

Improving current enteric viral surveillance through wastewater monitoring



Executive Summary

Enteric viruses are becoming increasingly commonplace in both community and healthcare settings. Clinical samples, collected from hospitals and diagnostic laboratories, are generally used to trace and identify viral outbreaks. However, by sampling wastewater, enteric viruses can be identified at a population level. This is an alternative to relying on medical/health data, which can be limited due to a high proportion of individuals not seeking medical attention for gastroenteritis. Not only does wastewater sampling provide information on the risk of water contamination, it can also be used to determine the effectiveness of pathogen removal in wastewater treatment systems.

Viral gastroenteritis

Diarrhoeal diseases continue to be a major global health problem despite the improved sanitation and hygiene around the world. It is the leading cause of death in children under the ages of five (1), with an estimated 1.3 million deaths each year (2). Bacteria are widely known as a major cause of diarrhoea in contaminated water. However, what is less recognised are viruses in water sources and their impact on human health. Norovirus is the leading cause of viral gastroenteritis (3, 4) and affects people of all ages worldwide (5). It is estimated to cause 677 million cases each year with 212,000 deaths around the globe (6). Another important viral agent of gastroenteritis is human mastadenovirus (HAdV). In addition to gastroenteritis, HAdV can cause a wide range of human diseases; such as respiratory illnesses, conjunctivitis and, in more severe cases, disseminated diseases (7). Noroviruses account for approximately 20% of global gastroenteritis cases (3), whilst enteric HAdV causes up to 15% of all acute gastroenteritis (8, 9). Both of these viruses are classified to have moderate to high health significance by the World Health Organisation (WHO).

Despite the self-limiting nature of viral gastroenteritis, severe outcomes such as dehydration and death can occur in vulnerable populations. Outbreaks commonly occur in semi-enclosed environments such as nursing homes, childcare centres, hospital wards and military camps (10-12). With the lack of preventative measures and treatments available for norovirus and HAdV infections, continuous surveillance is vital to identify circulating and emerging strains. This can help predict the emergence of a novel strain or potential epidemic/pandemic virus, and better precautions can be taken to prevent viral gastroenteritis outbreaks.

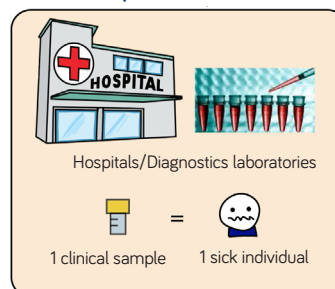
Current enteric viral surveillance system

Molecular surveillance of enteric viruses is commonly conducted using clinical samples collected from hospitals and diagnostic laboratories, where the patients are presented with clinical symptoms. However, norovirus and HAdV-associated gastroenteritis are generally self-limiting and therefore many do not seek medical advice. Consequently, the genetic diversity of norovirus and HAdV determined using clinical samples are only representative of symptomatic cases or individuals within the high-risk groups, i.e. children, elderly and immunocompromised

High-level facts

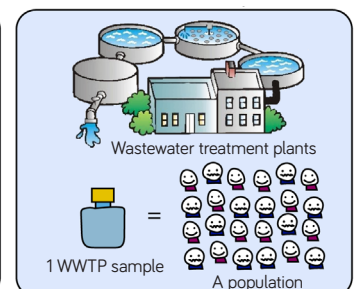
- Acute gastroenteritis-associated outbreaks commonly occur in semi-enclosed environments, such as cruise ships, hospital wards, childcare centres and nursing homes. The results of these can be damaging as they can easily affect hundreds of people.
- Norovirus is the leading cause of viral gastroenteritis around the globe, with an estimated 212,000 deaths each year and 677 million infections worldwide.
- Human mastadenovirus (HAdV) can cause a wide range of human diseases; in addition to gastroenteritis, it can cause respiratory illnesses, conjunctivitis and hepatitis.
- The use of wastewater samples for surveillance can provide a more comprehensive representation of circulating enteric viruses within a population, including both symptomatic and asymptomatic cases.
- This study found the ongoing presence of high levels ($> 10^7$ genome copies/L) of nucleic acid (norovirus RNA and HAdV DNA) in wastewater at three sewage treatment plants from Sydney and Melbourne, Australia.
- The viral nucleic acid of norovirus and HAdV were quantified in the wastewater system, however, it is not representative of the number of viable or infectious particles presented. Further studies will need to be conducted to examine their abilities to cause infections.

Clinical samples



▶ Representative of symptomatic cases only

Wastewater samples



▶ Representative of asymptomatic & symptomatic cases



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individuals, who are more likely to seek medical attention. Therefore, there is a need for a new approach to the current surveillance system where the symptomatic and asymptomatic cases can both be monitored at a population level.

Environmental norovirus and HAdV

Enteric viruses are commonly found in the external environments. The persistence of enteric viruses in the external environment is due to two main factors; (a) high levels of virions are shed from both symptomatic and asymptomatic individuals (13, 14), and (b) enteric viruses are resistant to commonly used disinfectants and degradation (15, 16). This results in an abundance of enteric viruses in the wastewater system, making it a suitable sample type for comprehensive molecular epidemiological study. Furthermore, enteric viruses have been commonly associated with waterborne outbreaks (17, 18). These viruses are transmitted through the faecal-oral route and when the quality of the water source is compromised, it can lead to a huge waterborne outbreak, affecting hundreds and thousands of individuals (19-21).

Quantification of norovirus and HAdV in Australia

Water treatment utilities routinely assess the presence of faecal coliform and other bacteria in water, however, the number of viruses present is rarely examined. The prolonged and high viral shedding from infected individuals after symptoms have subsided (14), allows norovirus and HAdV to persist in external environment and thus are both commonly detected in wastewater from sewerage systems around the globe (22-26). However, the level of norovirus RNA and HAdV DNA have not been systematically, routinely and widely measured in Australian wastewater systems. Using quantitative polymerase chain reaction (qPCR)-based methods, the total viral genome levels of norovirus and HAdV in the Australian wastewater system were determined.

Over the two-year period, an average of 1.5×10^7 norovirus genome copies/L was detected across three wastewater treatment plants (WWTPs) in Sydney and Melbourne, Australia. Despite the increase of norovirus-related outbreaks during the winter seasons, no seasonality was observed throughout the two-year period in the wastewater. HAdV was also measured across the three different WWTPs, with an average of 1.8×10^7 genome copies of HAdV DNA detected per litre of wastewater. Similarly, no seasonality was observed.

The genetic diversity of norovirus and HAdV in Australia

The advancement in next generation sequencing (NGS) technologies has revolutionised pathogen genomic sequencing, enabling the ability to generate a large amount of data at a much lower cost with increased turnaround time. Prolonged and high viral shedding of norovirus and HAdV is observed in infected individuals, making wastewater an ideal source for enteric virus molecular surveillance.

With this technology, the genetic diversities of norovirus and HAdV were characterised in complex wastewater samples collected from Sydney and Melbourne, Australia. Using the Illumina MiSeq sequencing platform, a more diverse range of norovirus genotypes ($n=19$) and HAdV

serotypes ($n=19$) were identified in wastewater, compared to clinical samples (norovirus=10, HAdV=7), highlighting the benefit of using NGS technology. In addition, the dominant strain of norovirus and HAdV observed in wastewater samples complemented those identified in clinical samples, showcasing the benefit of using wastewater samples for more comprehensive enteric virus surveillance.

Summary and future implications

This study demonstrated the ability to efficiently detect, identify and quantify enteric viruses in wastewater systems, thus providing a more complete picture of the circulating enteric viruses at a population level. The methodologies used can both be applied in the water industry and health sector for quantification of enteric viruses and viral diversity in wastewater samples. By understanding the viral concentration of enteric viruses in wastewater, not only does it provide information on the risks involved in the case of water contamination, it can also be used to determine the effectiveness of pathogen removal in wastewater treatment systems. Furthermore, it can mitigate health hazards for workers at wastewater treatment plants.

The genetic diversity of norovirus and HAdV identified in wastewater reflected greatly on what was observed in clinical samples, suggesting the molecular surveillance of enteric viruses at a population level can be enhanced by the use of the two samples types. The dominant norovirus genotype was observed in both wastewater and clinical samples, and in some cases, the genotype dominated in wastewater samples months prior to its dominance in clinical samples. This signifies wastewater can also be used to identify emerging viruses, demonstrating the practicality of using wastewater sample as a source for molecular epidemiological studies.

It should be noted that the studies only examined the viral nucleic acid to look at the pattern of occurrence and genotype prevalence. Future studies could consider viability and infectivity which, for some viruses, such as HAdV, is an option, although at present in vitro infectivity assays for norovirus are still not in routine use.

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