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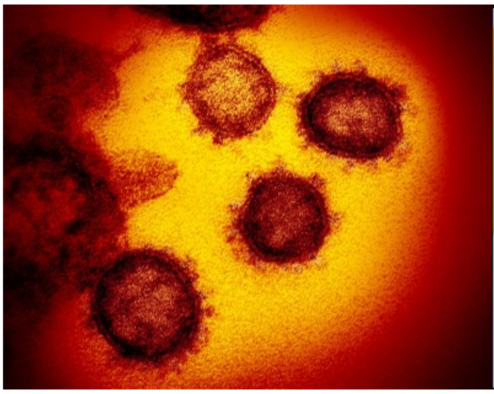
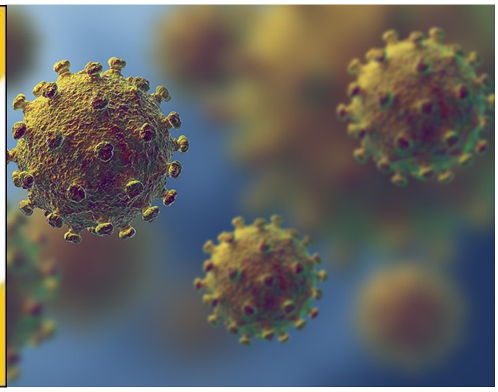
NZ Land Treatment Collective NEWSLETTER

Dedicated to improving and communicating research and technology for the land treatment of waste





Update from the NZLTC Chair and Technical Manager

Kia ora everyone

Our thoughts and best wishes are with you all as New Zealand and the rest of the world comes to terms with and unites against COVID-19.

With the health and safety of us all and the wider community in mind our annual conference was cancelled and the on-site wastewater workshop postponed. No decision has been made yet on if some conference presentations with be offered online sometime in the future.

We want to focus on providing COVID-19 peer reviewed journal papers to our NZ land treatment community. The NZLTC is fortunately enough to be members of Northwest Biosolids based at Washington University and managed by Prof Sally Brown. Sally was our 2018 keynote conference speaker and she has provided us with relevant COVID-19 journal papers. If you would like the full papers of any of these articles please get in touch with bronwyn.humphries@esr.cri.nz

Peer reviewed COVID-19 research papers related to wastewater

The AGM was scheduled to occur during the conference on the 31st March. With the cancelling of the conference we need to find a different way of sharing the year's events with the members. This will also include the election of new members to the Technical Committee. When the dust of the next few weeks settles down we will update you on the AGM process.

Please look after yourself, your family and friends and our wider community. We will be in touch when more papers become available.

Ngā mihi nui
Grant Northcott (NZLTC Chair) and Bronwyn Humphries (NZLTC Technical Manager)

NZLTC Resources

NORTHWEST BIOSOLIDS **https://nwbiosolids.org/**

The NZLTC is a member of Northwest Biosolids (University of Washington). Northwest Biosolids provide its members with exclusive access to up-to-date biosolids research and online resources. These resources are available on the NZLTC members only portal as a mix of abstracts and full papers. https://nzltc.wordpress.com/members-area/northwest-biosolids-resources/

The latest Northwest Biosolids library focus is on:
- COVID-19

If you are a member and have forgotten the members only password or would like more information about becoming an NZLTC member to gain access to these resources please contact the NZLTC Technical Manager bronwyn.humphries@esr.cri.nz

COVID-19

Title: Emerging investigators series: the source and fate of pandemic viruses in the urban water cycle

Author: Wigginton, K.R., Ye, Y. and Ellenberg, R.M
Source: Environ. Sci. Water Res. Tech. 2015 1:735-746
Abstract: Several recent high profile outbreaks such as SARS, MERS, Ebola and avian influenzas draw attention to the continued risk of a deadly viral pandemic. In general, these enveloped viruses are not considered a major threat for the wastewater and water industries due to their assumed low concentrations in municipal wastewater and high susceptibilities to degradation in aqueous environments. A number of clinical reports, however, suggest that certain enveloped viruses are excreted in human feces during infection. Furthermore, survivability studies show that many enveloped viruses are capable of retaining infectivity for days to months in aqueous environments. Here, we examine the potential presence and fate of enveloped viruses in the urban water cycle, with emphasis on coronaviruses (e.g., SARS and MERS) and avian influenza viruses. We identify a number of pressing research questions that must be answered before the water and wastewater industries can confidently assure the public, through the dissemination of evidence-based guidance, that irrigation waters, recreation waters, and drinking water sources are safe during a viral out- break or pandemic event.

Title: Enteric involvement of severe acute respiratory syndrome—associated coronavirus infection

Author: Leung, W.K., To, K.F., Chan, P.K.S., Chan, H.L.Y., Lee, W.N., Yuen, K.Y and Sung, J.J.Y
Source: Gastroenterology. 2003 125: 1011-1017
Abstract: Background & Aims: Severe acute respiratory syndrome (SARS) is a recently emerged infection from a novel coronavirus (CoV). Apart from fever and respiratory com- plications, gastrointestinal symptoms are frequently observed in patients with SARS but the significance re- mains undetermined. Herein, we describe the clinical, pathologic, and virologic features of the intestinal involvement of this new viral infection. Methods: A retrospective analysis of the gastrointestinal symptoms and other clinical parameters of the first 138 patients with confirmed SARS admitted for a major outbreak in Hong Kong in March 2003 was performed. Intestinal specimens were obtained by colonoscopy or postmortem examination to detect the presence of coronavirus by electron microscopy, virus culture, and reverse-transcription polymerase chain reaction. Results: Among these 138 patients with SARS, 28 (20.3%) presented with watery diarrhea and up to 38.4% of patients had symptoms of diarrhea during the course of illness. Diarrhea was more frequently observed during the first week of illness. The mean number of days with diarrhea was 3.7 ± 2.7, and most diarrhea was self-limiting. Intestinal biopsy specimens obtained by colonoscopy or autopsy showed minimal architectural disruption but the presence of active viral replication within both the small and large intestine. Coronavirus was also isolated by culture from these specimens, and SARS-CoV RNA can be detected in the stool of patients for more than 10 weeks after symptom onset. Conclusions: Diarrhea is a common presenting symptom of SARS. The intestinal tropism of the SARS-CoV has major implications on clinical presentation and viral transmission.

Title: Identification of viral pathogen diversity in sewage sludge by metagenome analysis

Author: Bibby, K and Peccia J
Source: Environ. Sci. Tech. 2013 47: 1945-1951
Abstract: The large diversity of viruses that exist in human populations are potentially excreted into sewage collection systems and concentrated in sewage sludge. In the U.S., the primary fate of processed sewage sludge (class B biosolids) is application to agricultural land as a soil amendment. To characterize and understand infectious risks associated with land application, and to describe the diversity of viruses in human populations, shotgun viral metagenomics was applied to 10 sewage sludge samples from 5 wastewater treatment plants throughout the continental U.S, each serving between 100 000 and 1 000 000 people. Nearly 330 million DNA sequences were produced and assembled, and annotation resulted in identifying 43 (26 DNA, 17 RNA) different types of human viruses in sewage sludge. Novel insights include the high abundance of newly emerging viruses (e.g., Coronavirus HKU1, Klassevirus, and Cosavirus) the strong representation of respiratory viruses, and the relatively minor abundance and occurrence of Enteroviruses. Viral metagenome sequence annotations were reproducible and independent PCR-based identification of selected viruses suggests that viral metagenomes were a conservative estimate of the true viral occurrence and diversity. These results represent the most complete description of human virus diversity in any wastewater sample to date, provide engineers and environmental scientists with critical information on important viral agents and routes of infection from exposure to wastewater and sewage sludge, and represent a significant leap forward in understanding the pathogen content of class B biosolids.

COVID-19

Title: Survivability, partitioning, and recovery of enveloped viruses in untreated municipal wastewater

Author: Ye, Y., Ellenberg, R.M., Graham, K.E and Wigginton, K.R

Source: Environ. Sci. Tech. 2016 50: 5077-5085

Abstract: Many of the devastating pandemics and outbreaks of the 20th and 21st centuries have involved enveloped viruses, including influenza, HIV, SARS, MERS, and Ebola. However, little is known about the presence and fate of enveloped viruses in municipal wastewater. Here, we compared the survival and partitioning behavior of two model enveloped viruses (MHV and φ6) and two nonenveloped bacteriophages (MS2 and T3) in raw wastewater samples. We showed that MHV and φ6 remained infective on the time scale of days. Up to 26% of the two enveloped viruses adsorbed to the solid fraction of wastewater compared to 6% of the two non-enveloped viruses. Based on this partitioning behavior, we assessed and optimized methods for recovering enveloped viruses from wastewater. Our optimized ultrafiltration method resulted in mean recoveries (±SD) of 25.1% (±3.6%) and 18.2% (±9.5%) for the enveloped MHV and φ6, respectively, and mean recoveries of 55.6% (±16.7%) and 85.5% (±24.5%) for the nonenveloped MS2 and T3, respectively. A maximum of 3.7% of MHV and 2% of MS2 could be recovered from the solids. These results shed light on the environmental fate of an important group of viruses and the presented methods will enable future research on enveloped viruses in water environments.

Title: Inactivation of an enveloped surrogate virus in human sewage

Author: Casanova, L.M

Source: Environ. Sci. Tech. Letters 2015 2:76-78

Abstract: Data are needed to provide guidance for handling of human sewage potentially containing infectious Ebola virus. The purpose of this research was to determine inactivation of enveloped viruses in sewage using bacteriophage Φ6 as a surrogate. Sewage was spiked with Φ6 and held at 22 or 30 °C, and the viral titer was measured over time. Inactivation was much more rapid at 30 °C than at 22 °C. At 30 °C, inactivation was approximately linear and reached 1.7 log10 in 24 h, 5 log10 by 48 h, and >7 log10 within 72 h. At 22 °C, the time to 5 log10 inactivation was 6 days and nonlinear. In sewage, Φ6 should be considered as a potential model for survival and inactivation of enveloped human viruses. The results suggest that enveloped viruses can undergo 6–7 log inactivation in sewage in 3–7 days, depending on temperature. Longer holding times may be desirable out of an abundance of caution at lower temperatures.

Title: Survival of coronaviruses in water and wastewater

Author: Pepper I.L., Gundy, P.M and Gerba, C.P

Source: Food Environ. Virol 2009 1:10-14

Abstract: The advent of severe acute respiratory syndrome and its potential environmental transmission indicates the need for more information on the survival of coronavirus in water and wastewater. The survival of representative coronaviruses, feline infectious peritonitis virus, and human coronavirus 229E was determined in filtered and unfiltered tap water (4 and 23°C) and wastewater (23°C).This was compared to poliovirus 1 under the same test conditions. Inactivation of coronaviruses in the test water was highly dependent on temperature, level of organic matter, and presence of antagonistic bacteria. The time required for the virus titer to decrease 99.9% (T99.9) shows that in tap water, coronaviruses are inactivated faster in water at 23°C (10 days) than in water at 4°C ([100 days). Coronaviruses die off rapidly in wastewater, with T99.9 values of between 2 and 4 days. Poliovirus survived longer than coronaviruses in all test waters, except the 4°C tap water.

Title: Survival of surrogate coronaviruses in water

Author: Casanova, L., Rutala, W.A., Weber, D.J and Sobsey, M.D

Source: Water Research 2009 43: 1893-1898

Abstract: The emergence of a previously unknown coronavirus infection, Severe Acute Respiratory Syndrome (SARS), demonstrated that faecally contaminated liquid droplets are a potential vehicle for the spread of a respiratory virus to large numbers of people. To assess potential risks from this pathway, there is a need for surrogates for SARS coronavirus to provide representative data on viral survival in contaminated water. This study evaluated survival of two surrogate coronaviruses, transmissible gastroenteritis (TGEV) and mouse hepatitis (MHV). These viruses remained infectious in water and sewage for days to weeks. At 25°C, time required for 99% reduction in reagent-grade water was 22 days for TGEV and 17 days for MHV. In pasteurized settled sewage, times for 99% reduction were 9 days for TGEV and 7 days for MHV. At 4°C, there was <1 log10 infectivity decrease for both viruses after four weeks. Coronaviruses can remain infectious for long periods in water and pasteurized settled sewage, suggesting contaminated water is a potential vehicle for human exposure if aerosols are generated.

Message from Prof Sally Brown
Washington University

NORTHWEST BIOSOLIDS

This Northwest Biosolids library provides articles on the fate of coronaviruses in wastewater treatment. Remember here that COVID- 19 is a new type of coronavirus. What has been reported before for this type of virus is likely to apply to COVID-19 but there are also likely some differences. This is all new and happening fast hopefully some background will help.

First the basics. With wastewater treatment and biosolids, we have typically focused on disease causing bacteria. With bacteria it is a question of good guys versus bad guys. There are a lot of great bacteria our bodies are full of them. There are a few bad bacteria. When we are attacked by the bad it is our good army versus their bad army.

You can think of this in terms of a previous blockbuster movie.

We use fecal coliform or salmonella to measure pathogen kill in biosolids. Below a certain number, their armies are way too weak to cause a problem for the good guys. Viruses are different. It only takes one or two viable bad guys to cause problems. They are not living per se, but contain DNA or RNA and start making our bodies work against us on entry. While not quite as well known as Lord of the Rings I would strongly recommend watching Osmosis Jones both for a good laugh and to better understand the way a virus can work.

Wigginton et al (2015) is a great overview paper. Coronaviruses are a subset of viruses that contain the meat of the virus - the DNA or RNA inside an outer protective shell or an envelope. A lot of recent outbreaks fall into this category including SARS, MERS and Ebola. SARS and MERs are both coronaviruses. A table from the article shows the different viruses that have come from animal sources and their fatality rates.

Table 1 Examples of viral diseases that rapidly emerged in humans, likely from an animal source. Viral diseases are listed chronologically with respect to the initial outbreak date

Outbreak/pandemic	Years	Most likely animal source	Deaths	Approximate case fatality rate	Genome type	Ref.
"Spanish" pandemic influenza H1N1	1918–1920	Unresolved	>40 million	2–3%	ssRNA	1, 5, 6
Ebola virus (EBOV)	1976–present	Unresolved	10 353[a]	50%	ssRNA	7
Avian influenza H5N1	1997–present	Birds	398[b]	60%	ssRNA	8
SARS-CoV	2002–2003	Bats	774	10%	ssRNA	9, 10
Pandemic influenza H1N1 2009	2009–2010	Unresolved	>284 500[c]	Up to 0.03%	ssRNA	11
MERS-CoV	2012–present	Unresolved	456[d]	40%	ssRNA	7
Avian influenza H7N9	2013–present	Poultry	177[e]	40%	ssRNA	8

[a] Data for 2014–15 outbreak only, as of March 24, 2015. [b] As of December 4, 2014. [c] In first 12 months of circulation. [d] As of March 26, 2015. [e] As of December 10, 2014.

In general, these viruses are not considered a threat for wastewater or wastewater treatment as most degrade easily and quickly in water. However, there are exceptions. Some avian flu viruses and some coronaviruses are able to persist in water. The authors make a critical point for these viruses to be a concern for wastewater treatment systems, they have to enter those systems. To do so people would need to shed the viruses in urine, feces or vomit. These viruses that give you the runs are enteric viruses ones that multiply in the gut and are transmitted through products of our guts. COVID-19 and all coronaviruses have a different outer structure than enteric viruses and are more susceptible to deactivation in water. These are also primarily respiratory viruses they get in your lungs, not your gut. The main way that they can get into wastewater is if you swallow your snot. Put in more scientific terminology '*the presence of respiratory virus genes in feces is thought to stem from a patient swallowing virus-laden nasal secretions*.'

The authors also point out here that just because you can find the DNA or RNA of a virus in wastewater, it doesn't mean that the virus is active. An intact and entire particle is required to be of concern. There is a section on coronaviruses with a focus on SARS. SARS was unique because although primarily a respiratory virus, diarrhea occurred in a portion of infected individuals. The other prior coronaviruses have not had a significant presence in fecal material. From what I've read about COVID-19, it is primarily a respiratory virus with little in the way of gastrointestinal symptoms being reported. Here is another table from the paper with survivability of different viruses in aqueous environments.

Table 3 Time for enveloped viruses to reach 90% inactivation (T_{90}) in aqueous environments compared to time for non-enveloped poliovirus to reach 90% inactivation

Virus	T_{90} (days)	Temp. (°C)	Matrix	Ref.
Avian influenza virus H5N1	84	20	Distilled water	75
Avian influenza virus H5N1	508	10	Distilled water	75
Avian influenza virus H5N1	19	20	Surface water	75
Avian influenza virus H5N1	61	10	Surface water	75
SARS-CoV	9	RT	Serum-free culture media	76
HCoV 229E	<1	RT	Serum-free culture media	76
HCoV 229E	2	RT	Dechlorinated tap water	77
FIPV (feline coronavirus)	<1	RT	Primary wastewater effluent	77
TGEV (swine coronavirus)	11	25	Reagent-grade water	78
TGEV	110	4	Reagent-grade water	78
TGEV	4	25	Pasteurized settled wastewater	78
TGEV	24	4	Pasteurized settled wastewater	78
MHV (murine coronavirus)	9	25	Reagent-grade water	78
MHV	>365	4	Reagent-grade water	78
MHV	3	25	Pasteurized settled wastewater	78
MHV	35	4	Pasteurized settled wastewater	78
Hantavirus	3	20	Cell culture media	79
HIV	<1	25	Primary wastewater effluent	80
Poliovirus	4	23	Primary wastewater effluent	77
Poliovirus	56	23	Mineral water	81
Poliovirus	342	4	Mineral water	81

Leung et al (2003) take an in depth look at the SARS virus. SARS is of interest here as it is a coronavirus just like COVID 19. However, it is different from COVID 19 in two critical ways the % mortality is much higher, and it is often accompanied by diarrhea. In fact, SARS was spread in one cluster (an apartment building in Hong Kong) through poorly constructed plumbing. Aerosolized fecal matter is what got them. This article talks about the presence of the virus in the intestinal tracts of victims, the prevalence of diarrhea (20%), and replication in the intestinal tract. In fact the Pepper et al (2009) paper on coronaviruses was a study on persistence in wastewater of other viruses used as surrogates for SARS.

Bibby and Peccia (2013) represent a 'sky is falling paper'. Here the authors use metagenomics to test for DNA/ RNA presence of a wide range of viruses in wastewater and sludge. They found plenty of them, including coronaviruses. The authors collected wastewater from different regions in the US and different sized plants and scoured the samples for virus DNA. The authors identified over 400,000 contiguous sequences of genetic data. Of those, 0.11% were likely associated with human viral pathogens. The most common viruses were no surprises here herpes and HPV. A reason to get the vaccine and wear a condom. Coronaviruses were also relatively common, found in 83% of the tested samples. The results are shown below in Figure 2.

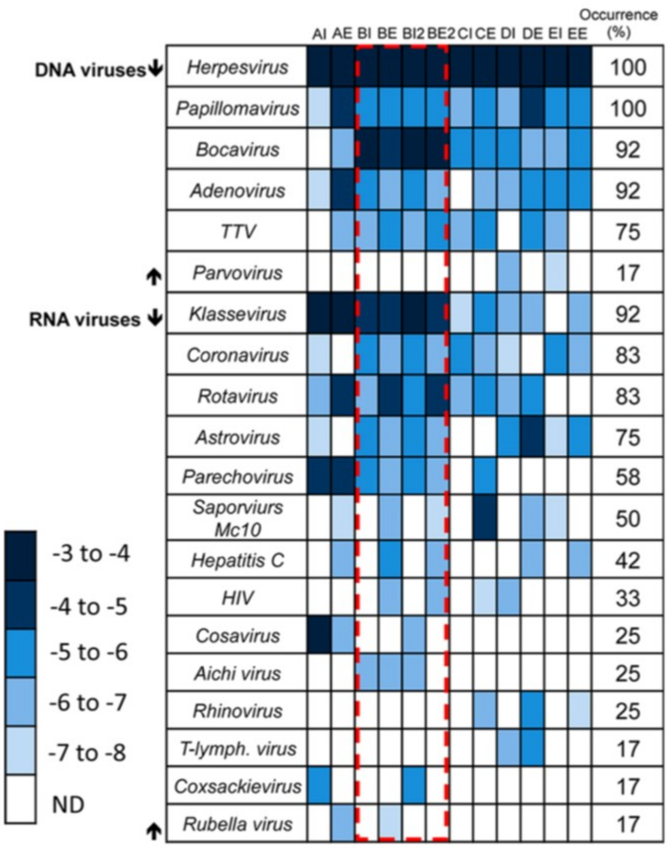
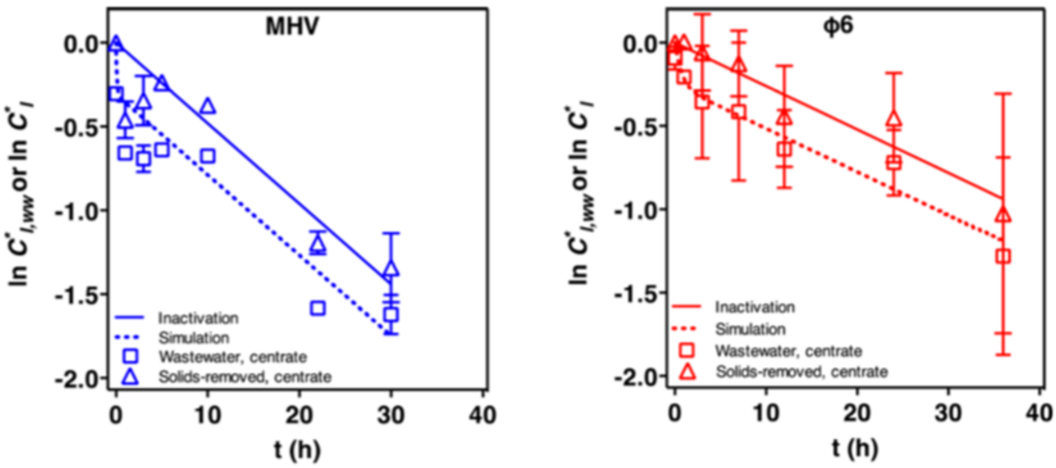


Figure 2. Heat map demonstrating the relative abundance and occurrence for human viral pathogens. Relative abundance is defined as the $\log_{10}$[reads mapped to a virus contig divided by the total reads in the sample]. The dashed box represents replicated samples. SI Tables S3 and S4 provide virus identification to the highest taxonomic level.

What is critical to remember here, and was discussed in Wigginton (2015), is that sequencing to find viruses does not have any relation to the viability of those viruses. So cry wolf all you want, let's look at viability of coronaviruses in the last two papers in the library.

Ye et al (2016) specifically addresses survivability of enveloped (includes corona) viruses in untreated municipal wastewater. They used two model enveloped viruses and two bacteriophages (viruses that infect bacteria). In unpasteurized wastewater at 25°C the two enveloped viruses lasted for 7 and 13 hours. Cool that water down and the viruses last a lot longer; 28-36 hours. Pasteurizing the water also slowed down the deactivation. The authors also found that the majority of the viruses partitioned to the liquids rather than the solids. When spiked into water containing solids, a rapid decrease was seen in the first hour. This means that viruses entering the plant in cooler climates may be active but they are almost certainly inactive in the solids and very likely inactive in the effluent. Below is the graph showing the decline of the two enveloped surrogates that they used.



Casanova (2015) confirms the results from the 4th paper. The hotter the better. But lifespan is days rather than weeks.

The take-home from these studies is that because of a number of factors wastewater and biosolids are not going to be a realistic concern for transmission of COVID-19. I hope that this is of some help and hope. Remember to social distance and to wash your hands!

If you would like to sign up for our quarterly newsletters or to become a member of the NZLTC please email: bronwyn.humphries@esr.cri.nz

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