Detection of human adenovirus F41 in wastewater and its relationship to clinical cases of acute hepatitis of unknown aetiology

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Short Communication

Detection of human adenovirus F41 in wastewater and its relationship to clinical cases of acute hepatitis of unknown aetiology

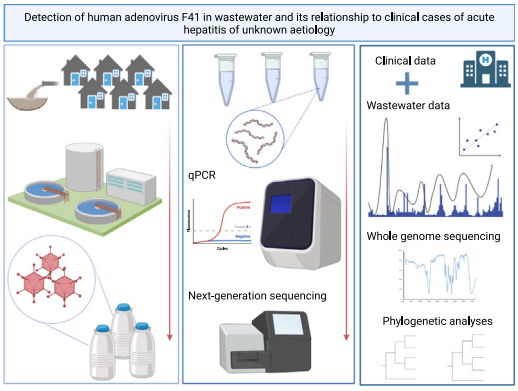
Marina I. Reyne [a,*], Danielle M. Allen [a], Ashley Levickas [a], Pearce Allingham [a], Jonathan Lock [a], Arthur Fitzgerald [a], Cormac McSparron [b], Behnam F. Nejad [b], Jennifer McKinley [b], Andrew Lee [a], Stephen H. Bell [a], Joshua Quick [c], Charlotte J. Houldcroft [d], Connor G.G. Bamford [e], Deirdre F. Gilpin [f], John W. McGrath [a]

[a] School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, Northern Ireland, UK
[b] School of Natural and Built Environment, Queen's University Belfast, Elmwood Avenue, Belfast BT9 6AZ, Northern Ireland, UK
[c] School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[d] Department of Genetics, University of Cambridge, Cambridge CB2 3EH, UK
[e] Wellcome-Wolfson Institute for Experimental Medicine (WWIEM), School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK
[f] School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

HIGHLIGHTS

- Correlation between Adenovirus (HAdV) F41 detection in wastewater and reported cases of acute hepatitis of unknown aetiology.
- HAdV-F41 sequences from wastewater belong to F41 lineage 2b.
- HAdV-F41 genome sequences from wastewater were homologous to sequences obtained from clinical cases.
- Wastewater detection presents an effective tool for community surveillance of HAdV-F41.

GRAPHICAL ABSTRACT



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ABSTRACT

As of 8 July 2022, the World Health Organization (WHO) have reported 1010 probable cases of acute hepatitis of unknown aetiology in children worldwide, including approximately 250 cases in the United Kingdom (UK). Clinical presentations have often been severe, with liver transplantation a frequent clinical outcome. Human adenovirus F41 (HAdV-F41) has been detected in most children with acute hepatitis, but its role in the pathogenesis of this infection has yet to be established. Wastewater-based epidemiology (WBE) has become a well-established tool for monitoring the community spread of SARS-CoV-2, as well as other pathogens and chemicals. In this study, we adopted a WBE approach to monitoring levels of HAdV-F40/41 in wastewater before and during an acute hepatitis outbreak in Northern Ireland. We report increasing detection of HAdV-F40/41 in wastewater, concomitant with increasing numbers of clinical cases. Amplicon whole genome sequencing further classified the wastewater-derived HAdV as belonging to the

* Corresponding author.
E-mail address: m.reyne@qub.ac.uk (M.I. Reyne).

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F41 genotype which in turn was homologous to clinically derived sequences. We propose that WBE has the potential to inform community surveillance of HAdV-F41 and can further contribute to the ongoing global discussion supporting HAdV-F41 involvement in acute hepatitis cases.

1. Introduction

As of 8 July 2022, 1010 probable cases of acute hepatitis of unknown aetiology in children have been reported to the World Health Organization (WHO). Clinical presentations have often been severe, with liver transplantation a frequent clinical outcome (Kelgeri et al., 2022; UKHSA, 2022; WHO, 2022); in the United Kingdom (UK) 15 of 274 cases (as of 4 July 2022), resulted in liver transplant (UKHSA, 2022). Although the aetiology contributing to this acute hepatitis has yet to be definitively identified and is under international investigation (ECDC, 2022; Mücke and Zeuzem, 2022), human adenovirus (HAdV) has, in many cases, been the most common pathogen detected (Gutierrez Sanchez et al., 2022). Within the UK, 65.9 % of samples from clinical cases of acute hepatitis in children were positive for HAdV-F41 (Benko et al., 2022), a genotype not previously linked to severe acute hepatitis in healthy children.

Wastewater-based epidemiology (WBE) has proved to be an effective tool to monitor the levels of circulating pathogens within a population, as has been most recently demonstrated for SARS-CoV-2 (Ahmed et al., 2020; Hemalatha et al., 2021; Wade et al., 2022). Indeed, studies have shown that SARS-CoV-2 RNA concentrations significantly correlate with clinical cases and hospitalisation within the same wastewater catchment area (Galani et al., 2022), while also showing that the appearance and spread of circulating variants within the community can be tracked over time (Gregory et al., 2022; Reynolds et al., 2022). WBE has also been used to track a range of other pathogens (e.g., norovirus, poliovirus, hepatitis A), antimicrobial resistance genes and chemical compounds such as illicit drugs and food toxins (Sims and Kasprzyk-Hordern, 2020).

In this study, we utilised a WBE approach to assess HAdV-F40/41 DNA concentrations in wastewater before and during an acute hepatitis outbreak in Northern Ireland (NI). Furthermore, using amplicon whole-genome sequencing we compared wastewater-derived HAdV-F41 sequences to those originating from clinical cases.

2. Materials and methods

HAdV-F40/41 concentrations were quantified monthly in samples of primary influent from nine wastewater treatment plants (WWTP), covering 36 % of the Northern Irish population, over the period March 2021–June 2022 (Fig. A.1). Sites were selected based on them being the largest WWTPs within the five NI Healthcare Trusts: four additional WWTPs were chosen to ensure greater geographical distribution across NI. Wastewater samples (50 ml) were concentrated using CP select™ ultrafiltration tips (InnovaPrep LLC) and nucleic acid was extracted using a MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Diagnostic Limited). HAdV-F40/41 DNA was detected in duplicate samples by qPCR (LightCycler 480 Roche Diagnostics Germany) as previously described by Rajal et al. (2007). A final reaction volume (25 μl) containing TaqMan™ Environmental Master Mix (Applied Biosystems™) (12.5 μl), Bovine Serum Albumin (Sigma-Aldrich) (0.25 μl), forward and reverse primers (400 nM), probe (80 nM), and DNA extract (3 μl) was used. Thermal cycling parameters were 95 °C for 10 min, 45 cycles at 95 °C for 15 s, and 60 °C for 1 min. A positive wastewater sample (Cp value: 24), was confirmed as HAdV-F40/41 via Sanger sequencing and quantified using digital droplet PCR (ddPCR; BioRAD QX200): final reaction volume (22 μl) containing Supermix for Probes (no dUTP) (Bio-Rad 1,863,024) (11 μl), primers (400 nM), probes (80 nM), and DNA extract (3 μl). ddPCR was performed using the Bio-Rad QX200 platform (with AutoDG Droplet Generator and C1000 Touch Thermal Cycler) using the following cycling conditions: 95 °C for 10 mins, 40 cycles at 94 °C for 30 s and 60 °C for 1 min, and 98 °C for 10 min. Thresholding was carried using the QX Manager Software (Bio-Rad, v1.2). Serial dilutions from the quantified wastewater sample were used to generate a standard curve and calculate the Limit of Detection (LOD) of the HAdV-F40/41 assay. HAdV-F401/41 DNA concentrations were normalised using wastewater flow and expressed as gene copies (gc) per 100,000 population equivalents (p.e.) per day. Clinical case data for NI was obtained from UKHSA technical report (UKHSA, 2022) which did not detail patient demographic information or geolocation. The relationship between HAdV-F40/41 DNA concentration in wastewater and clinical cases was determined using linear regression. Data visualization and analyses were performed in R v4.2.1 (R Core Team, 2022).

To delineate between HAdV-F40 and HAdV-F41 within the wastewater, five positive samples from May 2022 were selected for overlapping-amplicon whole-genome sequencing using the recently developed HAdV-F41 ARTIC primers (Maes et al., 2022); https://github.com/quick-lab/HAdV) following the Mini-XT SARS-CoV-2 protocol (Fuchs et al., 2022). Sequencing was performed with a MiSeq reagent kit v2 (500 cycles). Short reads were pre-processed by removing adaptors and low-quality reads (below Q30). Reads representing each sample were mapped onto the HAdV-F41 "Tak" genome (GenBank DQ315364) (Lemiale et al., 2007) to generate consensus sequences. All available HAdV-F41 genomes on GenBank >20 Kilobase pairs were downloaded (16 June 2022), including the Tak strain (GenBank DQ315364), as well as two sequences from paediatric cases of acute hepatitis of unknown aetiology: one from the UK (kindly provided by Prof. Emma Thomson and Dr. Richard Orton from the MRC-University of Glasgow Centre for Virus Research) and one from Spain (GenBank ON561778). All sequences were aligned using MAFFT (Katoh and Standley, 2013) and manually checked in MEGA11 (Tamura et al., 2021). All sequences were aligned to published genomes using MAFFT with the –add and –keep length options. Maximum likelihood trees were generated in IQ Tree with default settings and 1000 bootstraps (Hoang et al., 2017; Kalyaanamoorthy et al., 2017; Nguyen et al., 2014). [IQ Tree options: path_to_iqtree -s BelfastCVR_160622.fas -st DNA -m TEST -bb 1000 -alrt 1000]. The consensus ML tree was visualised using IcyTree (Vaughan, 2017).

3. Results and discussion

In total, 144 wastewater samples were retrospectively screened for the presence of HAdV-F40/41 DNA between March 2021 and June 2022 with 114 (79.2 %) testing positive. The LOD for HAdV-F40/41 DNA detection in wastewater was 3 copies per reaction. HAdV-F40/41 DNA was first detected in April 2021 (4.38×10^6 gc per 100 k population equivalents) with concentrations peaking in April 2022 after a 4-log increase (3.35×10^{10} gc per 100 k p.e.) when compared to those concentrations observed in November 2021 (Fig. 1.A; Table 1). Across this sampling time period, 23 clinical cases of HAdV-F41 were detected in NI with the clinical peak observed in May 2022 (11 cases) (UKHSA, 2022). This correlates with the temporal pattern of presumptive HAdV-F40/41 gene fragment detection within wastewater ($t = 3.132$, d.f. $= 15$, $p = 0.007$). The strength of this relationship was moderate ($R^2 = 0.64$) potentially as a result of the small sample size ($n = 16$). Prior to the first reported cases of acute hepatitis in children viral loads of 6 log were detected from April 2021 to November 2021. This likely indicates detection of sub-acute infections and the start of an HAdV outbreak across the province.

To compare the wastewater derived HAdV-F40/41 DNA with those sequences obtained from clinical cases, amplicon whole-genome sequencing was carried out. Sequencing depth (per base) and the number of reads mapped to the reference genome varied between samples with an average

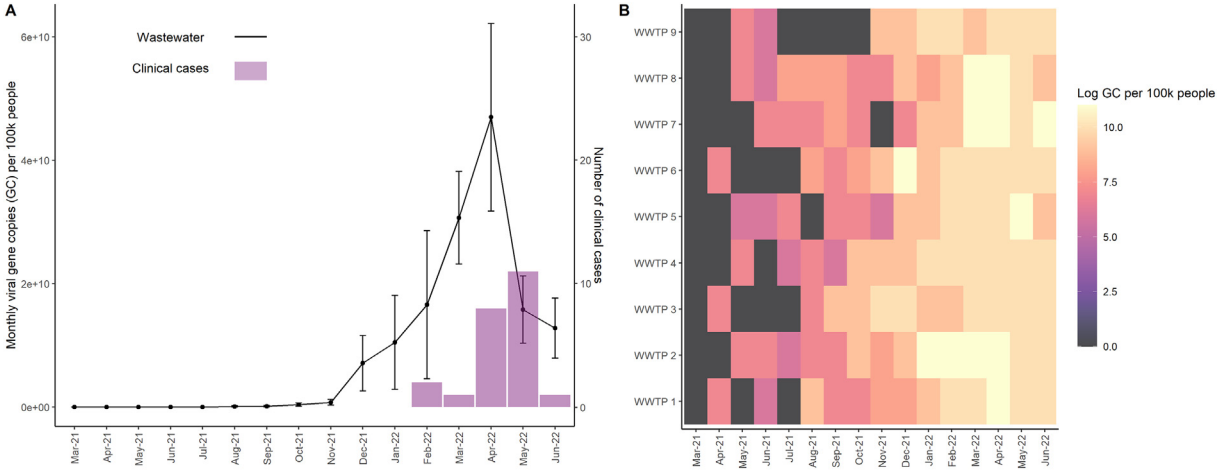

Fig. 1. Concentration of HAdV-F40/41 DNA (gene copies per 100 k people) in wastewater across Northern Ireland (NI) from March 2021 to June 2022. A) Monthly averages and error bars indicating standard error. Histogram showing the number of clinical cases for NI during the same period (UKHSA, 2022). B) Heatmap visualization of HAdV-F40/41 DNA gene copies per 100 k people at a log scale by Wastewater Treatment Plant (WWTP) versus month.

± SD of 995.4 ± 341.1 and 200,826 ± 76,408, respectively (Table A.1). Genome coverage was high (>80 % for 4 samples with only one sample showing lower coverage - 54.45 %). A drop in coverage around 18-20 kb (L3 hexon) and 21-24 kb (L4 hexon assembly protein) was observed in all samples, likely due to amplicon dropout. Annotated sequences generated within the study were deposited in GenBank (accession numbers ON923973-77). Phylogenetic analysis assigned these sequences to HAdV F41 lineage 2b alongside sequences obtained from clinical hepatitis-associated viruses, and several non-hepatitis clinical viruses sampled across Europe over recent years (Fig. 2). Further phylogenetic analyses of the immunogenic hexon, long fiber, penton and short fiber genes are shown in Fig. B.1–B.4: each show high similarity between wastewater and clinical sequences. Sequences of HAdV-F41 from May 2022 and an HAdV-F41 sequence obtained from an archived wastewater sample from November 2021 were also identical, with no non-synonymous changes present (data not shown).

The detection of increasing concentrations of HAdV-F40/41 DNA in wastewater via qPCR (and its subsequent assignment to the HAdV-F41 lineage 2b), alongside the concomitant increase in NI acute hepatitis cases from which HAdV-F41 lineage 2b was also detected, adds further weight to the ongoing global discussion supporting HAdV-F41 involvement in acute hepatitis of unknown aetiology in children. Several hypotheses are being actively explored as to why increased cases of acute hepatitis may have occurred including the removal of pandemic imposed "non-pharmaceutical interventions" (e.g., social distancing, masks, regular hand washing etc.) leading to increased HAdV-F41 exposure amongst susceptible individuals. Indeed, several studies have observed an increase in incidence rates of other infections post-pandemic, with mathematical models suggesting more intense epidemics in the coming years (Amar et al., 2022; Baker et al., 2020; Cohen et al., 2021).

As clinical surveillance of adenovirus at a community level is challenging, with laboratory testing often only occurring after the manifestation of usually severe symptoms, this data suggests that wastewater surveillance could provide a useful and holistic tool for the community surveillance of HAdV-F41. Moreover, this study further supports the growing evidence that WBE can be used as an effective public health strategy for the community-wide surveillance of pathogens other than just SARS-CoV-2, to provide ongoing evidence and timely information to help inform public health decision-making.

Table 1
Monthly average concentration of HAdV-F40/41 DNA (gene copies per 100 k people) in wastewater across Northern Ireland (NI) from March 2021 to June 2022.

Month	Monthly viral gene copies per 100 k people	Standard deviation	Standard error
Mar-21	0.00E+00	0.00E+00	0.00E+00
Apr-21	4.38E+06	7.41E+06	2.47E+06
May-21	3.33E+06	3.76E+06	1.25E+06
Jun-21	4.51E+06	7.12E+06	2.37E+06
Jul-21	8.95E+06	1.42E+07	4.73E+06
Aug-21	1.25E+08	3.23E+08	1.08E+08
Sep-21	1.62E+08	3.79E+08	1.26E+08
Oct-21	4.02E+08	7.72E+08	2.57E+08
Nov-21	7.87E+08	1.40E+09	4.67E+08
Dec-21	7.12E+09	1.35E+10	4.48E+09
Jan-22	1.05E+10	2.29E+10	7.62E+09
Feb-22	1.66E+10	3.61E+10	1.20E+10
Mar-22	2.73E+10	2.11E+10	8.12E+09
Apr-22	3.35E+10	3.33E+10	1.28E+10
May-22	1.65E+10	1.61E+10	5.76E+09
Jun-22	1.28E+10	1.46E+10	4.87E+09

CRediT authorship contribution statement

Marina I. Reyne: Conceptualization, Methodology, Investigation, Validation, Data curation, Formal analysis, Visualization, Writing – original draft. **Danielle M. Allen:** Conceptualization, Investigation, Validation, Data curation, Formal analysis, Writing – review & editing. **Ashley Levickas:** Data curation, Formal analysis, Writing – review & editing. **Pearce Allingham:** Data curation, Formal analysis, Writing – review & editing. **Jonathan Lock:** Investigation, Validation, Writing – review & editing. **Arthur Fitzgerald:** Investigation, Validation, Writing – review & editing. **Cormac McSparron:** Data curation, Formal analysis, Writing – review & editing. **Behnam F. Nejad:** Data curation, Formal analysis, Writing – review & editing. **Jennifer McKinley:** Funding acquisition, Supervision, Project administration, Writing – review & editing. **Andrew Lee:** Investigation, Validation, Writing – review & editing. **Stephen H. Bell:** Investigation, Validation, Writing – review & editing. **Joshua Quick:** Methodology, Writing – review & editing. **Charlotte J. Houldcroft:** Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – review & editing. **Connor G.G. Bamford:** Conceptualization, Methodology, Writing – review & editing. **Deirdre F. Gilpin:** Funding acquisition, Supervision, Project administration, Writing – review & editing. **John W. McGrath:** Conceptualization, Funding acquisition, Supervision, Project administration, Writing – review & editing.

Data availability

GenBank ON923973-77

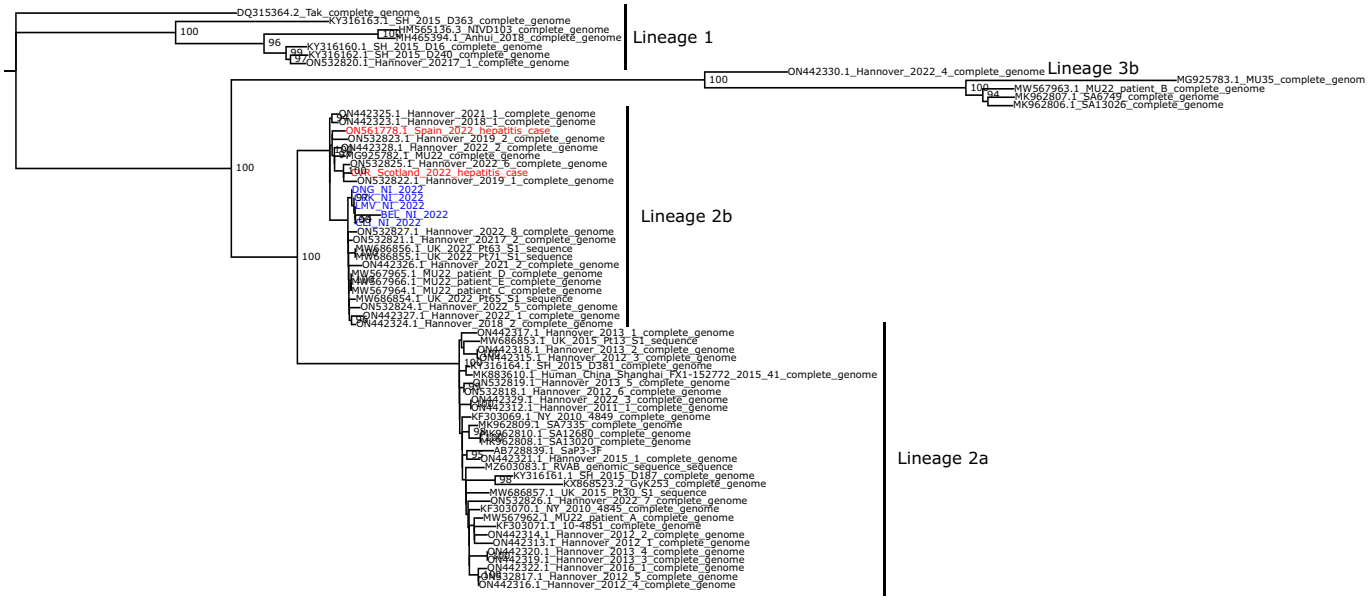

Fig. 2. Phylogenetic tree of whole genome sequences human adenovirus F41 with major lineages annotated. Viral genomes associated with cases of paediatric hepatitis are shown in red; sequences newly generated for this study are shown in blue. Only nodes with bootstrap support >90 are labelled.

Declaration of competing interest

None.

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Ethical statement

This study was conducted using environmental samples and publicly available data. Under UK law, there is no requirement for ethical approval for these samples or data.

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