sample]	-	-	Direct	4.09E+06	30
6H	Horizontal drain [cap open]	Valve	OFF	SKC	5.02E+03	40
7A	Virus solution [sample]	-	-	Direct	6.84E+06	30
7F	Branch end in chamber	Valve	ON	SKC	6.00E+03	40
8A	Virus solution [sample]	-	-	Direct	4.52E+06	30
8F	Branch end in chamber	Valve	ON	SKC		
9A	Virus solution [sample]	-	-	Direct	1.21E+07	29
9H	Horizontal drain [cap open]	Valve	ON	SKC		
10A	Virus solution [sample]	-	-	Direct	1.55E+07	28
10I	Stack [cap open]	Valve	ON	SKC		
11A	Virus solution [sample]	-	-	Direct	3.94E+06	30
12A	Virus solution [sample]	-	-	Direct	2.08E+06	30
12J	Branch to chamber [bag] – filled/65 sec	Valve	ON	SKC (Indirect)		
13J	Branch to chamber [bag] (Double flush)	Valve	ON	SKC (Indirect)		
14H	Horizontal drain [nozzled cap]	Valve	ON	SKC	5.45E+03	39
15A	Virus solution [sample]	-	-	Direct	4.49E+06	29
16A	Virus solution [sample]	-	-	Direct	1.48E+06	31
16K	Horizontal drain [bag]	Valve	OFF	Filter (Indirect)		
16L	Horizontal drain [rinsed bag surface]	Valve	OFF	Direct (Indirect)		
17M	Branch to chamber [bag] – filled to 40 litre/30 sec	Valve	ON	Filter (indirect)	4.09E+00	40
17N	Branch to chamber [rinsed bag surface]	Valve	ON	Direct (Indirect)		
18O	Positive filter test	-	-	Filter	2.93E+01	37
19P	Horizontal drain [cafetiere] (Double flush) – Exp 17-19	Valve	Mixed	Direct		
19Q	Stack [cafetiere] (Double flush) – Exp 16-19	Valve	Mixed	Direct	3.01E+05	33
19R	Branch to chamber [cafetiere] (Double flush) – Exp 19	Valve	Mixed	Direct		
20A	Virus solution [sample]	-	-	Direct	9.71E+05	32
21A	Virus solution [sample]	-	-	Direct	2.53E+05	33

Test ID	Description	Flush Type	Extract Fan	Sampler	Gen. eq/L	Cq
22A	Virus solution [sample]	-	-	Direct	1.07E+04	38
23A	Virus solution [sample]	-	-	Direct	4.43E+05	32
24S	Horizontal drain [cafetiere] (Double flush) + NaCl	Valve	OFF	Direct	1.13E+04	37
24T	Stack [passive sampler (Double flush) + NaCl]	Valve	OFF	Direct	1.16E+04	37
25A	Virus solution + NaCl [sample]	-	-	Direct	4.32E+05	32
26A	Virus solution + NaCl [sample]	-	-	Direct	3.05E+06	30
26I	Horizontal drain (triple flush)	Valve	OFF	SKC		
27U	Stack top (triple flush)	Valve	OFF	SKC		
28V	Stack top	Valve	OFF	Cyclone		
29V	Stack top (triple flush)	Valve	OFF	Cyclone		
30W	Horizontal drain	Valve	Off	Cyclone		
31W	Horizontal drain	Valve	Off	Cyclone	1.12E+05	36
32W	Horizontal drain (triple flush)	Valve	Off	Cyclone	7.25E+04	37
33A	Virus solution + NaCl [sample]	-	-	Direct	1.46E+06	31

Notes:

Direct sampling is where the sample is taken directly from the air stream inside the plumbing system.

Indirect sampling is where the air from the system was captured in a container and that captured air was then sampled.

Results in red are for samples which returned a result but it was at or below the PCR limit of detection.

Appendix C Publication

See next page.

frontiers in Built Environment

REVIEW
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What's in the Pipeline? Evidence on the Transmission of SARS-CoV-2 *via* Building Wastewater Plumbing Systems

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There is emerging evidence of the transmission of SARS-CoV-2 *via* the sanitary plumbing wastewater system, a known transmission pathway of SARS-CoV-1. These events can no longer be dismissed as isolated cases, yet a lack of awareness and of basic research makes it impossible to say just how widespread this mode of transmission might be. Virus is transmitted within wastewater systems by the aerosolisation of wastewater and subsequent transport of bioaerosols on naturally occurring airflows within the piped network. Central to the debate around risk to building occupants from SARS-CoV-2 spread *via* wastewater plumbing systems is the question of infectivity of faeces, urine and associated aerosols. This paper presents an examination of the processes which underlie this mode of transmission, and the existing epidemiological evidence, as well as existing mitigation strategies; significant gaps in the state of the knowledge are also identified. It is hoped that this review will cultivate a wider awareness and understanding of this most overlooked of threats, and to facilitate the selection and adoption of appropriate mitigation strategies. Key gaps in the knowledge span the rate of generation of bioaerosols within the building drainage system, their composition and transport properties, and the viability and infectivity of virions and other pathogens which they carry. While much of this work will be conducted in the laboratory, we also identify a dearth of field observations, without which it is impossible to truly grasp the scale of this problem, its character, or its solution.

Keywords: SARS-CoV-2, buildings, wastewater, plumbing, COVID-19

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INTRODUCTION

Concern has been raised that the building drainage system (BDS) may pose a risk of infection of SARS-CoV-2 (Gormley et al., 2020a; Patel, 2020), particularly in tall buildings where drainage systems can be subject to higher air pressures. In the Amoy Gardens SARS-CoV-1 outbreak, a cumulative effect was posited as contributing to spread by this route; the index case is said to have used the toilet "several times" during his visit (Hung et al., 2006), and the large number of flats connected to a common drainage stack would have led to much elevated levels of viral aerosol within the BDS once the outbreak was underway. "Watery diarrhoea", (Choi et al., 2003; Peiris et al., 2003), a common symptom, is believed to have generated a "diarrhoeal mist" in the building drainage system, which served as the vector for the Amoy Gardens outbreak (Gormley et al., 2013). Viral aerosols are believed to have entered other flats through depleted water traps, with "several" having floor-level

drains latterly found to have been depleted (Jack et al., 2006); Hung et al. (2006) noted that this was consistent with their experience working with building drainage in Hong Kong, and Gormley et al. (2017) reported on the problem of dry traps in a range of buildings, including hospitals, in Europe, Asia, and North America.

In SARS-CoV-1, diarrhoea was identified on admission in 10.6% (Hung et al., 2004) and 15% (Choi et al., 2003) of patients in two large cohorts in Hong Kong, while Booth et al. (2003) observed it in 23.6% of patients on admission to hospitals across Toronto. Hung et al. (2004) reported diarrhoea in 43.5% of patients during days 10–15 post-admission, while 53% of patients in Choi et al. (2003) developed diarrhoea during the course of the study, at a median of 3 days after admission. Peiris et al. (2003) reported that 73% of patients suffered diarrhoea, at a mean of 7.5 days after onset, and Booth et al. (2003) reported that the median time until onset was 8 days from admission. In SARS-CoV-1, diarrhoea was associated with the elevated presence of viral RNA in stool samples. Hung et al. (2004) reported median values of $10^{7.5}$/ml and $10^{5.0}$ /ml in patients with and without diarrhoea respectively; RNA was present in much lesser concentrations, and in fewer patients ($10^{4.4}$/ml, 28.2%) in urine. Lau et al. (2005) reported many stool samples with between 10^{8} and 10^{10} copies/ml of SARS-CoV-1 RNA, with some possibly exceeding these values. At Amoy Gardens, virus-laden aerosol are thought to have entered flats through dry floor drainage traps, driven by positive pressures generated within the system by the flow of wastewater and negative pressures generated by bathroom extract fans (Hung et al., 2006; Jack et al., 2006).

For there to be a similar risk with SARS-CoV-2, viable virus must enter the building drainage system. While at Amoy Gardens this was apparently associated with an index patient with diarrhoea, it has long been known that viable pathogens also diffuse from the stools of asymptomatic patients (Moore et al., 1952; Breathnach et al., 2012). This has been the basis for a substantial body of research, which has widely been translated into practice to monitor the presence of SARS-CoV-2, particularly in pre-symptomatic and asymptomatic populations, by wastewater sampling (Chavarria-Miró et al., 2020; Polo et al., 2020). It must be stressed that although related, this is a quite distinct field of inquiry. In this paper, the emphasis is on the "above-ground" drainage system, and the transmission of disease by the formation and transmission of viable bioaerosols.

One probable instance of the transmission of SARS-CoV-2 *via* the building drainage system has been identified in the peer-reviewed literature (Gormley, 2020; Kang et al., 2020), in a 30-storey residential building in Guangzhou. The building is served by separate blackwater and greywater stacks which share a common vent pipe. Residents of Flat 1502 had Covid; occupants of Flats 2502 and 2702, on the same drainage stack, subsequently developed Covid, despite stringent social distancing measures. No-one living elsewhere in the building became infected. Interpersonal contact was excluded as a means of transmission, and sampling of common areas, including lifts, did not identify any virus. Virus could not be identified in any of eleven environmental samples taken from Flats 2502 and 2702 shortly after a programme of disinfection. However, a swab comprising material from the washbasin trap, shower switch, and a tap, from the vacant Flat 1602, tested positive. Whereas the use of floor drains drew criticism in the wake of the Amoy Gardens outbreak (Hung et al., 2006), interviews here also identified a likelihood of dry bathtub traps. The potential for aerosol spread through the system was tested using a tracer gas injected into the drainage system at the WC discharge of Flat 1502. Bathroom doors and windows were left open, which was justified by interviews with residents; tracer gas was identified in the dry bathtub and floor drain traps of each of the five flats investigated. As in the Amoy Gardens outbreak, it is unclear whether the final transmission might have been airborne, or *via* fomites such as surfaces or hygiene products (Gormley et al., 2017).

Kang et al. (2020) cite two further likely examples of Covid transmission through the building drainage system in Hong Kong, from outwith the peer-reviewed literature.

LITERATURE REVIEW

We conducted systematic literature reviews to assess the prevalence of diarrhoea in Covid, and the prevalence of SARS-CoV-2 in stool and urine. PubMed and Scopus were searched up to August 31, 2020, with the default settings employed to specify synonyms and alternative spellings, and to search titles, abstracts and key words. Materials in English, French, Spanish and Russian were reviewed, to the exclusion of those in Chinese and Dutch. Titles and abstracts were used to exclude material which was clearly not relevant, with all remaining papers reviewed in full. Where only an abstract was available, this was considered acceptable for review.

Diarrhoea in SARS-CoV-2

The first part of the review addressed the prevalence of diarrhoea in Covid, using the terms (Covid OR SARS-CoV-2) AND (diarrhoea OR loose stool*). This yielded 1181 results, of which 236 were unique to PubMed, 614 were unique to Scopus, and 331 were common to both. The review of titles and abstracts was used to identify cohort studies or non-family case series presenting original research on the symptoms of Covid. 318 papers were reviewed in full, of which 213 met the inclusion criteria for further analysis.

Excluding cohorts of pregnant patients and those with underlying conditions, 89 unique groups were identified, which are presented in **Supplementary Appendix 1** The widely differing rates of diarrhoea reported, and the differences which underlie them, mean that any aggregation of results must be tret with extreme caution, although a reference value derived from the adult studies of 4506/28,180 (16.0%) will be useful for subsequent analysis. Notwithstanding the variations in prevalence reported, a comparison of different studies and groups of studies clarifies the role of diarrhoea in the course of Covid, which is necessary to understand the risk from virus in faeces.

Different studies report on different definitions of diarrhoea, and the definition was not stated in the majority of cases. A minimum of three episodes over the course of a day or other 24 h period is a common requirement (Ellington et al., 2020; Jin et al., 2020; Lo et al., 2020; Shang et al., 2020; Zhang H. et al., 2020), however Xiao Y. et al. (2020) reported that 40 of 63 cases with diarrhoea passed stool 1–3 times/day, and Pan et al. (2020) reported diarrhoea "typically up to thrice daily". Some authors required symptoms to persist for more than 1 day (Ai et al., 2020; Ishiguro et al., 2020), while others reported on shorter manifestations (Jin et al., 2020; Remes-Troche et al., 2020). Whereas the majority of studies used data recorded by healthcare workers, several relied on self-reporting. Menni et al. (2020) gathered symptom and Covid test data in the United Kingdom and United States using an app. Self-reported prevalence of diarrhoea was 509,174/2,600,461 (19.6%) among those not tested for Covid, 2359/11,493 (20.5%) among those with negative test results, and 1913/7178 (26.7%) among those with positive Covid tests. Clemency et al. (2020) recorded diarrhoea in 57/225 (25.3%) healthcare workers asked to self-report who tested positive for Covid, and in 26.1% of those with negative tests. Magnavita et al. (2020) additionally surveyed healthcare workers who were not tested; this control group reported diarrhoea in 15/361 (4.2%) cases, as compared to 13/152 (8.6%) among those who received negative tests, and 20/82 (24.4%) of those with positive tests.

In largest cohort included here, Abraham et al. (2020) reported one of the lowest rates of diarrhoea, with 396/22,425 (1.8%) reported to have suffered "loose stools", itself a relatively lenient marker. Although this study included 40,184 Covid cases verified by PCR, symptom status and description were missing in 17,759 cases. The 22,425 cases described comprised patients symptomatic following potential exposure (5727), those hospitalised with severe acute respiratory infections (4204), patients with flu-like symptoms in Covid hotspots (1199), and asymptomatic cases screened due to likely exposure (11,295). The high number of asymptomatic cases here seems likely to indicate an underrepresentation of asymptomatic cases in the other studies reviewed. Although this would suggest that the prevalence of diarrhoea across the entire population of those infected with Covid could be lower than reported elsewhere, there is considerable uncertainty around the figure of 1.8%, with unknowns including the bias among those whose data were missing, the efficiency of the contact tracing system, and how many cases here presented as asymptomatic would go on to develop symptoms. Other studies generally identified asymptomatic patients at much lower levels or not at all, although Magnavita et al. (2020) identified 24 asymptomatic cases among 84 healthcare workers with Covid (29.3%).

The reasons posed above with reference to the literature do not seem to fully account for the extreme variation in values reported. Influences not directly in evidence could include cultural factors leading to over-reporting or under-reporting of diarrhoea, particularly but not solely where symptoms are self-reported, and in the absence of a prescribed definition. Socioeconomic conditions also influence patients' readiness to seek medical attention. In addition to factors influencing the reporting of diarrhoea, prior health status affects the prevalence of diarrhoea; the impact of certain underlying health conditions is reviewed below and found to be significant, including in case-control studies. Gayam et al. (2020), who reported diarrhoea among 220/408 (53.9%) patients in a deprived area of New York, cited "prevailing relatively poor health" as likely to have been the major factor behind poor clinical course and prognosis in this cohort.

The Characterisation of Diarrhoea and its Significance

Where initial symptoms were documented across the adult and predominantly adult cohorts, diarrhoea was among them in 24/271 (8.9%) cases; by admission, it had developed in 7404/60,205 (12.3%) cases; several studies provided more description, demonstrating the development of diarrhoea at various times during the disease course, before (Xiao Y. et al., 2020) and following (Huang et al., 2020; Hung et al., 2020; Ishiguro et al., 2020; Nowak et al., 2020; Vacchiano et al., 2020; Yan et al., 2020; Zhang et al., 2020b) admission and treatment. In children, Zhang C. et al. (2020) reported diarrhoea as an initial symptom in 4/34 (11.8%), and Chen J. et al. (2020) in 1/12 (8.3%) patients, of whom a total of 4 (33.3%) would develop diarrhoea.

Shang et al. (2020) recorded "three or more loose or liquid stools per day" in 157/564 patients (27.8%). Most cases passed 3 or 4 stools per day, but in some cases this exceeded 10/day; of these 157, 79 (50.3%) patients' diarrhoea was "loose", and 78 (49.7%) "watery".

Xiao Y. et al. (2020) reported diarrhoea at presentation in 90/912 (9.9%) cases, and characterised it in 50 instances. It was "mushy" in 14 cases (28%), "loose" in 4 cases (8.0%), and "watery" in 32 cases (64.0%); the duration was given in 63 cases, being 1–3 days in 40 (63.4%), 4–6 days in 17 (27.0%), and >6 days in 6 (9.5%) cases.

In Huang et al. (2020), 3 of 8 (37.5%) young adults presented with diarrhoea, and a further 3 (37.5%) developed diarrhoea during hospitalisation; it occurred up to 6 times per day.

Ishiguro et al. (2020) reported diarrhoea for a mean of 7 days among 6/11 (55.6%) patients, with a maximum duration of 14 days. In this case, the patient had diarrhoea for 10 days in the community before hospitalisation.

Ai et al. (2020) reported diarrhoea at presentation in 2/142 (1.4%) patients, and throughout the disease in 6/142 (4.2%), although they only counted those with GI symptoms over ≥3 days in the inpatient setting. They recorded diarrhoea which was mostly watery, lasting up to 14 days.

Zhang et al. (2020a) described diarrhoea in 91/409 (21.0%) severe adult cases; in this population, the mean duration was 4.4 days, at a mean frequency of 4.5 episodes/day. Diarrhoea was described as "loose" in 37 (40.7%), and "watery" in 54 (59.3%) cases.

Zhang H. et al. (2020) tracked symptoms in 505 patients, documenting diarrhoea in 62 (12.3%). They reported that:

"Patients' diarrhoea frequency was between 3 and 10 times per day. Most of them passed thin pasty yellow or watery stools [. . .]".

Wei et al. (2020) reported on a cohort of 84 healthcare workers, 26 (31.0%) of whom experienced diarrhoea, defined as three or more loose or liquid stools per day. It occurred up to 14 times/day, with a mean of 5.7 episodes/day before treatment, and lasted a mean 3.7 and a maximum of 14 days, with a mean of 5.7, episodes/day before treatment. The mean Bristol score was 5.9, or visual analogue scale (VAS) mean 6.8, described by the authors as "pasty".

Pan et al. (2020) reported diarrhoea in 35/204 (17.2%) patients. "Cases of diarrhoea were usually not high volume or clinically severe, but more commonly presented as nondehydrating loose stools, typically up to thrice daily", indicating the inclusion of patients passing fewer than three loose stools/day. Similarly Lin et al. (2020) recorded diarrhoea in 5/95 (5.3%) patients at onset, and 23 (24%) in total, presenting as 2–10 loose or watery stools/day.

Han C. et al. (2020) reported on a cohort of 206 adults with mild disease, 67 (32.5%) of whom had diarrhoea lasting from 1 to 14 days (mean 5.4), comprising up to 18 (mean 4.3) episodes/day. 35 (52.2%) of those patients with diarrhoea described it as "watery", as opposed to "loose". 23 did not report any respiratory symptoms, and diarrhoea did not coincide with fever in 18 patients; it preceded or coincided with the onset of respiratory symptoms and fever in 13 and 44 cases respectively.

Other Subgroups

A number of studies looked at the manifestations of Covid among those with preexisting conditions. Methods varied, with some retrospectively analyzing a wider cohort, one matched case-control study, and some reporting data only on their selected group. Here, the significance of variations in the rate of diarrhoea is appraised using Fisher's exact test where either group in the cohort contains fewer than 500 cases (one-tailed unless stated); otherwise, Chi-square is used. In those cases with no form of control group, comparison is made to the adult reference figure of 4506/18,180 (16.0%). Studies investigating the same or related diseases or conditions are pooled in order to increase the statistical power of analysis.

Ellington et al. (2020) reported diarrhoea in 497/3474 (14.3%) pregnant women with Covid across the United States with symptoms described on the CDC database, as opposed to 10,113/43,855 (23.1%) of their non-pregnant peers; this is a significant difference (Chi square, $p = 1 \times 10^{-32}$). (Cao et al., 2020; Chen H. et al., 2020; Liu D. et al., 2020; Wu Y.-T. et al., 2020; Yin et al., 2020; Yu et al., 2020) gathered data on pregnant women hospitalised with Covid in Wuhan, among whom 7/70 (10.0%) had diarrhoea. Of 88 pregnant women in France with Covid-19 who responded to a survey, 18 of whom were hospitalised, 28 (31.8%) reported diarrhoea (Cohen et al., 2020).

Guerra et al. (2020) and Taxonera et al. (2020) reported on cohorts of irritable bowel disease (IBD) patients in Spain with Covid. They reported diarrhoea in 35/82 (42.7%) and 9/12 (75.0%) patients respectively, giving a pooled prevalence of 44/94 (46.8%), although a more stringent definition of diarrhoea was adopted than elsewhere.

Palaiodimos et al. (2020) investigated the impact of obesity on clinical course and prognosis in Covid in patients in the United States (NY). Patients were placed into groups of BMI <25 (healthy weight), 25 ≤ BMI <35 (overweight-obese), and 35 ≤ BMI (severely obese). The prevalence of diarrhoea in these groups was 8/38 (21.1%), 35/116 (30.2%), and 23/46 (50.0%) respectively. In each pair of adjacent groups, there was a significant positive association between obesity and the incidence of diarrhoea ($p = 4 \times 10^{-5}$, $p = 0.007$).

Li et al. (2020) analysed the association between cardiovascular disease, and the clinical course and prognosis of Covid patients. Within their cohort, 25/566 (4.4%) of those without cardiovascular disease had diarrhoea, compared to 8/89 (9.0%) of those with cardiovascular disease. This is not significantly different to those seen in the broader adult population ($p = 0.08$) or those seen in their control group ($p = 0.11$–both Fisher's exact test, two-tailed).

Du H. et al. (2020) compared children with Covid with and without allergies in Wuhan, 1/43 (2.3%) and 8/139 (5.8%) of whom had diarrhoea respectively; this is not a significant difference ($p = 0.688$–Fisher's exact test, 2-tailed).

Mathian et al. (2020) reported diarrhoea in 7/17 (41.2%) Covid patients with lupus erythematosus across France; this is significantly higher than among the wider adult population ($p = 0.0118$).

Wang F. et al. (2020) reported diarrhoea in 12/28 (42.9%) diabetic Covid patients in Wuhan; this is significantly higher than among the wider adult population ($p = 0.0007$).

Dhakal et al. (2020) and Gonzalez-Lugo et al. (2020) reported on Covid patients with multiple myeloma and monoclonal gammopathy respectively, in the United States (Wi. and NY). Each recorded diarrhoea in 1/7 patients, giving a pooled prevalence of 2/14 (14.3%). This is not significantly different to the general adult population ($p = 1.00$–Fisher's exact test, 2-tailed).

Wang R. et al. (2020), and Sachdeva et al. (2020) reported on renal patients with Covid. Respectively, 5/7 (71.4%) haemodialysis patients in Wuhan and 6/11 (66.7%) with end-stage kidney disease in the United States (NY) experienced diarrhoea; the pooled prevalence in this group was 11/18 (61.1%), significantly higher than the wider adult population ($p = 2 \times 10^{-5}$).

Wu et al. (2020b) studied a cohort of Covid patients in Wuhan with various haematological malignancies, and Hussain et al. (2020) reported on patients with sickle-cell anemia. Rates of diarrhoea in these cohorts were 0/6 and 1/4 (25.0%) respectively, with a pooled prevalence of 1/10 (10.0%); this was not significantly different to the general adult population ($p = 1.00$–Fisher's exact test, 2-tailed).

Benkovic et al. (2020) and Ridgway et al. (2020) reported on US patients with HIV, reporting diarrhoea in 1/4 (25.0%) patients in NY and 3/5 (60.0%) in Il. respectively, giving a pooled prevalence of 4/9 (44.4%). This is significantly higher than in the wider adult population ($p = 0.042$).

Several studies have addressed cohorts of solid organ transplant recipients; rates of diarrhoea were reported to be 10/14 (71.4%) in Italy (Cavagna et al., 2020), 16/53 (30.2%) in Sweden

(Felldin et al., 2020), 4/18 (22.2%) in Spain (Fernández-Ruiz et al., 2020), 7/21 (33.3%) in Switzerland (Tschopp et al., 2020), 1/7 (14.3%) in the United Kingdom (Banerjee et al., 2020); in the United States, rates were 26/47 (55.3%) (Mi.) (Chaudhry et al., 2020) and 8/36 (22.2%) (NY) (Akalin et al., 2020). The pooled prevalence of diarrhoea was 72/196 (36.7%), significantly higher than among the general population ($p = 3 \times 10^{-12}$). Chaudhry et al. (2020) included a control group of hospitalised Covid patients without solid organ transplants, of whom 17/100 (17%) had diarrhoea; this is significantly lower than in those with transplants ($p = 4 \times 10^{-6}$).

SARS–CoV-2 in Faeces and Urine

The presence of SARS-CoV-2 RNA in stool and urine has now been widely documented, and has been reviewed elsewhere (Jones et al., 2020); nevertheless, a systematic review was undertaken in order to identify those trends most relevant in this context. Using the same parameters set out in the previous section, PubMed and Scopus were searched for (Covid OR SARS-CoV-2) AND (stool OR "faeces" OR urine). 565 papers were identified, of which 96 were exclusive to Pubmed, 190 were exclusive to Scopus, and 279 appeared on both. Titles and abstracts were reviewed to identify 88 cohort studies. Of these, 49 and 20 included data on virus in faeces and urine, and are presented in **Supplementary Appendix 2, 3** respectively. Excluding studies in which the cohorts may have overlapped, 30 and 14 studies were included in a pooled analysis, in which viral RNA was detected in the stool of 328/1168 (28.1%) adults and 83/161 (51.6%) children, and in the urine of 9/233 (3.9%) adults and 2/31 (6.5%) children.

Concentration

Reported values of viral RNA in stool reached a maximum of $O(10^{10})$ copies/ml, although values of 10^6–10^8 were much more widespread. Lui et al. (2020) reported maximum and median concentrations of $10^{6.4}$ and $10^{4.1}$/ml. Hung et al. (2020) took stool samples at the beginning of their study, reporting concentrations as high as 10^{10} copies/ml, although typical concentrations appeared to be around $10^{3.3}$. Wang W. et al. (2020) reported cycle thresholds corresponding to median, 95th percentile, and maximum concentrations of $10^{4.0}$, $10^{4.6}$, and $10^{6.8}$/ml. Of 20 patients with viral RNA detected in the faeces, Wang X. et al. (2020) presented data on the concentration from those 11 patients whose stool remained positive after respiratory swabs. Of these, several produced samples with cycle thresholds of 25–27, corresponding to a viral RNA load of $10^{5.5}$–$10^{6.0}$/ml. Wölfel et al. (2020) reported that faecal viral RNA reached 10^7 copies/ml in 3 of 8 positive cases.

In children, Du W. et al. (2020), reported mean faecal viral RNA loads of $10^{6.5}$/ml, and a maximum of $10^{7.4}$. Han M. S. et al. (2020) observed the progression of concentrations, recording median and maximum concentrations in the 1st, 2nd and 3rd weeks of sampling of $10^{8.0}$ and $10^{10.3}$; $10^{7.3}$ and $10^{9.0}$, and $10^{7.6}$ and $10^{8.7}$; the values across all subsequent sampling were $10^{7.6}$ and $10^{8.6}$/ml.

Some authors reported only cycle thresholds, rather than concentrations (Young et al., 2019; Bonetti et al., 2020; Kujawski et al., 2020; Wu et al., 2020a). Of these, Wu et al. (2020a) provided the greatest detail, showing the cycle threshold of each test conducted. The cycle threshold of different genes within the same sample often varied sharply, with no consistent pattern discernible. Where other authors have provided less detail, it is impossible to say how much unexplained variation in the experimental data this might mask. Muenchhoff et al. (2020) compared the results from a selection of Covid PCR tests, and found that the concentrations corresponding to different cycle thresholds were similar, with variations not generally greater than a factor of three. However, this work also demonstrated that poorly designed tests can produce inconsistent and misleading results. The relationship between cycle threshold and copy number also depends on the dilution of the sample, which is not described in detail by all authors.

Jeong et al. (2020) found viral RNA in the urine of 5/5 adults tested, at concentrations between $10^{0.59}$ and $10^{2.09}$/ml. Peng et al. (2020) reported $10^{2.5}$/ml in the urine of 1/9 (11.1%) patients tested. Kim et al. (2020) reported viral RNA in the urine of 2/54 (3.7%) patients in a mixed cohort, having an average of $10^{4.9}$/ml.

Han M. S. et al. (2020) reported $10^{7.55}$ and $10^{3.82}$ copies/ml in the urine of two mildly symptomatic infants.

Duration

Viral RNA in stools was widely reported to outlast that detected in respiratory swabs (**Supplementary Appendix 2**). It is difficult to determine an average duration due to the infrequency of sampling and high numbers of patients who were still shedding virus at the end of their studies, however authors suggested values of 22.3 days (He et al., 2020), 19.3 days (Lo et al., 2020) and 22 days (Zheng et al., 2020), and 28.9 days in children, decreasing with age (Chen Z. et al., 2020). In extreme cases, virus continued to be shed up to 103 (He et al., 2020) and 49 days (Wu et al., 2020a) from onset in adults, and for up to 65 days (Liu P. et al., 2020) in children. Liu P. et al. (2020) found that viral RNA in stool outlasted that in respiratory samples by a median of 25 days among children.

Most patients whose stool samples contained viral RNA contained it from the commencement of sampling, although there were some exceptions. Wu et al. (2020a) reported that in a cohort of 74 patients, 12 (16.2%) had detectable faecal viral RNA only after respiratory swabs had turned negative, with a delay of up to 17 days; the same observation was made of 1 of 11 (9.1%) patients by Lui et al. (2020), and in 2 of 69 (2.9%) patients in Wang X. et al. (2020). There have been instances in which RNA becomes undetectable in stool samples before reappearing (Du W. et al., 2020; Lo et al., 2020). Viral RNA was detected in the stool of 6/18 (33%) asymptomatic children by Xiong et al. (2020), and three of three asymptomatic children by Han M. S. et al. (2020).

The pattern of small numbers of patients shedding virus for an extended period was also observed in SARS-CoV-1 (Leung et al., 2003; Peiris et al., 2003). As with assessments of the prevalence of diarrhoea, the programme of sampling significantly influenced the reported figures. Where investigators tested only once or twice in adult or mixed cohorts, SARS-CoV-2 RNA was reported in the faeces of 30/260 (11.5%) of patients. Where more intensive programmes of sampling were undertaken, RNA was detected in the faeces of 205/397 (57.8%) patients.

Association Between Diarrhoea and the Presence of SARS-CoV-2 in Faeces

Association between diarrhoea and the presence of viral RNA in stool has widely been taken as an indicator of active infection of the digestive tract, which would seem to increase the likelihood of viable virus in stools. Furthermore, the continued viability and aerosolisation of any virus may vary with the consistency of the stool, and so the concentrations anticipated in building drainage systems must be determined with reference to the characterisation of stool, and the virus within it.

Wei et al. (2020) reported that 18/26 (69.2%) and 10/58 (17.2%) of those with and without diarrhoea produced positive stool swabs respectively ($p = 8 \times 10^{-6}$–Fisher's exact test), and that stool swabs were significantly more likely to remain positive for longer than pharyngeal swabs among patients with diarrhoea (6/26 (23.1%), 2/58 (3.4%); $p = 0.01$). A further two papers presented data on prevalence in patients with and without diarrhoea: Chen Y. et al. (2020) reported the detection of viral RNA in the stool of 6/7 (85.7%) patients with diarrhoea and 22/35 (62.6%) of those without ($p = 0.39$), and Wang X. et al. (2020) reported the detection of viral RNA in 5/12 (41.6%) of those with diarrhoea and 15/57 (28.3%) of those without ($p = 0.31$).

Bonetti et al. (2020) noted an association between diarrhoea and the concentration of viral RNA in positive samples, although the observed positive association was not statistically significant ($p = 0.056$). Similarly, Yin et al. (2020) reported that the mean cycle threshold of positive samples from patients with diarrhoea was 31.37, as compared to 36.09 from those without.

Virus has been detected directly in diarrhoea (Holshue et al., 2020), and in firm stool (Park et al., 2020; Wang W. et al., 2020).

Presence of Viable Virus in Faeces or Urine

In reviewing the presence of viable virus in samples, important evidence was found outwith the papers presented in the systematic review; this is a rapidly advancing field.

Chen X. et al. (2020) presented the case of a seven-year-old girl with diarrhoea alongside "classical" Covid symptoms, with "abundant" viable virus in her faeces, although no further details on this were given.

Wei et al. (2020) and Xiao et al. (2020b) report the existence of data not published in full elsewhere in the literature, showing the isolation of SARS-CoV-2 from stool. Wei et al. (2020) state that viable virus was found in the faeces of 19 patients. Xiao et al. (2020a) subsequently published a report showing the successful culture of SARS-CoV-2, from the stool of two of three patients selected for the presence of viral RNA by PCR, on Vero E6 cells. One of these patients was studied in more detail, and later stool samples did not yield culturable virus, even as viral RNA remained detectable.

Wang W. et al. (2020) tested stool samples from four patients, of which samples from two patients without diarrhoea were said to contain viable virus.

Kim et al. (2020) used a CaCo-2 cell line (ultimately of human colorectal epithelial origin) to attempt to culture SARS-CoV-2 from 13 stool and two urine, as well as nine serum samples, containing viral RNA. Virus could not be isolated from any of these samples.

Jeong et al. (2020) attempted virus isolation from faecal suspension and urine on ATCC CCL-81 cells, however the samples were found to be cytopathic. 2/2 patient urine samples ($10^{1.51}$ and $10^{2.09}$/ml), and 1/1 patient faecal supernatant (faecal RNA concentration $10^{2.18}$/ml, diluted by a factor of 10; all inocula 500 µl) appeared to induce "moderate increases in body temperature, rhinorrhoea and decreased activity at 4 dpi [days post-infection] which persisted until 6 dpi" in intranasally inoculated ferrets. Viral loads were detected in ferret nasal wash between $10^{0.35}$–$10^{3.24}$/ml, with isolation on Vero cells successful only on those samples at $\geq 10^{1.68}$/ml. The observed symptoms and viral loads in ferrets are consistent with previous work by the same team, which did include a negative control (Kim et al., 2020), however contrast with the asymptomatic infection of ferrets reported by Kutter et al. (2020) and Schlottau et al. (2020). The viral loads in patient samples here are much lower than those reported elsewhere, and those in ferrets are much lower than in Schlottau et al. (2020) and Shi et al. (2020).

SARS-CoV-2 in Aerosol

There has been much controversy over the labeling of Covid-19 as an airborne disease, although this is now generally accepted as an important mode of transmission. In many contexts the term "airborne" is suggestive of virions becoming aerosolised in the human respiratory tract, and remaining suspended and viable for many hours. This has been at the crux of much of the wider debate on the adoption of the term "airborne", but has little bearing on the spread of SARS-CoV-2 through the building drainage system, and its designation as such in this context (Wilson et al., 2020; editorials in: CDC, 2020; Nature, 2020; WHO, 2020).

Liu Y. et al. (2020) measured viral RNA in droplets and aerosol in air sampled from two hospitals dedicated to the treatment of Covid patients. Viral RNA was detected in particles in all size ranges investigated, from <0.25 to >2.5 µm. The highest concentrations in patient areas were found in a WC, although the detection method employed here did not differentiate between particle sizes. This was an unventilated space, implying a local source for the droplets and aerosol detected, rather than transfer on building air flows. However, the lack of ventilation precludes comparison between the rate of particle generation here and in ventilated spaces.

van Doremalen et al. (2020) report that the half-life of viable SARS-CoV-2 in aerosolised tissue culture medium (Dulbecco's modified Eagle's medium; DMEM) is very similar to that of SARS-CoV-1. Particles of <5 µm were generated in a 3-jet Collison nebuliser and suspended in a Goldberg drum, wherein the half-life of SARS-CoV-1 was 1.18 h and that of SARS-CoV-2 was 1.09 h at 65% relative humidity (RH) and 21–23°C. However, under these experimental conditions, viable aerosolised SARS-CoV-2 was found at only one tenth the concentration of viable aerosolised SARS-CoV-1.

Smither et al. (2020) compared the aerosolisation and subsequent survival of SARS-CoV-2 (England-2 strain) in DMEM and simulated saliva. Aerosols of 1–3 µm were generated in a 3-jet Collison nebuliser and suspended in a

TABLE 1 | Half-life of aerosolised SARS-CoV-2 under different conditions–data from Smither et al., 2020.

	Medium RH	High RH
DMEM	1.3	0.7
Artificial saliva	0.5	2.8

Half-life (h).

dark Goldberg drum at RH 40–60% or 68–88%, at 19–22°C. The culture assay showed that the artificial saliva produced a density of viable aerosolised virus around ten times less than that of the DMEM [$TCID_{50}$ of O (10^1) as opposed to O (10^2)/L], which was attributed to a lower particle generation rate rather than virus inactivation. The half-life of the virus in different media and at different humidities is presented in **Table 1**. Increased humidity was associated with diminished recovery of viable virus in aerosol in DMEM, but with increased recovery in simulated saliva.

Pathogen Aerosolisation (Theory)

The concentration of suspended matter in aerosol can be characterised by an Enrichment Factor (EF); where applied to the recovery of viable microorganisms, this has often been found to be greater than unity (Blanchard and Syzdek, 1970; Blanchard and Syzdek, 1972). Many microbes and viruses exhibit surface-active effects. This leads to the accumulation of waterborne microbes at the liquid interface, including that at the surface of bubbles passing through the liquid and adjacent to suspended solids. The EF of bacteria has been observed to vary between droplets within a population, depending on their mode of formation; between different organisms, and between different strains of the same organism; and with the presence of other impurities in the water; interaction effects have also been noted between these factors (Blanchard, 1978; Blanchard and Syzdek, 1978; Baron and Willeke, 1986). Further influences include the generation fluid, the temperature, and the humidity, and radiation (Kim et al., 2007). Whereas much of this research has been conducted with aerosol generated by bubbles, other relevant modes of droplet production include spraying, and droplet breakup and impaction (Xu and Weisel, 2005). The partition of microbes and other contaminants by these modes has not been well described, and the contribution of each mode within the building drainage system is not known.

Many researchers have identified interacting factors which influence the tendency of viruses to flocculate or coagulate, including the nature of other solids present in suspension, the ionic strength and pH of the suspension, and the size of the virion (Xagoraraki et al., 2020). Additionally, increasing hydrophobicity–associated with lipid shells–increases the tendency of viruses to adsorb to solid substrates (Kinoshita et al., 1993). These effects are likely to play a role in the formation of bioaerosols, and their subsequent transport and ongoing viability, however this remains poorly characterized (Lin and Marr, 2017). SARS-CoV-2 virions are spherical, of 70–90 nm diameter (Kumar et al., 2020).

Pathogen Aerosolisation (Observed)

The role of the building drainage wastewater system as a pathway for disease transmission is supported by a body of evidence for the creation and diffusion of bioaerosols at and from sanitary fittings. WCs have attracted particular attention.

Gerba et al. (1975) studied the isolation of MS2 (c. 27 nm dia., unenveloped), poliovirus [c. 30 nm; unenveloped (Romero and Modlin, 2015)], and *Escherichia coli* [rods; 1.1–1.5 × 2.0–6.0 μm, often paired; often with flagella, multifarious fimbriae especially common in pathogenic strains (Scheutz and Strockbine, 2015)], from flushing WCs. All of these were recovered from gauze covering the WC bowl, and from exposed plates on bathroom surfaces. The form of the inoculum—culture, homogenised stool, or stool "pellet"—was found to exert little influence on the recovery of bacteria. This finding replicated that of Newsom (1972), working with a range of bacteria.

Barker and Jones (2005) used a single-stage impactor to detect viable MS2 and *Serratia marcescens* [rods; 0.5–0.8 × 0.9–2.0 μm; usually with flagella (Grimont and Grimont, 2015a)] in the air following a toilet flush; both were selected partially for their good environmental stability. c. 10^{10} MS2 virions or cells in a semisolid agar were seeded onto the exposed surfaces of a WC. Following flushing of the WC, viable MS2 was recovered from the air at 2420 PFU/m^3 after 1 min, 178 PFU/m^3 after 30 min, and 27 PFU/m^3 after 60 min, and culturable bacteria at around half that concentration. The reduction in the airborne bacteria with subsequent flushes was between 2.4 and 3.9 times, while bacteria retrieved from the toilet surfaces and water diminished by around two orders of magnitude per flush. This could be attributable to and illustrative of the enrichment of aerosol, although effects relating to the adsorption and elution of bacteria are also possible. Single-stage impactors are typically inefficient below around 4 μm, although this is less problematic when working with bacteria than with viruses. They were also used to demonstrate the diffusion by toilets of *Salmonella enteritidis* [rods; 0.7–1.5 × 2.0–5.0 μm; with flagella (Popoff and le Minor, 2015)] from a relatively inviscid inoculum (Barker and Bloomfield, 2000), and *Clostridium difficile* [rods; 0.5–1.9 × 3.0–16.9 μm, sometimes chained; typically with flagella (Rainey et al., 2015)] from faecal suspension (Best et al., 2012), the latter up to 90 min post-flush.

Moore et al. (2015) demonstrated the recovery of aerosolised MS2 from above a home spa. Given a concentration of 27,000 PFU/cm^3 in the pool water, 528 PFU/m^3 were present 10 cm above the pool edge; mean concentrations taken at sampling points ≥25 cm hence horizontally and/or 90 cm vertically, were no more than 11 PFU/m^3.

Gormley et al. (2017) modelled the spread of a pathogen through a building drainage system using *Pseudomonas putida* [typically rods; typically c. 5 μm long; >1 flagellum (Palleroni, 2015)]. The inoculum was disseminated by a simulated toilet flush into the ground floor level of a two-storey test rig constructed in accordance with (BS EN 12056-2, 2000), and air flow was induced by a typical extract fan from a chamber at the level of the first floor. Viable organism was retrieved from the air in the test chamber using a single stage impactor, and cultured from the interior surfaces of the dry WC.

Newsom (1972) compared the aerosolisation by flushing toilets of several strains of bacteria; the numbers of CFUs per unit air sampled were highest for *Achromobacter* [rods 0.8–1.2 × 2.5–3.0 μm; 1–20 flagella (Busse and Auling, 2015)] and *Pseudomonas* spp. [rods, 0.5–1.0 × 1.5–5.0 μm; fimbriae more common in pathogenic strains; typically ≥1 flagellum (Palleroni, 2015)], intermediate for *Enterobacter cloacae* [rods; 0.6–1.0 × 1.2–3.0 μm; fimbriae more common in pathogenic strains; 4–6 flagella (Grimont and Grimont, 2015b)], *Proteus* spp. [rods, 0.4–0.8 × 1.0–3.0 μm; fimbriae common, sometimes involved in pathogenesis; typically ≥1 flagellum (Penner, 2015)], and *Shigella sonnei* [rods; 1–3 × 0.7–1.0 μm; nonmotile (Strockbine and Maurelli, 2015)], and lowest for *E. coli*, *Klebsiella pneumoniae* [rods; 0.3–1.0 × 0.6–6.0 μm; often paired or in short chains; hydrophilic capsule, sometimes with fimbriae, nonmotile (Grimont and Grimont, 2015c)], *Salmonella typhimurium* (as *S. enteritidis*), and *Serratia* spp. (as *S. marcescens*).

Lai et al. (2018) reported much higher EFs from a toilet flush for *Staphylococcus epidermis* (spherical, 0.96 μm, nonmotile) than for *Escherichia coli* and *Pseudomonas alcaligenes*; the authors suggested that the latters' larger size may have been responsible for this. Their experiments with each bacterium encompassed a range of initial concentrations, and demonstrated an inverse association between initial concentration and EF in nine of ten datasets presented.

Work with viruses has been more limited. The influence of surface-active effects was demonstrated by Morrow (1969), who showed that the accumulation of foot-and-mouth-disease virus (c. 25 nm, non-enveloped) at the air-water interface could be driven by bubble generation. Baylor et al. (1977) showed the aerosolisation of TS2 and TS4 bacteriophages (both protein-sheathed) on jet droplets, with EF around 50. In Gerba et al. (1975), the bioaerosols generated by toilet flushing contained more culturable units of poliovirus than *E. coli* under the same conditions, even though the number of *E. coli* seeded into the toilet was greater. Fischer et al. (2016) found that different strains of Zaire ebolavirus formed viable bioaerosols at differing rates. Kim et al. (2007) found that the recovery of Transmissible Gastroenteritis Virus, an α-coronavirus around 100 nm diameter (Salanueva et al., 1999; Escors et al., 2001) was minimally sensitive to nebuliser design and pressure, suggesting that physical stresses do not significantly degrade viruses during the aerosolisation process in this context. The recovery of viable bioaerosols decreased with increasing relative humidity.

Lin and Marr (2017) showed modest levels of viable bioaerosol generation at converging near-horizontal pipes using bacteriophages MS2 and Phi6 (c. 75 nm dia.; lipid envelope) in digested sewage sludge. The rate of isolation of Phi6 from the air was two orders of magnitude less than that of MS2 in both tests conducted, given the same concentration in the bulk liquid. When tested in a Collison nebuliser, the number of bioaerosols generated varied only by a factor of two.

Aerosol Generation and Size Distributions

In Lin and Marr (2017), the peak aerosol concentrations were in the region 0.03–0.3 μm, across converging near-horizontal pipes, a model aeration basin, and toilet plume. Similarly, Lai et al. (2018), investigating four different toilet flushes, reported that in all cases most particles were of diameter less than 0.6 μm, given a minimum size for detection of 0.3 μm.

Baron and Willeke (1986) measured the particles above the surface of a spa whirlpool under different operating conditions, in the range 0.7–16 μm. The particle concentration increased sharply toward the lower limit of detection, at 0.7 μm whether the pool was on or off, and at a range of water temperatures. No particles above 9 μm were detected, and in all cases at least 90% of particles were of diameter <4 μm. This finding was replicated by Moore et al. (2015).

Xu and Weisel, (2005) used an optical particle sampler sensitive down to 0.1 μm to measure the aerosols present during a hot shower, at breathing height. Particles between 0.1 and 0.3 μm initially comprised around 60% of those detected, rising to and stabilising at around 75% from the second minute of the 10-min shower. Zhou et al. (2007) also investigated particles in the in-shower breathing zone, using an erodynamic particle sampler stated to have been effective for erodynamic diameters of 1–30 μm. Their shower contained a mannequin, and was tested with hot and cool water, and with three different shower heads associated with different flow rates. The emphasis of this study was on mass fraction, and no particles below 1.8 μm were reported using warm water. The use of cold water reduced the total mass of particles recovered, however a much greater proportion was associated with smaller aerosol, which were reported down to diameters of 0.5 μm; median particle diameters were around 1 μm diameter, with 90% of particles below 2 μm. In all cases, more particles were generated at higher flow rates. As in Xu and Weisel (2005), the distribution of particle sizes varied little during a 10 min experiment.

Gormley et al. (2020b) produced the only known result in the literature documenting the size of airborne particles within a model building drainage system, down to a lower limit of detection of 0.5 μm. In all presented datasets, the peak concentration occurred below 1 μm, with a dropoff at the lower end of this scale. They were able to demonstrate the transit of viable *Pseudomonas putida* the equivalent of one storey, taking from 48 to 155 s under the same configuration as in Gormley et al. (2017).

It must be noted that in the foregoing, populations of particles below the limits of detection could play an important role in the transmission of virions of the order of 10 nm, if present in sufficient concentrations.

Gormley et al. (2014) demonstrated the transit of a smoke particle tracer through the drainage system of a house under naturally occurring conditions, with a simulated trap failure. Hung et al. (2006) showed that sulphur hexafluoride tracer gas was drawn up through the building drainage system of a building similar to Amoy Gardens by a domestic extract fan, rising eleven storeys in 3 min. They further demonstrated that the tracer was entrained by water flowing down the stack, and could be driven through a depleted trap near the base of the stack where an offset in the pipework contributed to positive pressure generation.

Conditions in Building Drainage Systems

Gormley et al. (2013) investigated the conditions in a hospital drainage stack in Scotland and found temperatures of around 24°C, with relative humidity at or very near to 100%. Transitory air flows in both directions were observed at the top of the stack, with upwards flow in one stack perhaps driven by air entrainment in an adjacent stack, this suggesting another, unsteady-state mechanism by which aerosol might be driven upwards through drainage systems. Conditions in the stack were broadly constant over the course of 1 week, and a literature review identified studies of conditions in sewers, which suggested little variation globally. However, this review identified a lack of data on conditions within the BDS, where many factors might influence the conditions between and even within different buildings. For example, in contrast with the stack examined here, that at Amoy Gardens was external to the building, and under the generally accepted failure conditions, would have been drawing large volumes of air in from indoors (Jack et al., 2006).

Mitigation–Regulation and Practice

American, European and British regulation have historically been written to avoid the loss of trap seals due to siphonage or blowout (Swaffield et al., 2005a); evaporation is often afforded less attention (CIBSE, 2014: Guide G; BS EN 12056-2, 2000), or ignored (Department of Health (UK), 2013). Evaporation is not mentioned in the main text of BS:EN 12056, however the National Annex cites the risk of evaporation specifically from floor gullies, suggesting that they should only be sited where they would be adequately replenished. These documents also seem to understate the risk attendant on trap failure, referring not to the spread of pathogens but to "odours", "vapours", and "foul air", framing the integrity of traps as a matter of comfort rather than life safety. The Health Building Note HBN 00-09: Infection Control in the Built Environment (Department of Health (UK), 2013), addressing the spread of pathogens *via* other building services, cites risks from bacteria and protists in the water supply; no mention whatsoever is made of viruses.

Given the state of the knowledge on the spread of pathogens *via* the BDS, there are several simple, established technologies which seem likely to effectively mitigate this risk, particularly with the development of a more amenable regulatory environment.

Early BDS were generally "two-pipe" systems, with separate stacks and ventilation for the disposal of blackwater (that containing human excreta), and greywater (e.g. from sinks and baths) (Swaffield et al., 2005b). One-pipe systems were generally adopted from the mid-20th century for reasons of economy, but two-pipe systems are acknowledged as an acceptable configuration in BS:EN 12056, and remain in use in many older buildings. Two-pipe systems are however proscribed in some jurisdictions.

Hung et al. (2006) noted the widespread practice of using one trap to service multiple appliances in Hong Kong; this arrangement can conveniently be retrofitted where regulations permit, as was seen in the aftermath of the Amoy Gardens outbreak. Typically, all the greywater fittings within a bathroom are connected to the same trap, which consequently is replenished by the use of any of the appliances. Low-evaporation floor drain traps have also been developed, which retain the intended functionality of conventional floor drain traps given infrequent use (Chan et al., 2008).

There are also now waterless traps, typically consisting of a silicone sheath which opens under the weight of wastewater, or in response to negative air pressure in the drainage system (Swaffield et al., 2005a); these have found extensive use in practice (Gormley and Beattie, 2010; Gormley et al., 2017). However, their function is not regulated by any standard, which may decrease confidence in their adoption; they are also vulnerable to blockage by solid matter (CIBSE, 2014). The main text of BS:EN 12056 specifies that appliances must be fitted with a "trap", defined as a "device that prevents the passage of foul air by means of a water seal"; however, the National Annex suggests their use particularly in floor gullies in closely controlled environments where their condition can be adequately monitored. This raises a legitimate concern about their use in domestic environments. Furthermore, it is not clear whether this permits common trapping as described in Hung et al. (2006); this is generally avoided in practice in Europe.

CIBSE (2014) suggests the use of self-replenishing traps for condensate drains, which are liable to dry out over long periods of inactivity. BS EN 12056-2 (2000) provides for the use of stub stacks, which can help to avoid trap blowout due to large pressures in the drainage stacks of tall buildings.

The risk of trap blowout due to transient pressure waves caused by the sudden interruption of air flows, such as by backup, water curtain formation, or branch discharge into the stack, can be mitigated by attenuating the pressure waves. (Swaffield et al., 2005a, Swaffield et al., 2005b) developed a positive air pressure transient attenuator (PAPA) for this purpose, the use of which has been demonstrated experimentally and in the field. Kelly et al. (2008) demonstrated the use of pressure waves as relatively low-amplitude vibrations to identify vacant trap seals on a BDS.

CONCLUSION

Although the prevalence of diarrhoea in SARS-CoV-2 is less than that in SARS-CoV-1, there are nevertheless a large number of patients in the community who develop gastrointestinal symptoms, some of whom may never be recognised as having Covid-19. Viral RNA in stool may persist for weeks or months, however it is most abundant around the second week of illness. Concentrations are probably similar to those found in SARS-CoV-1, although the difficulties of quantification mean that comparisons to historical data must be drawn with caution. Culturable virus was less persistent. Many groups of patients with pre-existing conditions were more likely to develop diarrhoea with Covid 19, however no evidence could be found comparing the prevalence and concentration of virus detectable by PCR or culturable from faecal matter. Although diarrhoea has generally been cited as a causative factor in the SARS-CoV-1 outbreak at Amoy Gardens, several investigators have shown that the aerosolisation of viable bacteria in toilet plumes occurs at

similar rates from solid stool; there is no comparable evidence from within the BDS.

Limited data suggest that SARS-CoV-2 aerosolises less readily than SARS-CoV-1 in a Collison nebuliser, and the ongoing viability of those aerosols remains poorly characterised. Existing evidence has been gathered in a controlled laboratory setting, and while building drainage systems are persistently warm, damp and dark, other factors such as the gases, solutes, fluids and suspended solids present may also play a decisive role in bioaerosol formation and inactivation; it must also be noted that existing evidence has been gathered over the course of hours, whereas bioaerosols can transit the BDS in a matter of minutes.

Although existing evidence of virus transmission through the building drainage system pertains mostly to particles of above 5 μm, this appears to be due to limitations in the experimental methods employed. The generation of finer aerosol from sanitary and wastewater has been demonstrated from appliances and in "ex-building" wastewater transport and treatment. Independently, viable SARS-CoV-2 bioaerosols (≤5 μm) have been demonstrated, including from aqueous suspension in a nebuliser, and viral RNA has been detected on aerosol in the submicrometre range.

The available evidence would support the possibility of SARS-CoV-2 transmission through building drainage systems, however significant gaps in the research remain. The generation of viral bioaerosols has been demonstrated from many water appliances, and in a model sewer, by a range of mechanisms. Similarly, the generation and transport of bacterial bioaerosols has been demonstrated in a model building drainage system. No attempt has been found to generate viral bioaerosols in this context, but the available evidence from related studies suggests that this is likely to be possible. There are however important factors which are inadequately addressed by the existing literature. Notably, the enrichment factor of bioaerosols has been shown to be influenced by the choice of organism, the mode of droplet creation, and the presence of impurities in the water. In addition, much of the existing research has relied on sampling techniques which omit or under-report fine bioaerosols, particularly in the submicron range. The role of diarrhoea in virus aerosolisation and disease transmission also merits closer attention.

There is now at least one outbreak of Covid 19 which, like the Amoy Gardens outbreak of SARS-CoV-1, can only plausibly be explained by transmission *via* the building drainage system. There exist a range of inexpensive mitigation measures which are suitable for new-build projects and retrofitting, however their adoption is often overlooked, or even impeded, due to regulation which is contradictory, outdated and varies even within nations.

Without developing a better understanding of the underlying processes, it is impossible to say how widespread this mode of transmission might be, from Covid 19, from other viruses, and from other classes of pathogen, and what measures might best mitigate the risks from each of these. What is clear in the residential sphere at least, is that designers must take a thoughtful approach which recognises the unpredictable behaviour of building occupants, and that the regulatory environment must both facilitate and require this.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

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